# CENTER FOR DRUG EVALUATION AND RESEARCH

***APPLICATION NUMBER:***

# 205003Orig1s000

# PHARMACOLOGY REVIEW(S)

**DEPARTMENT OF HEALTH AND HUMAN SERVICES**
**PUBLIC HEALTH SERVICE**
**FOOD AND DRUG ADMINISTRATION**
**CENTER FOR DRUG EVALUATION AND RESEARCH**

# PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

| | |
|---|---|
| Application number: | 205003 |
| Supporting document/s: | 1 and 8 |
| Applicant's letter date: | 3/21/2014 |
| CDER stamp date: | 4/25/2014 |
| Product: | Prestalia (perindopril arginine/amlodipine besylate) tablets |
| Indication: | Treatment of hypertension |
| Applicant: | Symplmed Pharmaceuticals, LLC (Symplmed) |
| Review Division: | Division of Cardiovascular and Renal Products |
| Reviewer: | Baichun Yang, PhD, DABT |
| Supervisor/Team Leader: | Thomas Papoian, PhD, DABT |
| Division Director: | Norman L Stockbridge, MD, PhD |
| Project Manager: | Wayne S Amchin |

## Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA205003 are owned by Symplmed or are data for which Symplmed has obtained a written right of reference. Any information or data necessary for approval of NDA205003 that Symplmed does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA205003.



Reference ID: 3604317

## TABLE OF CONTENTS





Reference ID: 3604317

Reference ID: 3604317

## Table of Tables





Reference ID: 3604317

## Table of Figures





Reference ID: 3604317

# 1 Executive Summary

## 1.1 Introduction (and Clinical Rationale)

The calcium channel blocker (CCB) amlodipine and angiotensin-converting enzyme (ACE) inhibitor perindopril have both been widely used for more than 15 years in countries worldwide. Both CCBs and ACE inhibitors lower blood pressure by reducing peripheral resistance. Blockade of calcium influx and reduction of angiotensin II-mediated vasoconstriction are complementary mechanisms. The combination of perindopril and amlodipine is recommended by the medical practice guidelines as being safe and effective (1). Both perindopril erbumine (ACEON®) and amlodipine besylate (NORVASC®) were approved in the US for the treatment of hypertension and coronary artery disease. The perindopril arginine/amlodipine besylate combination drug product COVERAM® is approved and is currently marketed in the European Union. The sponsor is developing the perindopril arginine/amlodipine besylate combination drug product Prestalia for treatment of hypertension in the US.

## 1.2 Brief Discussion of Nonclinical Findings

Because perindopril arginine had never been marketed in the US, bridging preclinical studies were performed to evaluate pharmacokinetic (PK) and safety profiles of perindopril arginine (PERa) with a comparison to perindopril erbumine (PERe). Similar to PERe, PERa was hydrolyzed to the active metabolite perindoprilat. Other metabolites of both PERe and PERa are perindopril glucuronide (Y 1303) and perindoprilat glucuronide (Y 1304). These metabolic steps occur with both oral and IV administration of PERa. PK profiles are similar between PERa and PERe following single- and multiple-dose oral administration in rats and dogs. In 28-day repeat dose study in dogs, oral PERa resulted in decreases in red blood cell counts and hemoglobin, and mild medulla and/or papilla mineralization in kidneys, which were not seen in PERe-treated dogs (NOAEL 0.83 mg/kg/day, ~2 times the recommended human maximal dose of PERa 14 mg in Prestalia 14/10 mg tablet for a 60-kg human on a body surface basis). Other findings in stomach (erosions and/or ulcerations, in rats), bronchus (minimal to mild bronchopneumonia or chronic peri-bronchiolitis, in dogs), serum chemistry, and urinalysis were similar between treatments with PERa or PERe in 28-day repeat dose oral studies in rats and dogs. PERa was not mutagenic in either in vitro or in vivo genotoxic assays.

In a 13-week repeated oral dose toxicity rat study with PERa, amlodipine besylate (AMLb), or PERa/AMLb, higher incidences of the following were observed in males and/or females at PERa/AMLb 15/8 mg/kg/day, PERa 15 mg/kg/day, and/or AMLb 8 mg/kg/day: sporadic sialism, lower mean body weights, higher urine volume associated with lower urine specific gravity, higher urinary chloride excretion and lower serum chloride concentrations associated with a minimal increase in serum urea concentrations, lower serum calcium concentrations, higher kidney weights, minimal hypertrophy of the juxtaglomerular apparatus, thickening of the wall of the afferent and efferent arterioles of the glomeruli in the kidneys, higher incidence of foci of basophilic (regenerating) tubules in the kidneys, and increased incidences and severity of diffuse hypertrophy/vacuolation of the zona glomerulosa of the adrenal glands . However, neither new target organs nor relevant additive effects were identified with the combination of PERa and AMLb, there were no TK drug-drug interactions between PERa and AMLb. NOAEL in the 13-week study was 7.5/4 mg/kg of PERa/AMLb, which is ~4-5 times the recommended human maximal dose of Prestalia 14/10 mg tablet for 60-kg human on a body surface basis.

Qualification studies were performed for impurities [redacted] in AMLb. AMLb spiked with impurities [redacted] each showed similar toxicological profile (dilation of small intestines with or without ulcerative ileitis and enteritis, changes in electrolytes, and higher heart weight) to that of AMLb in a 4-week oral repeat dose study in rats. Impurities [redacted] spiked in to parent compound at [redacted] each, were not mutagenic in in vitro genotoxic assays.

Qualification studies were also performed for impurities [redacted] in PERa. PERa spiked with impurities [redacted], or spiked with impurity [redacted] did not show mutagenic potential in the AMES test or the mouse lymphoma thymidine kinase gene mutation assay. In the 4-week repeated oral dose toxicity study in rats, treatment with PERa containing impurities [redacted] was associated with minimal but dose-related lower glucose levels in both sexes (about -19% at 33 mg/kg/day, $p<0.05$ vs control). Treatment of rats with oral PERa or PERa containing [redacted] for 4 weeks resulted in higher incidences of minimal hypertrophy of kidney juxtaglomerular apparatus and minimal to slight focal erosion of glandular stomach at the dose 33 mg/kg/day. The changes were similar between PERa alone and PERa containing [redacted]. NOAELs of PERa alone, PERa containing [redacted], and PERa containing [redacted] in these repeat oral dose studies in rats were 8 mg/kg/day, which is 5.5 times the recommended human maximal dose of PERa 14 mg in Prestalia 14/10 mg tablet for a 60-kg human on a body surface basis.

Thus, the nonclinical studies demonstrated the similarity, in general, between the arginine and erbumine salts of perindopril in terms of PK and safety profile. No new target organs or relevant additive effects were identified with the combination of PERa and AMLb, and there were no TK drug-drug interactions between PERa and AMLb. The safety profile of AMLb with impurities [redacted] each was similar to that of AMLb. PERa containing impurities [redacted] or containing impurity [redacted] did not demonstrate mutagenic potential, and showed toxicity profiles generally similar to PERa alone in rats. No new issue of safety concern was identified from the results of these preclinical studies. Providing no structural alerts for genotoxicity are shown for these 5 impurities, specifications for drug substance impurities [redacted] (3.2.S.4.1) and specifications for drug product impurities [redacted] are qualified.

## 1.3 Recommendations

### 1.3.1 Approvability

Yes

### 1.3.2 Additional Non Clinical Recommendations

None

### 1.3.3 Labeling

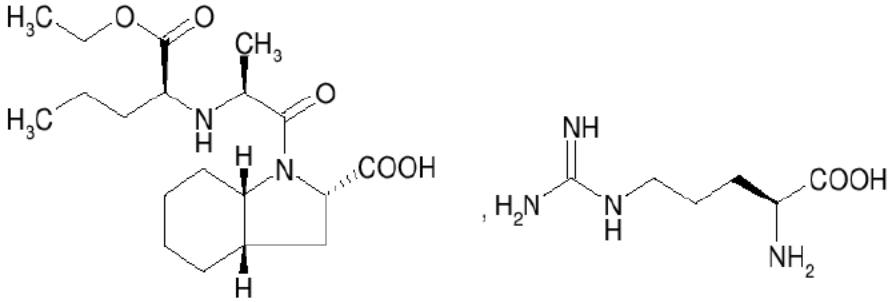
None

## 2 Drug Information

### 2.1 Drug - Prestalia (perindopril arginine/amlodipine besylate) tablets
**Drug code: XOMA 985**
**Drug class: Anti-hypertensive agent**

Generic Name: **perindopril arginine**
Chemical Name: (b) (4)

CAS Registry Number: 612548-45-5
Code Name: S 9490-6, S 06490, S 6490, S 09490-6, PERa
Molecular Formula/Molecular Weight: $C_{19}H_{32}N_2O_5$, $C_6H_{14}N_4O_2$/542.7 (salt form) or 368.5 (free acid)
Structure or Biochemical Description:



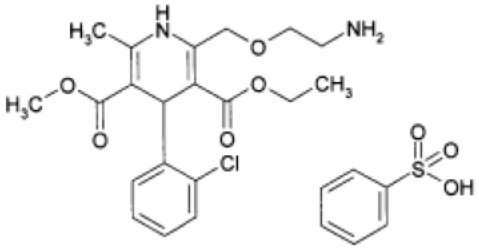
Pharmacologic Class: Angiotensin Converting Enzyme Inhibitor

Generic Name: **amlodipine besylate**
Chemical Name: 3-ethyl-5-methyl (±)-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylate, monobenzenesulphonate

(b) (4)

CAS Registry Number: 111470-99-6
Code Name: AMLb, AMLO
Molecular Formula/Molecular Weight: $C_{20}H_{25}ClN_2O_5 \cdot C_6H_6O_3S$/ 567.1 (besylate salt form); (b) (4)

Structure or Biochemical Description:



Pharmacologic Class: Calcium channel blocker

### 2.2 Relevant INDs, NDAs, BLAs and DMFs
IND108233, NDA020184, NDA019787

## 2.3 Drug Formulation

Prestalia (perindopril arginine/amlodipine besylate) tablets 3.5/2.5 mg, 7/5 mg, and 14/10 mg, with following quantitative compositions

| Component | Reference to Quality Standards | Function | Quantity per Unit (mg) | | |
|---|---|---|---|---|---|
| | | | 3.5/2.5 mg | 7/5 mg | 14/10 mg |
| Perindopril arginine [amount of perindopril] | In-House | Active Ingredient | **3.5** [2.38] | **7** [4.76] | **14** [9.51] |
| Amlodipine besylate [amount of amlodipine] | USP | Active Ingredient | 3.47 **[2.5]** | 6.94 **[5]** | 13.87 **[10]** |
| Lactose (b) (4) | NF | (b) (4) | | | |
| Magnesium stearate | NF | | | | |
| Microcrystalline cellulose | NF | | | | |
| Colloidal silicon dioxide | NF | | | | |
| | | Total Tablet Mass | 52 | 104 | 208 |

## 2.4 Comments on Novel Excipients

None

## 2.5 Comments on Impurities/Degradants of Concern

None

## 2.6 Proposed Clinical Population and Dosing Regimen

Proposed clinical population includes hypertensive patients who have not achieved adequate blood pressure control with their current therapeutic regimen. The recommended initial dose of PRESTALIA is 3.5/2.5 mg once daily. The recommended maximal dose of PRESTALIA is 14/10 mg once daily.

## 2.7 Regulatory Background

A type B pre-NDA meeting was held on November 4, 2013. Regarding the approach of providing the final nonclinical reports with the NDA, FDA recommended that all nonclinical study reports submitted to IND108233 and the report for study 6678 which was not previously submitted should be submitted with the NDA. FDA also agreed that if no novel toxicities of the XOMA 985 were identified in the 13-week, oral, repeat-dose toxicity study (study 6678), no further nonclinical studies will be needed.

# 3 Review Information of Submitted Studies

## 3.1 Studies Not Reviewed

Analytical methods and validation reports

chm-05985-001-deu chm-06490-002-deu pb-01-9490-014-drva
pb-02-06490-004-dsva pb-02-06490-005-ddva pb-03-06490-011-ddst
pb-03-06490-012-dsst

## 3.2 Previous Reviews Referenced

3.2.1 IND108233, Baichun Yang, 09/26/2011, REV-NONCLINICAL-03 (General Review) Original-1

3.2.2 IND108233, Baichun Yang, 01/06/2012, REV-NONCLINICAL-03 (General Review) SD5

### 3.3 Disclaimer

Tabular and graphical information are constructed by the reviewer unless cited otherwise. Drug doses/concentrations are expressed as free base or free acid.

## 4 Pharmacology

No pharmacology study was submitted under this submission. The sponsor intends to rely on previous findings of the listed drugs perindopril erbumine (ACEON®, PERe) and amlodipine (NORVASC®, AMLb) for the individual active components of Prestalia.

The renin–angiotensin system (RAS) participates significantly in the pathophysiology of hypertension via increasing vascular resistance and blood volume. ACE, one component of the RAS, is a peptidyl dipeptidase that catalyzes the conversion of angiotensin I to angiotensin II, the most active angiotensin peptide. Angiotensin II is a vasoconstrictor substance, and also stimulates aldosterone secretion by the adrenal cortex. Inhibition of ACE results in decreased plasma angiotensin II, which leads to decreased vasopressor activity and to decreased aldosterone secretion, and clinically expressed as lowering blood pressure and enhancing natriuresis (2). Perindopril, a pro-drug, is hydrolyzed to perindoprilat, which inhibits ACE in human subjects and in animals (2). Perindopril was more efficacious in patients with higher plasma renin activity, and plasma renin activity has a predictive value in determining the effectiveness of perindopril treatment in patients with primary hypertension (3). However, angiotensin II-induced increases in blood pressure has negative feedback effect on renin secretion, and decreased plasma angiotensin II secondary to ACE inhibition leads to higher plasma renin activity (2).

ACE is identical to kininase II, an enzyme that inactivates bradykinin (2). Whether increased levels of bradykinin, a potent vasodepressor peptide, play a role in the therapeutic effects of Prestalia remains to be elucidated.

Hypertension generally is the result of increased peripheral vascular resistance attributable to the contraction of vascular smooth muscle (2). The contractile processes of cardiac muscle and vascular smooth muscle are dependent upon the free intracellular concentration of $Ca^{2+}$, an outcome of the movement of extracellular calcium ions into these cells through specific ion channels. Inhibition of transmembrane movement of $Ca^{2+}$ through voltage-sensitive $Ca^{2+}$ channels can decrease the total amount of $Ca^{2+}$ that reaches intracellular sites. Amlodipine is a dihydropyridine calcium antagonist (calcium ion antagonist or slow channel blocker), and inhibits selectively the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle, with a greater effect on vascular smooth muscle cells than on cardiac muscle cells. Thus, amlodipine leads to peripheral arterial vasodilation via a direct action on vascular smooth muscle, and causes a reduction in peripheral vascular resistance and reduction in blood pressure (2).

As a consequence of a decrease in peripheral vascular resistance, the $Ca^{2+}$ channel blockers, including amlodipine, evoke a baroreceptor-mediated sympathetic discharge. Tachycardia and RAS activation may occur (2). ACE inhibitors block the $Ca^{2+}$ channel antagonist-associated RAS activation. The combination of perindopril and amlodipine was demonstrated to be an effective and well tolerated antihypertensive treatment (2, 4, 5).

## 5. Pharmacokinetics

Because perindopril arginine (PERa) had never been marketed in the US, two bridging preclinical pharmacokinetic (PK) studies were performed to evaluate PK profiles of PERa with a comparison to perindopril erbumine (ACEON®; PERe).

### 5.1 Pharmacokinetics of S 9780 and its glucuronide Y1304 in male Wistar rats after single oral and intravenous administration of S9490-6 (PB-02-06490-007-DRPK or RB-03-06490-007-DRPK or NP15043)

This study (NP15043) was performed in [redacted] (b) (4) during Dec 2002 – Jan 2003, and previously reviewed (9/26/2011) under IND108233 (Appendix I).

Male Wistar rats received a single dose of S 9490-6 (perindopril arginine, PERa) at a dose of 0.8 mg/kg by oral (PO) gavage or intravenous (IV) infusion (over 10 minutes). Blood and urine samples were collected and determined for S 9780 (perindoprilat) and its glucuronide Y 1304 using liquid scintillation chromatography [redacted] (b) (4) mass spectrometric detection (LC-MS/MS) after solid-phase extraction.

After oral administration of PERa, Tmax for perindoprilat and Y 1304 were 1 h and 30 min, respectively. This delayed Tmax of perindoprilat compared to Y 1304 suggests that Y 1304 is directly formed from perindopril via its glucuronide Y 1303 rather than from perindoprilat itself. The plasma AUC ratio of Y 1304 to perindoprilat after IV dosing was about 15 times lower than after PO dosing. This was possibly related to a pre-systemic formation of Y 1303 from perindopril, and subsequent hydrolysis to Y 1304.

After oral administration of PERa, perindoprilat and Y 1304 AUCs were 1017 and 50.9 ng•h/ml, respectively, indicating direct hydrolysis of PERa into perindoprilat as being the main transformation route in rats. Bioavailability was ~44% for the PERa oral dose.

Perindoprilat was rapidly eliminated with a short principal half-life of about 0.6 h representing about 88 % of the AUC. The urinary excretion of perindoprilat was 46.3 % after IV and 39.8 % after PO administration of PERa, whilst the urinary excretion of Y 1304 was less than 4 % of the dose after IV and PO administration.

### 5.2 Pharmacokinetics of S 9490 and S 9780 and their glucuronides Y 1303 and Y 1304 during repeated once daily oral administration of S 9490-3 or S 9490-6 for 7 days in male beagle dogs - Comparative study between the two salt forms of Perindopril (PB-02-06490-002-DDPK or RB-03-06490-002-DDPK or NP15052)

This study (NP15052) was performed in [redacted] (b) (4) uring Sept 2002 – Dec 2002, and previously reviewed (9/26/2011) under IND108233 (Appendix I).

Four male beagle dogs in 2 treatment phases using a cross over design with a 7-day wash out period were used. Each animal received an oral dose of S 9490-6 (PERa) or S 9490-3 (perindopril erbumine, PERe) at 0.834 mg/kg/day, once daily for 7 days. Blood and urine samples collected on days 1 and 7 were determined for S 9490 (perindopril), S 9780

(perindoprilat), and their respective glucuronides Y 1303 and Y 1304 using LC-MS/MS after solid-phase extraction.

After oral administration of each perindopril salt, no differences were observed between the two salts regarding PK parameters of perindopril, perindoprilat, Y 1303, or Y 1304. After repeated once daily oral administration, both PERe and PERa were rapidly absorbed, with a perindopril Tmax of 0.5 h. Tmax of perindoprilat (2 h) follows its glucuronide Y 1304 (1 h), indicating the formation of this glucuronide directly from perindopril rather than from perindoprilat itself. Perindopril was rapidly and extensively metabolized into perindoprilat and Y 1304, which were the main metabolites determined in plasma and in urine.

Perindopril glucuronide (Y1303) was formed to a less extent, and perindopril was the minor circulating component. The urinary excretion of these two compounds was negligible (at most 3% of the oral dose). These data indicate that both perindopril hydrolysis into perindoprilat and glucuronidation pathway occur at the same extent in dogs.

After oral administration of PERe or PERa, the mean terminal half-life was ~3 h for perindopril and Y 1303, 6-7 hours for perindoprilat, and ~5 h for Y 1304. No accumulation was observed after repeated oral dosing for 7 days for both compounds.

## 6 Toxicology

The sponsor intends to rely on the Agency's previous findings of safety and efficacy for the individual active components of Prestalia: the listed drugs perindopril erbumine (ACEON®, PERe) and amlodipine (NORVASC®, AMLb) for approval of this 505(b)(2) NDA. Bridging preclinical studies were performed to evaluate safety profiles of perindopril arginine (PERa) with a comparison to perindopril erbumine (PERe). The sponsor also conducted a 13-week, repeat-dose, oral toxicity study in rats comparing the toxicokinetics and toxicity of PERa and AMLb administered alone and in combination (Study 6678). Except for Study 6678, single-dose and repeat-dose toxicity studies were previously reviewed (Sept 26, 2011) under IND108233 (Appendix I), and summarized below (Table 1). Generally, PERa and PERe had similar safety and TK profiles in rats and dogs, except for slightly greater decreases in red blood cell count and hemoglobin (Hb), and kidney medulla and/or papilla mineralization in high dose PERa-treated dogs.



Reference ID: 3604317

Table 1. Summary of the bridging single and repeat-dose toxicity studies

| Study No. | Type of Study | Route | Duration | Doses mg/kg/day | Noteworthy findings |
|---|---|---|---|---|---|
| 5124 (NP15064) | Perindopril arginine: single dose toxicity study in OF1 mice | Oral gavage | Single dose | Vehicle PERa 2000 | No death, No target organs identified |
| 5123 (NP15065) | Perindopril arginine: single dose toxicity study in Wistar rats | Oral gavage | Single dose | Vehicle PERa 2000 | No death, excessive salivation for all rats within 30 minutes post-dose |
| 4642 (NP08100, NP15015) | Perindopril arginine and Perindopril erbumine: Comparative Toxicity Study by Repeated Oral Administration for 4 Weeks in Wistar Rats [GLP] | Oral gavage | Once a day for 4 weeks | Vehicle PERa*: 0.8, 8, or 33 PERe: 8 or 33 | ☐ No death<br>☐ Slight increases in water intake, urinary volume, urinary chloride and sodium elimination, associated with minimal decreases in serum sodium and chloride concentrations, in all the treated groups (similar magnitude for both salt)<br>☐ Dose-dependent higher incidence of erosions and/or ulcerations in the glandular stomach mucosa in treated groups, slightly higher incidence in PERe groups than in PERa groups<br>☐ TK profiles of perindopril and perindoprilat were similar between PERa and PERe rats. |
| 5003 (NP15081) | Perindopril arginine and Perindopril erbumine: Comparative Toxicity Study by Repeated Oral Administration for 4 Weeks in Beagle Dogs [GLP] | Oral gavage | Once a day for 4 weeks | Vehicle PERa: 0.83, 4.17, 20.87 PERe: 4.17 20.87 | ☐ No death<br>☐ Decreases (~20% vs pre-test) in red blood cell count and Hb in 1/3 females at PERa 4.17 mg/kg and 1/3 females at PERa 20.87 mg/kg. Similar severe effects were not seen with PERe.<br>☐ Medulla and/or papilla mineralization in kidneys of the PERa-treated dogs at all dose levels with similar incidence (1/3) and severity (minimal to mild) among doses<br>☐ Three PERa-treated and four PERe-treated animals (at doses ☐ 4.17 mg/kg) showed minimal to mild bronchopneumonia or chronic peri-bronchiolitis, with similar incidence and severity between the two salts<br>☐ TK profiles of perindopril and perindoprilat were similar between PERa- and PERe-treated dogs. |

* PERa contained various impurities including (b) (4)

## 6.2 Repeat-Dose Toxicity

### 6.2.1 S 9490-6/Amlodipine Besylate: Toxicity Study by Repeated Oral Administration for 13 Weeks in Wistar Rats

Conducting laboratory and location: (b) (4)
Study number(s): 6678
Date of study initiation: Sept 8, 2010
Drug lot/batch number/Purity: S 9490-6 (Perindopril Arginine), L0026952, 99.7%; Amlodipine besylate, L0031667, 100.3%
GLP compliance: Yes
QA statement: Yes

**Key Study Findings**

When compared with controls, there were higher incidences of sporadic sialism within 2 hours of dosing in both sexes at PER/AMLO 7.5/4, 15/8 mg/kg, and 0/8 mg/kg, and lower mean body weights in AMLO alone (0/8 mg/kg)-treated males. AMLO alone (0/8 mg/kg) for 3 months resulted in higher urine volume associated with lower urine specific gravity in males, higher urinary chloride excretion, and lower serum chloride concentrations associated with a minimal increase in serum urea concentrations in both sexes. Serum calcium concentrations were lower in male group at PER/AMLO 15/8 and 15/0, which may be attributed to PER. Kidney weights for males at 15/8 mg/kg were higher than controls. Minimal hypertrophy of the juxtaglomerular apparatus, characterized by thickening of the wall of the afferent and efferent

arterioles of the glomeruli, was present in the kidneys of 6/10 males and 5/10 females given the combination 15/8 mg/kg PER/AMLO and a female given 15 mg/kg PER alone. In addition, the incidence of foci of basophilic (regenerating) tubules in the kidneys of both sexes given the combination of 15/8 mg/kg PER/AMLO was greater than in controls (all males and 4/10 females versus 6/20 control animals). There were increased incidences and severity of diffuse hypertrophy/vacuolation of the zona glomerulosa of the adrenal glands of both sexes given 8 mg/kg AMLO alone.

No new target organs or relevant additive effects were identified with the combination of PER and AMLO. The No Observed Adverse Effect Level (NOAEL) was 7.5/4 mg/kg of PER/AMLO. Corresponding mean values for Cmax and AUC24 in Week 13 (males-females) were 3.32 - 4.54 µg/ml and 8.18 - 7.36 µg·h/ml, respectively for S 9780 and 75.5 - 90.0 ng/ml and 526.1 - 570.5 ng·h/ml, respectively for AMLO.

**Methods**

Wistar rats (approximately 6 weeks old on Day 1, 10/sex/group for main study and 2-6/sex/group for TK study) were orally gavaged with S 9490-6 (PER) and Amlodipine besylate (AMLO) once a day at dose levels of 0 [1% (w/v) hydroxyethylcellulose in purified water], 3.75/2, 7.5/4, 15/8, 15/0 or 0/8 mg/kg under a dosing volume of 4 ml/kg for 13 weeks. There was not a justification for the dose selection. Dosing formulations were confirmed to be in the acceptable ranges: Concentrations ranged 98.3 - 103.6% of the intended values for PER and 89.8 - 103.5% of the intended values for AMLO; The pH was 5.7, 6.3, 6.7, 6.9, 7.1 and 5.6 for control, for the combination PER/AMLO 3.75/2, 7.5/4, 15/8, 15/0 and 0/8 mg/kg/day preparations, respectively

All rats were killed after 13 weeks of dosing. Evaluations and measurements in the main study included changes in appearance and behavior (checked daily), bodyweight (once during the acclimation phase, twice a week during the first four weeks of dosing and once a week thereafter), feed and water intake (once a week), hematology and clinical chemistry (samples were collected after overnight fast during the week preceding the killing, Table 2 and Table 3), urinalysis (samples were collected overnight for approximately 16 hours during the week preceding the killing, Table 4), ophthalmology (in the acclimation phase and in Week 13), organ weights (Table 5), macroscopic and histomorphological examination (Table 6). Microscopic examination was performed externally by (b) (4) on:

- all organs and macroscopic anomalies of all animals from the control group, high combination dose (15/8 mg/kg), high dose of PER (15/0 mg/kg) and high dose of AMLO (0/8 mg/kg) groups.
- all macroscopic anomalies, adrenal glands and kidneys of all animals from the low and intermediate combination dose (3.75/2, 7.5/4 mg/kg) groups.

A peer review was performed internally.

Bone marrow smears were performed from the left femur and stained with May-Grünwald Giemsa for all animals at necropsy.

Table 2. Parameters of hematology

| | |
|---|---|
| Red Blood Cell Count | Mean Corpuscular Haemoglobin |
| Packed Cell Volume | Mean Corpuscular Haemoglobin Concentration |
| Mean Corpuscular Volume | Total and Differential Leucocyte Counts |
| Haemoglobin Concentration | Platelet Count |

Table 3. Parameters of clinical chemistry

| | | |
|---|---|---|
| Electrolytes ($Na^+$, $K^+$, $Cl^-$) | Total Cholesterol | Protein fractions by electrophoresis |
| Calcium | Triglycerides | Albumin |
| Inorganic Phosphorus | Total Bilirubin | Alpha-1 globulins |
| Glucose | Alkaline Phosphatase | Alpha-2 globulins |
| Urea | Alanine Aminotransferase | Beta globulins |
| Creatinine | Aspartate Aminotransferase | Gamma globulins |
| Albumin | Glutamate Dehydrogenase | Albumin to globulin ratio |
| Total Proteins | | |

Table 4. Parameters of urinalysis

- Quantitative parameters:

| | |
|---|---|
| Volume | Proteins |
| Creatinine | Specific gravity |
| Electrolytes (Na+, K+, Cl) | |

- Semi-quantitative (reagent strips) and microscopic evaluations:

| | |
|---|---|
| Appearance | Occult blood |
| Colour | Urobilinogen |
| Glucose | Nitrites |
| Bilirubin | Leucocytes |
| Ketones | Sediments |
| pH | |

Table 5. List of organs for weighing

| | | | | |
|---|---|---|---|---|
| Adrenal glands | Kidneys | Pituitary gland | Seminal vesicles | Thymus |
| Brain | Liver | Prostate | Spleen | Thyroid glands |
| Epididymides | Lungs | Salivary glands | Testes | Uterus |
| Heart | Ovaries | (mandibular/sublingual) | | |

Table 6. Organ list for macroscopic and histomorphological examinations

| | | | |
|---|---|---|---|
| Adrenal glands | Heart | Ovaries | Spinal cord |
| Aorta | Ileum | Oviducts | Spleen |
| Brain | Jejunum | Pancreas | Sternum |
| Caecum | Kidneys | Parathyroid glands | Testes |
| Colon | Larynx | Pituitary gland | Thymus |
| Duodenum | Liver | Prostate | Thyroid glands |
| Epididymides | Lungs | Salivary glands (parotid | Tongue |
| Eyes | Lymph nodes | and mandibular/sublingual) | Trachea |
| Femur and stifle joint | (mandibular, mesenteric) | Sciatic nerve | Ureters |
| Forestomach | Mammary gland | Seminal vesicles | Urinary bladder |
| Glandular stomach | Oesophagus | Skeletal muscle | Uterus |
| Harderian gland | Optic nerves | Skin | Vagina |



Reference ID: 3604317

The animals of the toxicokinetic phase (including the controls) were only weighed for dosing (data not shown), checked for morbidity and observed once a week outside of their cage for clinical monitoring. On Day 91, these animals were subjected to blood sampling (n=3/sex/group/time point, unfasted) at 0.5, 1, 3, 6, 8, and 24 hours post-dose. Plasma levels of S 9780 (Perindoprilat) and AMLO were determined in [redacted] (b) (4).

**Results**

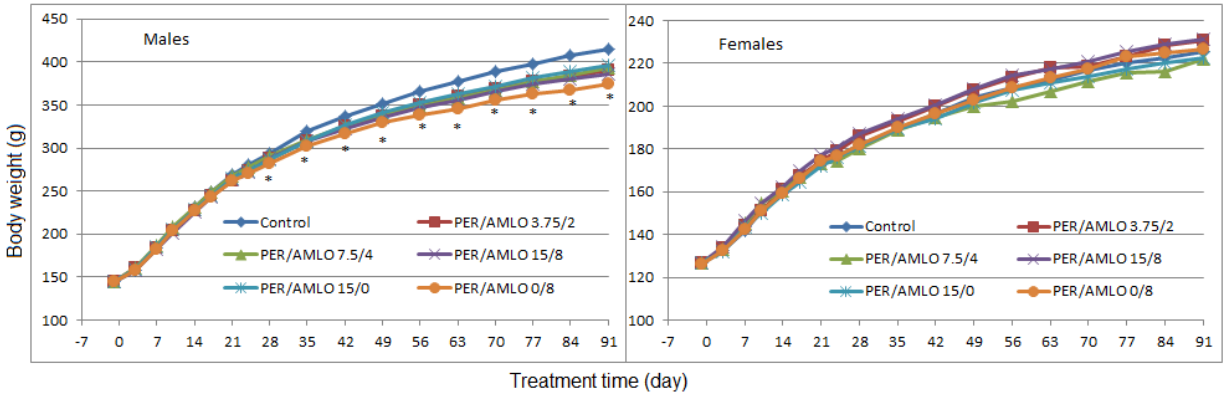
No deaths were observed in animals of this study (main and satellite groups). No relevant changes were noted in feed, water intake, ophthalmology, and hematological parameters for both sexes.

When compared with controls, higher incidence of sporadic sialism within 2 hours of dosing occurred in both sexes at PER/AMLO 7.5/4, 15/8 mg/kg, and 0/8 mg/kg (Table 7), and lower mean body weights in AMLO alone (0/8 mg/kg)-treated males were noted during days 28-91(Figure 1). AMLO alone (0/8 mg/kg) for 3 months resulted in higher urine volume associated with lower urine specific gravity in males, higher urinary chloride excretion, and lower serum chloride concentrations associated with a minimal increase in serum urea concentrations in both sexes (Table 8). Slightly lower serum calcium concentrations than controls were observed in all treated male groups (Table 8), which were not seen in treated females. Values for serum calcium concentrations in the males at PER/AMLO 3.75/2, 7.5/4, and 0/8 were similar to those in female controls, and were considered to be within normal variation. Lower serum calcium concentrations in male group at PER/AMLO 15/8 and 15/0 were attributed to PER. Other minimal variations observed for some parameters were considered not to be toxicologically relevant as the differences were of very low magnitude, isolated, in one sex only and/or not dosed-related.

Table 7. Findings in clinical signs

| | PER/AMLO (mg/kg) | 0/0 | 3.75/2 | 7.5/4 | 15/8 | 15/0 | 0/8 |
|---|---|---|---|---|---|---|---|
| | Animal number/group | 10 | 10 | 10 | 10 | 10 | 10 |
| Sialism | | | | | | | |
| Male | Number of Observations | . | . | 5 | 54 | 2 | 31 |
| | Number of Animals | . | . | 4 | 9 | 1 | 10 |
| | Days from - to | . | . | 19 61 | 2 88 | 15 20 | 1 86 |
| Female | Number of Observations | . | . | 1 | 15 | . | 21 |
| | Number of Animals | . | . | 1 | 7 | . | 8 |
| | Days from - to | . | . | 18 18 | 2 86 | . | 6 85 |

Figure 1. Body weight (mean)




* p<0.05 vs control

Table 8. Findings in serum chemistry and urinalysis at the end of 3-month treatment

| | PER/AMLO (mg/kg) | | 0/0 | 3.75/2 | 7.5/4 | 15/8 | 15/0 | 0/8 |
|---|---|---|---|---|---|---|---|---|
| | Animal number/group | | 10 | 10 | 10 | 10 | 10 | 10 |
| Clinical chemistry | | | | | | | | |
| Ca mmol/l | Male | Mean | 2.602 | 2.515* | 2.540* | 2.479** | 2.489** | 2.523* |
| | | S.D. | 0.074 | 0.088 | 0.056 | 0.061 | 0.055 | 0.104 |
| | Female | Mean | 2.552 | 2.533 | 2.529 | 2.522 | 2.510 | 2.547 |
| | | S.D. | 0.043 | 0.111 | 0.073 | 0.063 | 0.056 | 0.092 |
| Cl mmol/l | Male | Mean | 101.41 | 101.42 | 101.39 | 101.68 | 101.80 | 98.31** |
| | | S.D. | 1.16 | 1.02 | 1.37 | 1.58 | 1.27 | 2.29 |
| | Female | Mean | 102.77 | 102.06 | 102.21 | 103.94 | 102.78 | 98.33** |
| | | S.D. | 1.31 | 1.18 | 1.53 | 1.39 | 1.87 | 2.19 |
| Urea mmol/l | Male | Mean | 4.82 | 4.31 | 4.60 | 6.67 | 4.61 | 6.26** |
| | | S.D. | 0.58 | 0.67 | 0.48 | 2.04 | 0.58 | 1.44 |
| | Female | Mean | 6.40 | 5.99 | 5.86 | 6.36 | 6.20 | 7.98** |
| | | S.D. | 0.95 | 1.36 | 0.86 | 1.01 | 0.69 | 0.65 |
| Urinalysis | | | | | | | | |
| Vol/24h ml | Male | Mean | 7.711 | 23.292 | 11.017 | 13.928 | 15.582 | 18.019* |
| | | S.D. | 7.546 | 18.052 | 6.424 | 11.165 | 8.498 | 10.017 |
| | Female | Mean | 10.623 | 8.027 | 9.285 | 14.165 | 6.217 | 11.487 |
| | | S.D. | 7.843 | 4.675 | 8.084 | 10.726 | 6.579 | 10.253 |
| Specific gravity | Male | Mean | 1.0446 | 1.0303 | 1.0388 | 1.0362 | 1.0250** | 1.0191** |
| | | S.D. | 0.0184 | 0.0259 | 0.0233 | 0.0156 | 0.0099 | 0.0066 |
| | Female | Mean | 1.0261 | 1.0293 | 1.0317 | 1.0266 | 1.0436 | 1.0226 |
| | | S.D. | 0.0138 | 0.0112 | 0.0148 | 0.0147 | 0.0285 | 0.0091 |
| Cl mmol/24h | Male | Mean | 0.29 | 0.47 | 0.52 | 0.52 | 0.36 | 0.52* |
| | | S.D. | 0.15 | 0.14 | 0.20 | 0.32 | 0.18 | 0.25 |
| | Female | Mean | 0.27 | 0.29 | 0.40 | 0.37 | 0.20 | 0.37* |
| | | S.D. | 0.11 | 0.12 | 0.14 | 0.12 | 0.09 | 0.11 |

* p<0.05 vs control; ** p<0.01 vs control

When compared with controls, lower heart weights were seen for the males given PER, either in combination with AMLO at 3.75/2, 7.5/4 and 15/8 mg/kg or alone at 15/0 mg/kg, and for the females given PER alone at 15/0 mg/kg (Table 9). AMLO alone (0/8 mg/kg) showed higher heart weights in both sexes (Table 9). There were also higher liver weights for females given AMLO, either in combination with PER at 3.75/2, 7.5/4 and 15/8 mg/kg or alone at 0/8 mg/kg, higher ovary weights for females at PER/AMLO 15/8 mg/kg, lower epididymis weights for males at 0/8 mg/kg, and lower adrenal gland weights for males at the combinations 3.75/2, 7.5/4 and 15/8 mg/kg (Table 9). Although these differences in organ weight between control and treated groups were statistically significant, the weight differences were slight (<20%), not associated with microscopic findings, and were not considered to be toxicologically significant.

Kidney weights for males at 15/8 mg/kg were higher than controls (Table 9).

Table 9. Findings in organ weights at the end of 3-month treatment

| | | MALES (n=9-10 per group) | | | | | | FEMALES (n=10 er group) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PER/AMLO (mg/kg) | | 0/0 | 3.75/2 | 7.5/4 | 15/8 | 15/0 | 0/8 | 0/0 | 3.75/2 | 7.5/4 | 15/8 | 15/0 | 0/8 |
| HEART ..... | Mean (g) | 1.0402 | 0.8901** | 0.8794** | 0.8465** | 0.8462** | 1.0530** | 0.6293 | 0.6379 | 0.5791 | 0.6320 | 0.5715* | 0.7120** |
| | S.D. | 0.1040 | 0.0802 | 0.0864 | 0.0792 | 0.0551 | 0.0835 | 0.0606 | 0.0390 | 0.0238 | 0.0777 | 0.0555 | 0.0656 |
| | Mean (%) | 0.266 | 0.243** | 0.237** | 0.233** | 0.228** | 0.307** | 0.302 | 0.300 | 0.282 | 0.295 | 0.278* | 0.348** |
| | S.D. | 0.018 | 0.021 | 0.022 | 0.016 | 0.010 | 0.017 | 0.025 | 0.024 | 0.011 | 0.020 | 0.036 | 0.021 |
| LIVER ..... | Mean (g) | 9.118 | 8.297 | 8.554 | 8.553 | 8.324 | 8.671 | 4.961 | 5.427* | 5.240* | 5.385* | 5.092 | 5.288* |
| | S.D. | 1.183 | 0.820 | 1.226 | 1.168 | 0.641 | 1.158 | 0.573 | 0.625 | 0.316 | 0.567 | 0.365 | 0.637 |
| | Mean (%) | 2.326 | 2.257 | 2.296 | 2.344 | 2.241 | 2.530 | 2.375 | 2.540* | 2.553* | 2.517* | 2.477 | 2.573* |
| | S.D. | 0.152 | 0.115 | 0.167 | 0.091 | 0.096 | 0.334 | 0.152 | 0.156 | 0.168 | 0.156 | 0.258 | 0.116 |
| KIDNEYS ..... | Mean (g) | 2.117 | 2.091 | 2.155 | 2.275* | 2.124 | 2.095 | 1.317 | 1.397 | 1.341 | 1.378 | 1.344 | 1.310 |
| | S.D. | 0.200 | 0.151 | 0.266 | 0.259 | 0.157 | 0.196 | 0.132 | 0.112 | 0.127 | 0.133 | 0.163 | 0.129 |
| | Mean (%) | 0.545 | 0.570 | 0.581 | 0.627* | 0.572 | 0.613 | 0.632 | 0.656 | 0.653 | 0.645 | 0.651 | 0.639 |
| | S.D. | 0.073 | 0.035 | 0.057 | 0.062 | 0.031 | 0.076 | 0.047 | 0.036 | 0.060 | 0.041 | 0.059 | 0.029 |
| ADRENAL GLANDS ..... | Mean (g) | 0.0658 | 0.0565* | 0.0544* | 0.0546* | 0.0571 | 0.0602 | 0.0632 | 0.0693 | 0.0658 | 0.0708 | 0.0619 | 0.0663 |
| | S.D. | 0.0124 | 0.0067 | 0.0094 | 0.0098 | 0.0085 | 0.0075 | 0.0118 | 0.0127 | 0.0057 | 0.0136 | 0.0080 | 0.0118 |
| | Mean (%) | 0.017 | 0.015* | 0.015* | 0.015* | 0.015 | 0.018 | 0.030 | 0.032 | 0.032 | 0.033 | 0.030 | 0.032 |
| | S.D. | 0.003 | 0.002 | 0.002 | 0.003 | 0.002 | 0.003 | 0.004 | 0.005 | 0.003 | 0.005 | 0.003 | 0.003 |
| EPIDIDYMIDES ... | Mean (g) | 1.3508 | 1.2672 | 1.2884 | 1.2683 | 1.2523* | 1.2239* | | | | | | |
| | S.D. | 0.0912 | 0.1306 | 0.1178 | 0.1487 | 0.1073 | 0.0759 | | | | | | |
| | Mean (%) | 0.348 | 0.347 | 0.349 | 0.351 | 0.338* | 0.358* | | | | | | |
| | S.D. | 0.049 | 0.049 | 0.048 | 0.047 | 0.031 | 0.032 | | | | | | |
| OVARIES ..... | Mean (g) | | | | | | | 0.0908 | 0.1053 | 0.0970 | 0.1129** | 0.0926 | 0.0969 |
| | S.D. | | | | | | | 0.0179 | 0.0148 | 0.0145 | 0.0140 | 0.0121 | 0.0143 |
| | Mean (%) | | | | | | | 0.043 | 0.049 | 0.047 | 0.053** | 0.045 | 0.048 |
| | S.D. | | | | | | | 0.007 | 0.006 | 0.007 | 0.006 | 0.006 | 0.007 |

* p<0.05 vs control; ** p<0.01 vs control

There were increased incidences and severity of diffuse hypertrophy/vacuolation of the zona glomerulosa of the adrenal glands of both sexes given 8 mg/kg AMLO alone (Table 10). Minimal hypertrophy of the juxtaglomerular apparatus, characterized by thickening of the wall of the afferent and efferent arterioles of the glomeruli, was present in the kidneys of 6/10 males and 5/10 females given the combination 15/8 mg/kg PER/AMLO and a female given 15 mg/kg PER alone. In addition, the incidence of foci of basophilic (regenerating) tubules in the kidneys of both sexes given the combination of 15/8 mg/kg PER/AMLO was greater than in controls (all males and 4/10 females versus 6/20 control animals) (Table 10).

Table 10. Microscopic findings

| | Male | | | | | | Female | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Dosage of PER (mg free acid/kg)** | **0** | **3.75** | **7.5** | **15** | **15** | **0** | **0** | **3.75** | **7.5** | **15** | **15** | **0** |
| **Dosage of AMLO (mg base/kg)** | **0** | **2** | **4** | **8** | **0** | **8** | **0** | **2** | **4** | **8** | **0** | **8** |
| **KIDNEYS** | | | | | | | | | | | | |
| number examined | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| **Hypertrophy of the juxtaglomerular apparatus** | | | | | | | | | | | | |
| (minimal) | 0 | 0 | 0 | 6 | 0 | 0 | 0 | 0 | 0 | 5 | 1 | 0 |
| **Focus(i) of basophilic (regenerating) tubules** | | | | | | | | | | | | |
| (minimal) | 6 | 6 | 3 | 9 | 4 | 2 | 0 | 1 | 0 | 4 | 2 | 1 |
| (slight) | 0 | 0 | 0 | 1 | 1 | 1 | | | | | | |
| Total | 6 | 6 | 3 | 10 | 5 | 3 | 0 | 1 | 0 | 4 | 2 | 1 |
| **Focal cortical inflammatory cell infiltration** | | | | | | | | | | | | |
| (minimal) | 0 | 1 | 3 | 3 | 3 | 1 | 0 | 1 | 1 | 0 | 0 | 0 |
| **ADRENAL CORTEX** | | | | | | | | | | | | |
| number examined | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| **Diffuse hypertrophy/vacuolation - zona glomerulosa** | | | | | | | | | | | | |
| (minimal) | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 0 | 0 | 0 | 0 | 2 |
| (slight) | 0 | 0 | 0 | 0 | 0 | 8 | 0 | 0 | 0 | 0 | 0 | 8 |
| Total | 0 | 0 | 0 | 0 | 0 | 10 | 1 | 0 | 0 | 0 | 0 | 10 |

Mean plasma concentrations of S 9780 (Perindoprilat) and amlodipine on dosing Day 91 are shown Figure 2 and Figure 3 respectively, and toxicokinetics are summarized in Table 11. On dosing Day 91, plasma exposures ($AUC_{24}$ and Cmax) to S 9780 were approximately dose-proportional. There were no appreciably sex-related differences in systemic exposure to S 9780 (Figure 2). For AMLO systemic exposure, $AUC_{24}$ appeared to increase greater than dose-proportionally between each dose level in male and female animals, Cmax appeared to increase slightly greater than dose proportionally between the low and medium dose in male and female animals, and there were no obvious gender differences (Figure 3). TK data on Day 91 suggested no appreciably interaction between PER and AMLO.

Figure 2. Mean plasma concentrations of perindoprilat on Day 91 (n=3, modified from the submission)

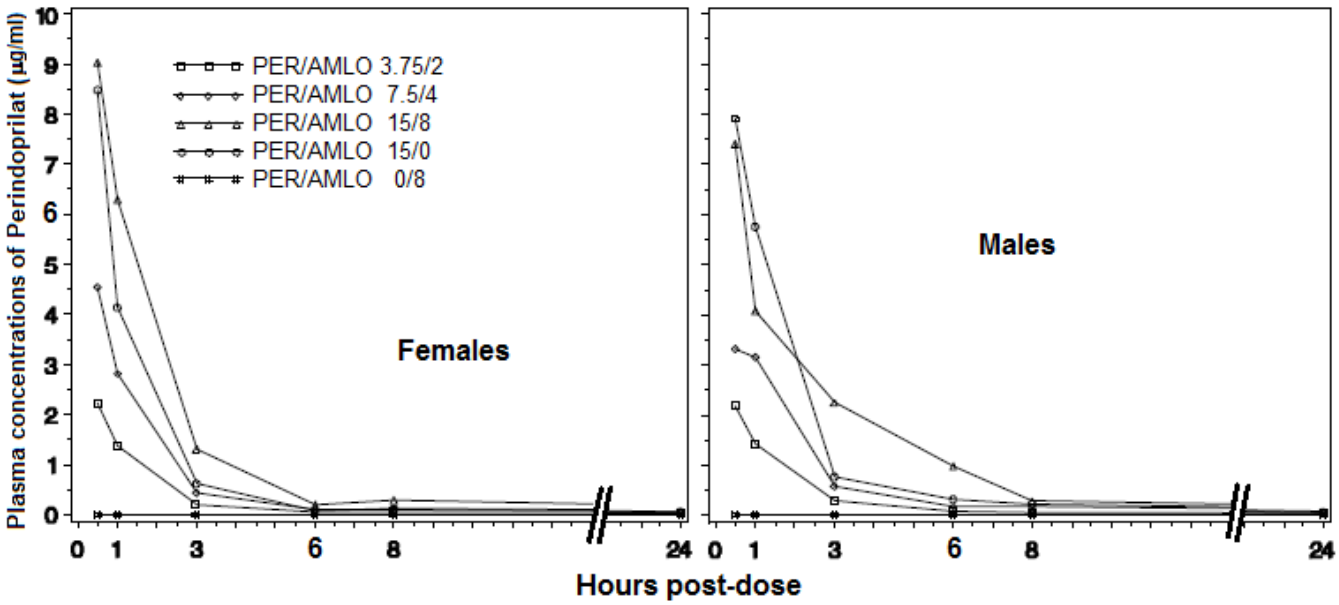


Figure 3. Mean plasma concentrations of amlodipine on Day 91 (n=3, modified from the submission)

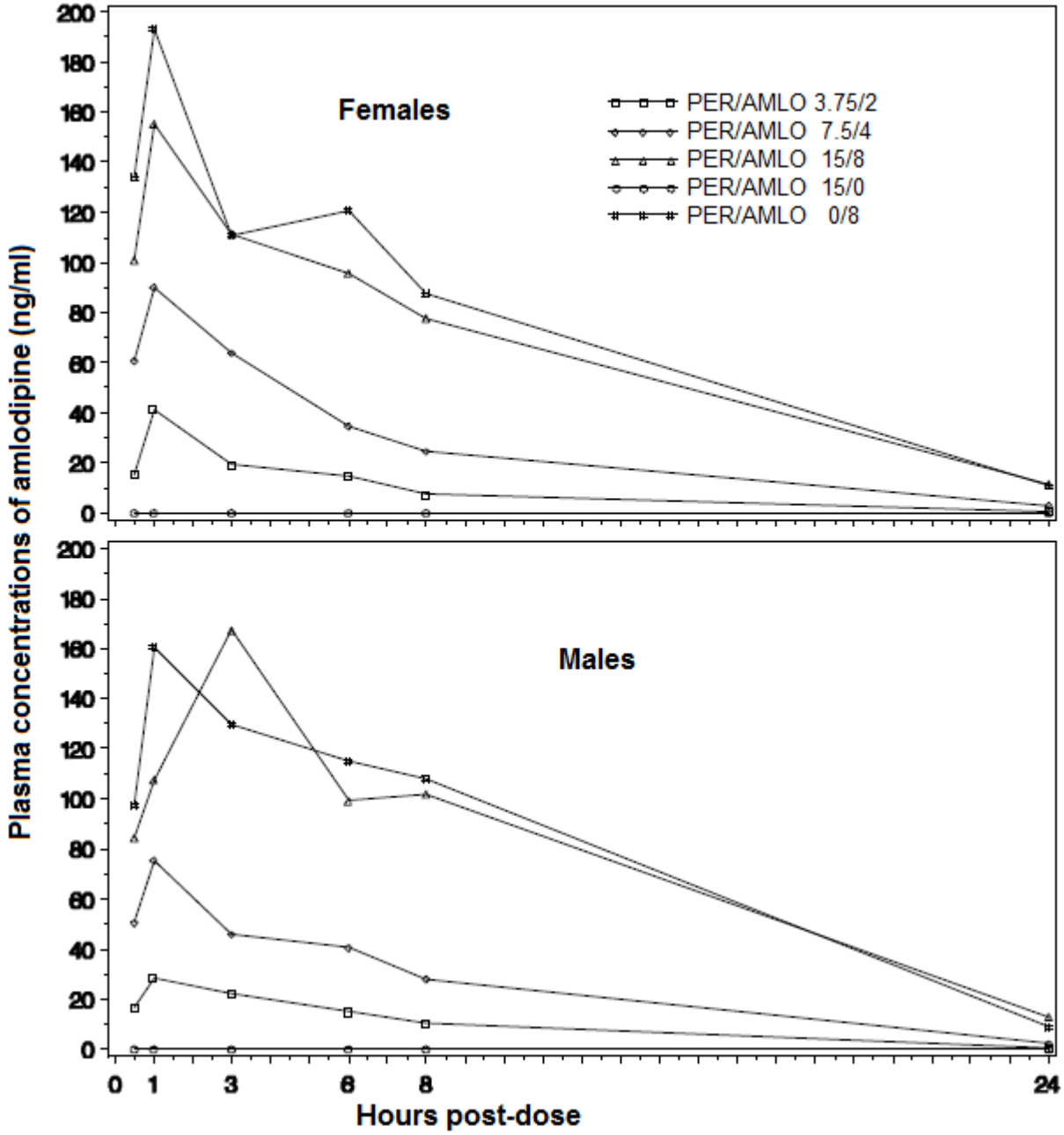



Table 11, Toxicokinetics on Day 91 (mean of 3, modified from the submission)

| Dose PER (mg/kg/day) | 3.75 | | 7.5 | | 15 | | 15 | | 0 | |
|---|---|---|---|---|---|---|---|---|---|---|
| AMLO (mg/kg/day) | 2 | | 4 | | 8 | | 0 | | 8 | |
| Number of Animals (satellite groups) | M: 6 | F: 6 | M: 6 | F: 6 | M: 6 | F: 6 | M: 6 | F: 6 | M: 6 | F: 6 |
| S 9780: $C_{max}$ (µg/ml) | 2.19 | 2.22 | 3.32 | 4.54 | 7.42 | 9.05 | 7.92 | 8.50 | 0.0037 [b] | 0.0015 [b] |
| S 9780: $AUC_{24}$ (µg.h/ml) | 4.10 | 3.64 | 8.18 | 7.36 | 18.88 | 16.96 | 14.41 | 11.21 | NA | NA |
| AMLO: $C_{max}$ (ng/ml) | 28.5 | 41.2 | 75.5 | 90.0 | 167.3 | 155.3 | BLQ | BLQ | 160.3 | 193.0 |
| AMLO: $AUC_{24}$ (ng.h/ml) | 206.0 | 189.7 | 526.1 | 570.5 | 1625.8 | 1393.2 | NA | NA | 1605.0 | 1556.0 |

NA: Not Applicable; M/F: Male/Female; BLQ: Below the Limit of Quantitation; [b]: max individual values reported. Plasma S 9780 concentrations were quantifiable (≤ 3.7 ng/ml) at all sampling times up to 24 h after dosing in male and female animals. Concentrations observed were close to the LLOQ (< 0.5 ng/ml) and do not display a toxicokinetic profile. Thus, they were considered to be due to accidental *ex vivo* contaminations and had no impact on the validity and integrity of the study.

In conclusion, under the conditions of this study, no new target organs or relevant additive effects were identified with the combination of PER and AMLO. The No Observed Adverse Effect Level (NOAEL) was 7.5/4 mg/kg of PER/AMLO. Corresponding mean values for Cmax and $AUC_{24}$ in Week 13 (males-females) were 3.32 - 4.54 µg/ml and 8.18 - 7.36 µg.h/ml, respectively for S 9780 and 75.5 - 90.0 ng/ml and 526.1 - 570.5 ng.h/ml, respectively for AMLO.

## 7 Genetic Toxicology

Potential genotoxicity of perindopril arginine (PERa) was evaluated in the in vitro and in vivo assay systems. These studies were summarized in Table 12, and previously reviewed (Sept 26, 2011) under IND108233 (Appendix I). The results of these studies document that PERa does not demonstrate genotoxic potential under the conditions studied.

Table 12. Summary of genotoxicity studies with perindopril arginine (PERa)

| Study No. | Type of Study | GLP | Species/Strain | Route/Method of Administration | Concentration/ Doses | Noteworthy Findings |
|---|---|---|---|---|---|---|
| 4643 (NP07996) | Detection of Reverse Mutation | Yes | *S. typhimurium TA98, TA100, TA1535, TA1537;* and *E. coli WP2 (pKM101), WP2 uvrA (pKM101)* | 3 days, direct plating, and preincubation methods | 0 µg/plate to 5000 µg/plate ± Aroclor 1254 induced rat liver S9 | Negative |
| 303/278 (NP08568) | Mutation at the Thymidine Kinase Locus | Yes | Mouse Lymphoma L5178Y Cells | Microtitre® Fluctuation Technique | 0 µg/mL to 3685 µg/mL ± Aroclor 1254 induced rat liver S9 | Negative |
| 303/276 (NP08569) | Chromosome Aberrations | Yes | Cultured human peripheral blood lymphocytes | Cell culture | 0 µg/mL to 3685 µg/mL ± Aroclor 1254 induced rat liver S9 | Not a valid study due to mitotic accumulation |
| 5011 (NP08649) | Micronucleus Cytogenic Assay | Yes | Mouse/OFI | Oral gavage Single dose | Vehicle 500 mg/kg, 1000 mg/kg, 2000 mg/kg | Negative |

## 8 Carcinogenicity

None

## 9 Reproductive and Developmental Toxicology

None

## 10 Special Toxicology Studies

Impurities were found in batches of PERa [redacted] (b) (4) and AMLb [redacted] (b) (4) [redacted] To determine if these degradation products had potential toxicity, studies comparing the general toxicity of PERa spiked with [redacted] (b) (4)

(b) (4) with those of PERa without added impurities were conducted. Similarly, the general toxicity of AMLb spiked with (b) (4) were determined. These studies were previously reviewed (09/26/2011 and 01/06/2012) under IND108233 (Appendix I and appendix II, respectively). The results of these studies revealed no significant differences between the spiked materials and PERa and AMLb without added impurities. These data therefore qualify the levels of impurities proposed for the drug product.

### 10.1 S 9490-6 spiked with Y31: Toxicity study by repeated oral administration for 4 weeks in Wistar rats (Study 6782)

This GLP study (#6782) was conduct at (b) (4), initiated in March 2011. The safety profile of S 9490-6 spiked with the impurity (b) (4) (PER SPIKED; batch L0037679) was compared with that of S 9490-6 alone (PER, batch L0009275) in Wistar rats (9/sex/group) following once daily oral gavage at dose levels of 0 (purified water), 0.8, 8 or 33 mg /kg of PER or PER SPIKED (10 mL/kg) for 4 weeks. Evaluations and measurements included changes in appearance and behavior, bodyweight, food intake, hematology, clinical chemistry, urinalysis, ophthalmology, organ weights, and macroscopic and microscopic examinations.

No death occurred and no relevant clinical signs were observed. No differences were noted in bodyweight, food intake, ophthalmoscopy, hematology, clinical chemistry and urinalysis between the PER and PER SPIKED-dosed groups. Minimally lower heart weights and slightly higher epididymis weights were seen in all PER or PER SPIKED dosed groups, which were not dose-related, similar between the PER or PER SPIKED dosed groups, and of no toxicological relevance.

At 33 mg/kg PER or PER SPIKED, minimal hypertrophy of the juxtaglomerular apparatus was seen at microscopic examination in the kidneys of some rats and a minimally higher incidence of minimal to slight focal erosions of the glandular stomach were observed, occasionally correlating with macroscopic changes (depressed dark red foci). These kidney and glandular stomach histopathological changes were considered as being similar between PER and PER SPIKED-dosed groups.

NOAELs in this study were 8 mg/kg/day for both PER and PER SPIKED. No toxicity was attributable to the presence of (b) (4) (details in appendix II).

### 10.2 4-Week toxicity study by oral route (gavage) in Rats (Study TOX-06490-001-FRA, NP15086)

This GLP study (NP15086) was conducted at (b) (4); initiated in Feb 2003. The potential toxicity of S 9490-6 (Batch FI 150) containing impurities (b) (4) PERa-Spiked) was evaluated Wistar rats (10/sex/group) with oral administration of PERa-Spiked at 0 (control, purified water), 0.8, 8, or 33 mg/kg/day for 4 weeks. Evaluations and measurements included mortality and clinical signs, body weight, food consumption, hematology, clinical chemistry, urinalysis organ weights, macroscopic and microscopic examinations.

Minimal but dose-related lower glucose levels were noted in treated animals of both sexes, which were statistically significant at 33 mg/kg/day (about -19%). No other abnormalities were observed in the treated animals. NOAEL was at 8 mg/kg/day in this study (details in appendix II).

### 10.3 Amlodipine spiked with (b) (4): Toxicity study by repeated oral administration for 4 weeks in Wistar Rats (Study 6579, NP30527)

This GLP study (# 6579) was conduct at (b) (4) initiated in March 2010. The safety profile of AMLb spiked with two impurities (b) (4) each (AMLb SPIKED, batch L0031791) was compared with that of AMLb (batch L0020227) in Wistar rats (10/sex/group) following once daily oral gavage at dose levels of 0 [1% hydroxyethylcellulose in purified water], 7, or 14 mg/kg of AMLb or AMLb SPIKED for 4 weeks. Evaluations and measurements included changes in appearance and behavior, bodyweight, food intake, hematology, clinical chemistry, urinalysis, ophthalmology, organ weights, and macroscopic and microscopic examinations.

1/10 females of the AMLb and 1/10 females of the AMLb SPIKED at the dose 14 mg/kg/day were prematurely dead or killed in week 3-4. The two premature death females and two surviving females in the 14 mg/kg AMLb group had distended abdomen (dilation of the small intestine), less or absence of stools, dyspnea, hunched posture, decreased motor activity, partial blepharoptosis and piloerection, low feed intake, and /or major changes in various clinical pathology parameters. Histopathological observation revealed moderate ulcerative enteritis of the ileum in some of these animals. Dilation of the small intestine, with or without any microscopic changes, was considered to be the probable cause of morbidity and to be the cause of the poor clinical status at the dose 14 mg/kg of AMLb or AMLb SPIKED. No relevant differences of gastro-intestinal tract effects were noted between AMLb and AMLb SPIKED.

AMLb and AMLb SPIKED induced minimal and dose-related decreases in serum sodium and chloride concentrations (both sexes), increases in serum urea concentration (males only) and increases in urinary volume (high-dosed females only). Those changes were associated with a diffuse hypertrophy of the zona glomerulosa of the adrenal cortex with similar intensity whatever the batch.

Higher relative heart weight was observed for both sexes with dose-related incidence and severity. Severity of these findings was not enhanced when the AMLb was spiked with the two impurities (b) (4).

NOAEL was not established in this study. Under the condition of this study, toxicological profiles were similar between AMLb and AMLb SPIKED (details in appendix I).

### 10.4 Genotoxicity potential of impurities

Genotoxicity of impurities, (b) (4) from PERa and (b) (4) from AMLb, were assessed in Ames test, chromosome aberration test, and a test of mutation at the thymidine kinase locus using PERa spiked with (b) (4), PERa spiked with (b) (4) or AMLb spiked with (b) (4) each. These studies were previously reviewed (09/26/2011 and 01/06/2012) under IND108233 (Appendix I

and appendix II, respectively), and are summarized in Table 13. All tests were negative. The reviewer is aware that impurities spiked in a parent compound may yield false negative due to loss of test sensitivity with impurity spiking, and the FDA chemist Dr. Charles Jewell was consulted regarding structures of these impurities. Dr. Jewell stated (4/3/2014) that DEREK analysis for impurities (b) (4) showed no structural alerts for genotoxicity. Impurities (b) (4) are degradation products of AMLb, are respectively an (b) (4) derivative of the drug substance (b) (4) derivative of the drug substance (b) (4), and are predicted to have no structural alerts for genotoxicity. Thus, the results of these studies and chemical structure analysis revealed no genotoxic potential for these 5 impurities.

Table 13. Summary of genotoxicity tests with impurities

| Study No. Type and Impurity | Species/Strain | Route/Method of Administration | Concentration / Doses | Results | Details |
|---|---|---|---|---|---|
| 6620 (NP30727) Ames Test, GLP Amlodipine spiked with (b) (4) | *S. typhimurium TA98, TA100, TA1535, TA1537;* and *E. coli WP2 (pKM101), WP2 uvrA (pKM101)* | 3 days, direct plating, preincubation methods | 0 µg/plate to 5000 µg/plate ± Aroclor 1254 induced rat liver S9 | Negative | **Appendix I Appendix II** |
| 5110 (NP15054) Ames Test, GLP Perindopril arginine Spiked (b) (4) | | | | | **Appendix II** |
| 6790 (NP30972) Ames Test, GLP Perindopril arginine Spiked (b) (4) | | | | | **Appendix II** |
| 8224400 (NP30608) Chromosome Aberrations, GLP Amlodipine spiked with (b) (4) | Primary human lymphocytes | Cell culture | 0 µg/mL to 1.0 µg/mL ± Aroclor 1254 induced rat liver S9 | | **Appendix I** |
| TOX- 06490- 002- GBR (NP15013) Mutation at the Thymidine Kinase Locus, GLP Perindopril arginine Spiked (b) (4) | Mouse Lymphoma L5178Y Cells | Microtitre® Fluctuation Technique | 0 µg/mL to 3685 µg/mL ± Aroclor 1254 induced rat liver S9 | | **Appendix II** |
| 6795 (NP31039) Mutation at the Thymidine Kinase Locus, GLP Perindopril arginine Spiked (b) (4) | | Microtiter cloning technique | | | **Appendix II** |

# 11 Integrated Summary and Safety Evaluation

## Brief Background / Introduction

Prestalia is a fixed-dose tablet for oral administration of perindopril arginine and amlodipine besylate for the treatment of hypertension. The proposed dosage strengths of Prestalia, the perindopril arginine/amlodipine fixed-dose combination product, are 3.5/2.5 mg, 7/5 mg, and 14/10 mg.

## Pharmacology

The calcium channel blocker (CCB) amlodipine and angiotensin-converting enzyme (ACE) inhibitor perindopril have both been widely used for more than 15 years in countries worldwide. Both CCBs and ACE inhibitors lower blood pressure by reducing peripheral resistance. Blockade of calcium influx and reduction of angiotensin II-mediated vasoconstriction are complementary mechanisms. The combination of perindopril and amlodipine is recommended by the medical practice guidelines as being safe and effective (1). Both perindopril erbumine (ACEON®) and amlodipine besylate (NORVASC®) were approved in the US for the treatment of hypertension and coronary artery disease. The perindopril arginine/amlodipine besylate combination drug product COVERAM® is approved and is currently marketed in the European Union. However, perindopril arginine has never been marketed in the US.

To support the marketing application for Prestalia in US, two bridging preclinical PK studies were performed to evaluate PK profiles of perindopril arginine (PERa) with a comparison to perindopril erbumine (ACEON®; PERe). The PKs of perindoprilat, the active metabolite of both perindopril salts, were determined in rats following single dose oral or intravenous administration of PERa. The PKs of the 2 salt forms (PERa and PERe) and their glucuronide metabolites were compared in dogs after 7 days of dosing. These studies demonstrated that the PKs of perindopril and the active metabolite perindoprilat were similar following administration of PERa or PERe. Similar to PERe, PERa was hydrolyzed to the active metabolite perindoprilat. Other metabolites of both PERe and PERa are perindopril glucuronide (Y 1303) and perindoprilat glucuronide (Y 1304). These metabolic steps occur with both oral and IV administration of PERa. PK profiles are similar between PERa and PERe following single-dose oral administration and multiple-dose administration in rats and dogs.

The comparative toxicokinetics of PERa and PERe were determined in 2 repeat-dose toxicity studies with PERa or PERe in rats and dogs and in a 13-week comparative toxicity study with PERa/AMLb in rats. These studies demonstrated that there were no differences in TKs of PERa and PERe and no TK drug-drug interactions between PERa and AMLb.

## Toxicology

Safety profiles of PERa/AMLb were evaluated using bridging studies with PERa and PERe in mice, rats, and dogs, and using a 13-week, repeat-dose, oral toxicity study in rats with PERa and AMLb administered alone and in combination. Single oral dose of PERa at 2000 mg/kg was well tolerated in OF1 mice and Wistar rats. The repeated oral dose toxicity studies revealed approximately similar findings for PERa and PERe and no additional toxicity for PERa/AMLb comparing to PERa or AMLb alone:

- In a 4-week repeated oral toxicity study with PERa 0, 0.8, 8, or 33, or PERe 8, or 33 mg/kg/day in Wistar rats -
  - Slight increases in water intake, urinary volume, urinary chloride and sodium elimination, associated with minimal decreases in serum sodium and chloride concentrations, in all the treated groups (similar magnitude for both salt)
  - Dose-dependent higher incidence of erosions and/or ulcerations in the glandular stomach mucosa in treated groups, slightly higher incidence in PERe groups than in PERa groups
- In a 4-week repeated oral toxicity study with PERa 0, 0.83, 4.17, or 20.87, or PERe 4.17, or 20.87 mg/kg/day in Beagle dogs -
  - Decreases (~20% vs pre-test) in red blood cell count and Hb in 1/3 females at PERa 4.17 mg/kg and 1/3 females at PERa 20.87 mg/kg. Similar severe effects were not seen with PERe.
  - Medulla and/or papilla mineralization in kidneys of the PERa-treated dogs at all dose levels with similar incidence (1/3) and severity (minimal to mild) among doses
  - Three PERa-treated and four PERe-treated animals (at doses ≥ 4.17 mg/kg) showed minimal to mild bronchopneumonia or chronic peri-bronchiolitis, with similar incidence and severity between the two salts
- In a 13-week repeated oral toxicity study with PERa/AMLO at 0/0, 3.75/2, 7.5/4, 15/8, 15/0 or 0/8 mg/kg/day in Wistar rats -
  - Higher incidences of sporadic sialism within 2 hours of dosing in both sexes at 7.5/4, 15/8 mg/kg, and 0/8 mg/kg
  - Lower mean body weights in AMLO alone (0/8 mg/kg)-treated males
  - Higher urine volume associated with lower urine specific gravity in males, higher urinary chloride excretion, and lower serum chloride concentrations associated with a minimal increase in serum urea concentrations in both sexes at AMLO alone (0/8 mg/kg)
  - Lower serum calcium concentrations in male group at 15/8 and 15/0
  - Higher kidney weights for males at 15/8 mg/kg; minimal hypertrophy of the juxtaglomerular apparatus, thickening of the wall of the afferent and efferent arterioles of the glomeruli in the kidneys of 6/10 males and 5/10 females at 15/8 mg/kg and 1/10 female at 15/0 mg/kg; higher incidence of foci of basophilic (regenerating) tubules in the kidneys of both sexes at 15/8 mg/kg (all males and 4/10 females versus 6/20 control animals)
  - Increased incidences and severity of diffuse hypertrophy/vacuolation of the zona glomerulosa of the adrenal glands of both sexes at AMLO alone (0/8 mg/kg)
  - Neither new target organs nor relevant additive effects were identified with the combination of PER and AMLO.
  - NOAEL was 7.5/4 mg/kg of PER/AMLO. Corresponding mean values for Cmax and AUC24 in Week 13 (males-females) were 3.32 - 4.54 µg/ml and 8.18 - 7.36 µg.h/ml, respectively for S 9780 and 75.5 - 90.0 ng/ml and 526.1 - 570.5 ng.h/ml, respectively for AMLO.

Potential genotoxicity of perindopril arginine (PERa) was evaluated in the Ames test, mutation test at TK locus, chromosome aberration assay and in vivo micronucleus cytogenic assay. The results of these studies document that PERa does not demonstrate genotoxic potential under the conditions studied.

Impurities found in batches of PERa (b) (4) were also assessed for potential general and genetic toxicity with impurities spiked into parent compounds:

- In a 4-week repeated oral dose toxicity study with PERa alone (0, 0.8, 8, or 33 mg/kg/day) or PERa (0.8, 8, or 33 mg/kg/day) spiked with (b) (4) in Wistar rats -
  - No differences were noted in bodyweight, food intake, ophthalmoscopy, hematology, clinical chemistry and urinalysis between the PER and PER SPIKED-dosed groups. Minimally lower heart weights and slightly higher epididymis weights were seen in all PER or PER SPIKED dosed groups, which were not dose-related, similar between the PER or PER SPIKED dosed groups, and of no toxicological relevance.
  - At 33 mg/kg PER or PER SPIKED, minimal hypertrophy of the juxtaglomerular apparatus was seen at microscopic examination in the kidneys of some rats and a minimally higher incidence of minimal to slight focal erosions of the glandular stomach were observed, occasionally correlating with macroscopic changes (depressed dark red foci). These kidney and glandular stomach histopathological changes were considered as being similar between PER and PER SPIKED-dosed groups.
  - NOAELs in this study were 8 mg/kg/day for both PER and PER SPIKED. No toxicity was attributable to the presence of (b) (4)
- In a 4-week repeated oral dose toxicity study with PERa spiked with (b) (4) and (b) (4) (0, 0.8, 8, or 33 mg/kg/day) in Wistar rats -
  - Minimal but dose-related lower glucose levels were noted in treated animals of both sexes, which were statistically significant at 33 mg/kg/day (about -19%).
  - NOAEL was at 8 mg/kg/day in this study
- In a 4-week repeated oral dose toxicity study with AMLb (0, 7, or 14 mg/kg/day) or AMLb spiked with (b) (4) each (7 or 14 mg/kg/day) in Wistar rats -
  - 1/10 females of the AMLb and 1/10 females of the AMLb SPIKED at the dose 14 mg/kg/day were prematurely dead or killed in week 3-4. The two premature death females and two surviving females in the 14 mg/kg AMLb group had distended abdomen (dilation of the small intestine), less or absence of stools, dyspnea, hunched posture, decreased motor activity, partial blepharoptosis and piloerection, low feed intake, and /or major changes in various clinical pathology parameters. Histopathological observation revealed moderate ulcerative enteritis of the ileum in some of these animals. Dilation of the small intestine, with or without any microscopic changes, was considered to be the probable cause of morbidity and to be the cause of the poor clinical status at the dose 14 mg/kg of AMLb or AMLb SPIKED. No relevant differences of gastro-intestinal tract effects were noted between AMLb and AMLb SPIKED.
  - AMLb and AMLb SPIKED induced minimal and dose-related decreases in serum sodium and chloride concentrations (both sexes), increases in serum urea concentration (males only) and increases in urinary volume (high-dosed females only). Those changes were associated with a diffuse hypertrophy of the zona glomerulosa of the adrenal cortex with similar intensity whatever the batch.
  - Higher relative heart weight was observed for both sexes with dose-related incidence and severity. Severity of these findings was not enhanced when the AMLb was spiked with the two impurities (b) (4)
  - NOAEL was not established in this study. Under the condition of this study, toxicological profiles were similar between AMLb and AMLb SPIKED.

- Genotoxicity of PERa spiked with [redacted], PERa spiked with [redacted] or AMLb spiked with [redacted] each was assessed using Ames test, chromosome aberration test, and a test of mutation at the thymidine kinase locus. All test results were negative.

**Conclusions**

Safety assessment for Prestalia was conducted with a serial of bridging nonclinical PK and toxicology studies with PERa, PERe, and PERa/AMLb. The nonclinical studies were generally well designed and conducted. Generally, PERa and PERe had similar PK, safety and TK profiles in rats and dogs, except for slightly greater decreases in red blood cell count and Hb, and kidney medulla and/or papilla mineralization in PERa-treated dogs (NOAEL 0.83 mg/kg/day, ~2 times the recommended human maximal dose of PERa 14 mg in Prestalia 14/10 mg tablet for a 60-kg human on a body surface basis). There were adverse findings in the 13-week repeated oral dose toxicity rat study at high doses of PERa, AMLb, and PERa/AMLb, but no new target organs or relevant additive effects were identified with the combination of PERa and AMLb. There were no TK drug-drug interactions between PERa and AMLb. NOAEL in the 13-week study was 7.5/4 mg/kg of PERa/AMLb, which is ~4-5 times the recommended human maximal dose of Prestalia 14/10 mg tablet for 60-kg human on a body surface basis. These results provide a scientific 'bridge' to allow the sponsor to rely upon the Agency's previous findings of safety and efficacy for Prestalia active components PERa and AMLb: the listed drugs Aceon (NDA 020184) and NORVASC® (NDA019787) for approval of this 505(b)(2) NDA.

Impurities found in batches of PERa [redacted] and AMLb [redacted] were also assessed for potential general and genetic toxicity with impurities spiked into parent compounds. Oral daily administration of PERa spiked with [redacted] and [redacted] for 4 weeks in rats resulted in minimal but dose-related lower glucose levels, which was not seen in other studies with PERa and reached statistical significance 33 mg/kg/day (-19%). NOAEL for PERa spiked with [redacted] and [redacted] was 8 mg/kg/day, which is 5.5 times the recommended human maximal dose of PERa 14 mg in Prestalia 14/10 mg tablet for a 60-kg human on a body surface basis. Repeated oral dose toxicity studies in rats comparing PERa spiked with [redacted], or AMLb spiked with [redacted] each) with those of PERa or AMLb without added impurities revealed no significant differences between the spiked materials and PERa or AMLb without added impurities. In vitro genotoxicity studies with PERa spiked with [redacted] PERa spiked with [redacted] or AMLb spiked with [redacted] each showed negative results. Providing no structural alerts for genotoxicity, these 5 impurities are not considered to possess genotoxic potential. Based on these aforementioned results, specifications for drug substance impurities [redacted] (3.2.S.4.1) and specifications for drug product impurities [redacted] (3.2.P.5.1) are qualified.

## 12 References

1. Giuseppe Mancia G. et al. 2007 ESH-ESC Practice Guidelines for the Management of Arterial Hypertension - ESH-ESC Task Force on the Management of Arterial Hypertension, Journal of Hypertension 25:1751–1762, 2007.

2. Randa Hilal-Dandan. Chapter 26. Renin and Angiotensin. In Goodman & Gilman's The Pharmacological Basis of Therapeutics. 12th Edition. The McGraw-Hill Companies, Inc. 2011. ISBN 978-0-07-162442-8.
3. Elisaf MS, Theodorou J, Pappas H, Papagalanis N, Katopodis K, Kalaitzidis R and Siamopoulos KC. Effectiveness and metabolic effects of perindopril and diuretics combination in primary hypertension. Journal of Human Hypertension 13: 787–791, 1999.
4. Bahl VK, Jadhav UM, Thacker HP. Management of hypertension with the fixed combination of perindopril and amlodipine in daily clinical practice: results from the STRONG prospective, observational, multicenter study. Am J Cardiovasc Drugs. 9: 135-142, 2009.
5. Dahlöf B, Sever PS, Poulter NR, et al.; ASCOT Investigators. Prevention of cardiovascular events with an antihypertensive regimen of amlodipine adding perindopril as required versus atenolol adding bendroflumethiazide as required, in the Anglo-Scandinavian Cardiac Outcomes Trial-Blood Pressure Lowering Arm (ASCOT-BPLA): A multicentre randomised controlled trial. Lancet 366: 895–906, 2005.

## 13 Appendix/Attachments

Appendix I, pages 30-103
Appendix II, pages 104-116

**DEPARTMENT OF HEALTH AND HUMAN SERVICES**
**PUBLIC HEALTH SERVICE**
**FOOD AND DRUG ADMINISTRATION**
**CENTER FOR DRUG EVALUATION AND RESEARCH**

# PHARMACOLOGY/TOXICOLOGY IND REVIEW AND EVALUATION

| | |
|---|---|
| Application number: | 108233 |
| Supporting document/s: | 0000 |
| Sponsor's letter date: | Aug 3, 2011 |
| CDER stamp date: | Aug 4, 2011 |
| Review Completion Date: | Sept 26, 2011 |
| Product: | XOMA 985 (Fixed-dose Combination of Perindopril arginine and Amlodipine besylate) |
| Indication: | Treatment of Hypertension |
| Sponsor: | XOMA (US) LLC |
| Review Division: | Cardiovascular and Renal Products |
| Reviewer: | Baichun Yang, PhD, DABT |
| Supervisor/Team Leader: | Thomas Papoian, PhD, DABT |
| Division Director: | Norman Stockbridge, MD, PhD |
| Project Manager: | Michael V. Montekeone |

*Template Version: September 1, 2010*


Reference ID: 3020370

# TABLE OF CONTENTS





Reference ID: 3020370

# Table of Tables

## Table of Figures

# 1 Executive Summary

## 1.1 Introduction

The calcium channel blocker (CCB) amlodipine and angiotensin-converting enzyme (ACE) inhibitor perindopril have both been widely used for more than 15 years in countries worldwide. Both CCBs and ACE inhibitors lower blood pressure by reducing peripheral resistance. Blockade of calcium influx and reduction of angiotensin II-mediated vasoconstriction are complementary mechanisms. The combination of perindopril and amlodipine is recommended by the medical practice guidelines as being safe and effective (1). Both perindopril erbumine (ACEON®) and amlodipine besylate (NORVASC®) were approved in the US for the treatment of hypertension and coronary artery disease. The perindopril arginine/amlodipine besylate combination drug product is approved and is currently marketed in the European Union. The sponsor is developing the perindopril arginine/amlodipine besylate combination drug product XOMA 985 for treatment of hypertension in the US.

## 1.2 Brief Discussion of Nonclinical Findings

Because perindopril arginine had never been marketed in the US, bridging preclinical studies were performed to evaluate pharmacokinetic (PK) and safety profiles of perindopril arginine (PERa) with a comparison to perindopril erbumine (PERe). Similar to PERe, PERa was hydrolyzed to the active metabolite perindoprilat. Other metabolites of both PERe and PERa are perindopril glucuronide (Y 1303) and perindoprilat glucuronide (Y 1304). These metabolic steps occur with both oral and IV administration of PERa. PK profiles are similar between PERa and PERe following single-dose oral administration and multiple-dose administration in rats and dogs. In 28-day repeat dose study in dogs, oral PERa resulted in decreases in red blood cell counts and hemoglobin, and mild medulla and/or papilla mineralization in kidneys, which were not seen in PERe-treated dogs. Other findings in stomach (erosions and/or ulcerations, in rats), bronchus (minimal to mild bronchopneumonia or chronic peri-bronchiolitis, in dogs), serum chemistry, and urinalysis were similar between treatments with PERa or PERe in 28-day repeat dose oral studies in rats and dogs. PERa was not mutagenic in either *in vitro* or *in vivo* genotoxic assays.

Qualification studies were performed for impurities (b) (4) in amlodipine besylate (AMLb). AMLb spiked with impurities (b) (4) showed similar toxicological profile (dilation of small intestines with or without ulcerative ileitis and enteritis, changes in electrolytes, and higher heart weight) to that of AMLb in a 4-week oral repeat dose study in rats , and was not mutagenic in the in vitro genotoxic assays.

Thus, the nonclinical studies demonstrated the similarity, in general, between the arginine and erbumine salts of perindopril in terms of PK and safety profile. The safety



Reference ID: 3020370

profile of AMLb with impurities [redacted] (b) (4) each was similar to that of AMLb.

## 1.3 Recommendations

### 1.3.1 Clinical Study Safe to Proceed: Yes

### 1.3.2 If Not Safe to Proceed

N/A

### 1.3.3 Additional Recommendation(s) (Non-hold comments/advice to sponsor)

NONE

## 2 Drug Information

## 2.1 Drug

**XOMA 985 (perindopril arginine/amlodipine besylate combination drug product)**

**Drug Substances**

*Generic Name:* **perindopril arginine**

*Code Name*: S 9490-6, S 06490, S 6490, 09490-6, PERa

*Chemical Name*: [redacted] (b) (4)

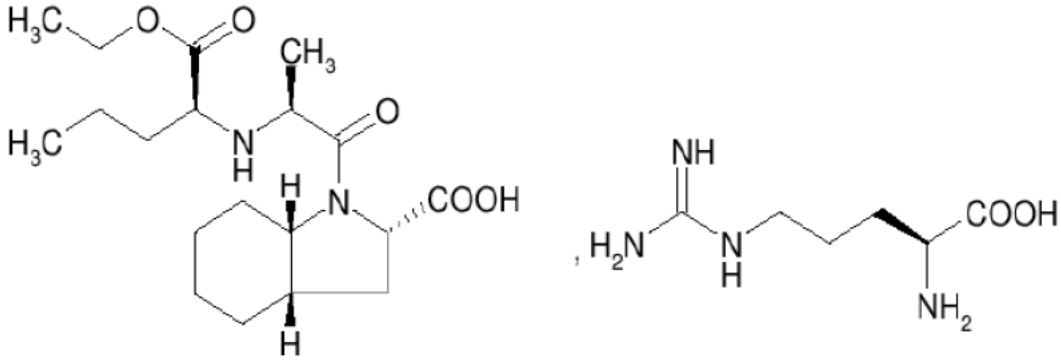
*CAS Registry Number:* CAS-612548-45-5

*Molecular Formula/Molecular Weight:* $C_{19}H_{32}N_2O_5$, $C_6H_{14}N_4O_2$/542.7 (salt form) or 368.5 (free acid)



Reference ID: 3020370

*Structure or Biochemical Description:*



*Pharmacologic Class:* Angiotensin Converting Enzyme Inhibitor

*Generic Name:* **amlodipine besylate**

*Code Name:* AMLb

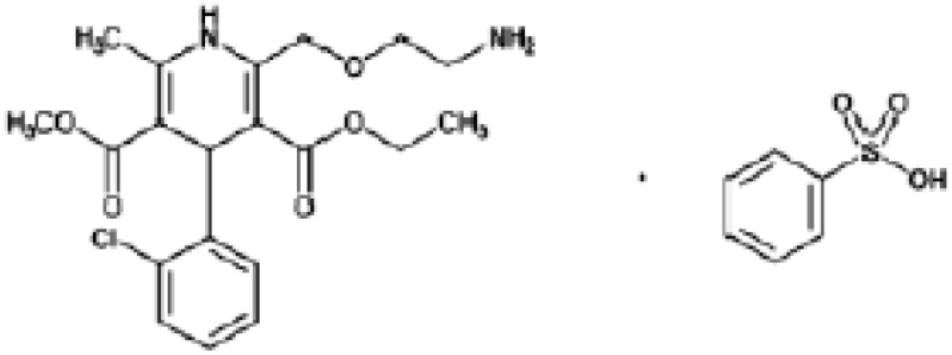
*Chemical Name*: 3-ethyl-5-methyl (±)-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylate, Monobenzenesulphonate

(b) (4)

*CAS Registry Number*: CAS-111470-99-6

*Molecular Formula/Molecular Weight:* $C_{20}H_{25}ClN_2O_5 \cdot C_6H_6O_3S$/ 567.1 (besylate salt form); (b) (4)

*Structure or Biochemical Description:*



*Pharmacologic Class:* Calcium Channel Blocker



Reference ID: 3020370

## 2.2 Relevant INDs, NDAs, BLAs and DMFs

NDA019787, NDA020184

## 2.3 Drug Formulation

Components and Composition of XOMA 985 Tablets, 14/10 mg

| Component | Quality | Function | Quantity per Unit (mg) | Weight % per Tablet |
|---|---|---|---|---|
| Perindopril arginine [amount of perindopril] | In-House | Active Ingredient | **14.00** [9.512] | 6.73 |
| Amlodipine besylate [amount of amlodipine] | USP | Active Ingredient | 13.87 **[10]** | 6.67 |
| Lactose (b) (4) | NF | (b) (4) | | |
| Magnesium stearate | NF | | | |
| Microcrystalline cellulose | NF | | | |
| Colloidal silicon dioxide | NF | | | |
| | | Total Tablet Weight | 208 | 100% |

Components and Composition of the Over-Encapsulated XOMA 985 Tablets, 14/10 mg

| Component | Quality | Function | Quantity per Unit |
|---|---|---|---|
| XOMA 985 Tablet (14/10 mg) | In-House | Drug Product | 1 |
| Lactose (b) (4) | NF | (b) (4) | QS |
| | | | 1 |
| | | | (b) (4) |

## 2.4 Comments on Novel Excipients

N/A

## 2.5 Comments on Impurities/Degradants of Concern

None

## 2.6 Proposed Clinical Protocol

**Perindopril Amlodipine for the Treatment of Hypertension (PATH, X985400)**

This is a phase 3, multicenter, randomized, double-blind, parallel-group trial in subjects with essential hypertension to evaluate the efficacy of the fixed-dose combination of



Reference ID: 3020370

PERa/AMLb compared to AMLb and PERe, and to assess the safety and tolerability of PERa/AML. This trial includes a screening visit, a 3-week washout period, and a 6-week double-blind treatment period. Approximately 816 subjects with essential hypertension will be enrolled into the trial and randomized in a 1:1:1 ratio to receive the fixed-dose combination of PERa/AMLb 14/10 mg once daily, AMLb 10 mg once daily, or PERe 16 mg once daily. The primary efficacy variable is the change from baseline (Day 0) to the end of treatment (Day 42) in the mean seated trough cuff diastolic blood pressure. The secondary efficacy variable is the change from baseline (Day 0) to the end of treatment (Day 42) in the mean seated trough cuff systolic blood pressure. Safety variables include adverse events and changes in physical examinations, laboratory parameters, vital signs, and electrocardiograms (ECGs).

## 2.7 Previous Clinical Experience

There is no previous human experience with XOMA 985 (perindopril arginine/ amlodipine besylate) in the US. However, there is extensive previous human experience with perindopril erbumine and amlodipine besylate since both products have been approved in the US and marketed worldwide for decades. Perindopril arginine and the fixed-dose combination of PERa/AMLb is approved in the European Union (EU) and currently marketed in more than 63 countries worldwide.

The initial US approval of NORVASC® (amlodipine besylate) Tablets for oral administration was in 1987. Amlodipine besylate may be used alone or in combination with other antihypertensive and antianginal agents for the treatment of Hypertension and Coronary Artery Disease [specifically, Chronic Stable Angina, Vasospastic Angina (Prinzmetal's or Variant Angina), Angiographically Documented Coronary Artery Disease in patients without heart failure or an ejection fraction < 40%]. The approved dosage form and strengths are 2.5 mg, 5 mg, and 10 mg Tablets. The recommended adult starting dose of NORVASC® is 5 mg once daily with a maximum dose 10 mg once daily. Amlodipine besylate is available from more than 25 generic drug manufacturers. It is also available in combination with benazepril hydrochloride, valsartan, telmisartan, aliskiren, and olmesartan medoxomil. Amlodipine besylate can cause symptomatic hypotension and worsen angina and acute myocardial infarction. The reported adverse effects of amlodipine besylate include headache, edema, fatigue, nausea, abdominal pain, and somnolence.

Perindopril erbumine (also called perindopril *tert*-butylamine) was approved in the US as ACEON® initially in 1993. Several generic versions are also marketed. Perindopril erbumine is indicated for the treatment of patients with essential hypertension and for treatment of patients with stable coronary artery disease to reduce the risk of cardiovascular mortality or nonfatal myocardial infarction. The approved dosage form and strengths are 2, 4 and 8 mg Tablets. The recommended initial dose for hypertension is 4 mg once a day with a maximum of 16 mg per day. The recommended initial dose for stable coronary artery disease is 4 mg once daily with a maximum of 8 mg once daily. Perindopril erbumine can cause injury to or death of the developing

fetus. Other most common adverse events of perindopril erbumine are cough, dizziness and back pain, drug intolerance, and hypotension. In 2008, perindopril arginine salt was approved in EU considering the better stability of perindopril arginine (2).

The fixed-dose combination of perindopril arginine/amlodipine besylate at doses of 5/5 mg, 10/5 mg, 5/10 mg, and 10/10 mg has been approval in EU under the product names COVERAM® and associated names for treatment of hypertension and coronary heart disease since 2008. The recent 12-month Periodic Safety Update Report (5th PSUR) covering the period from 26 March 2010 to 25 March 2011 for perindopril arginine/amlodipine tablets provided overall product marketing and usage information. Based on the unit sales data, the estimated number of patients exposed to perindopril arginine/amlodipine tablets since the marketing authorization is 8,708,282 patient-months. During the reported period (3/26/2010 – 3/25/ 2011), the estimate is 6,369,793 patient-months.

The 5th PSUR for perindopril arginine/amlodipine tablets stated –

* No specific regulatory or company actions were taken for safety reasons during this report period in any country.

* 109 patient-cases reports were received during this report period, including: 97 issued from Health Care Professionals (HCP) (75 spontaneous, 20 regulatory authorities, 2 literature, 0 clinical trial), and 12 issued from non-HCP.

* 9 patients experienced 14 serious unlisted events. Among them, the following events were the most frequently reported: 2 cardiac failures in the System Organ Class Cardiac disorders.

* 3 clinical trials were ongoing. No serious adverse reactions were reported in these studies. There was one case of pregnancy and induced abortion.

* No specific event was identified as a new signal. No new safety issue was identified.

## 2.8 Regulatory Background

A type B pre-IND/NDA meeting with the sponsor was held on October 20, 2010. Pharmacologists Drs. Baichun Yang, John Koerner, and Al Defelice attended the meeting. Nonclinical safety study-related questions, responses, and discussion (from the meeting minutes) are quoted here in the box –

***Question 13:***

*XOMA does not plan further pre-clinical testing of the proposed perindopril/amlodipine fixed-dose combination product. Is this acceptable to the Agency?*

**FDA preliminary response:**

Please remember that if you intend to rely on the Agency's finding of safety and/or effectiveness for a listed drug (s) or published literature that describes a specific listed drug(s), you should identify the listed drug(s) in accordance with the Agency's regulations at 21 CFR 314.54. The regulatory requirements for a 505(b)(2) application (including, but not limited to, an appropriate patent certification or statement) apply to each listed drug upon which a sponsor relies.

Perindopril arginine and perindopril erbumine are different molecules. There is no toxicology information available for perindopril arginine. If perindopril arginine is dissociated from each other soon after absorbed, perindopril and arginine are unlikely to cause additional toxicity than approved perindopril erbumine. You should demonstrate and state (1) perindopril arginine is dissociated from each other soon after being absorbed, or (2) perindopril arginine and perindopril erbumine are similar in toxicology.

Impurity limits > (b) (4) % in drug substance or > (b) (4) % in drug product will need to be qualified (including genotoxicity studies and general toxicity studies).

**Discussion during the meeting:**

The sponsor asked about the Division's last comment regarding impurities. The sponsor commented that in their calculations according to ICH Q3B, the impurity limits would be (b) (4) % for the high dose and (b) (4) % for low dose. Dr. Srinivasachar commented that if the sponsor is consistent with the ICH Q3B (R2) recommendations there should not be an issue. The sponsor also commented that they may propose higher impurity limits for the lowest strength.. Dr. Srinivasachar stated that the sponsor would have to present their case for review.

## 3 Studies Submitted

## 3.1 Studies Reviewed

| | | | |
|---|---|---|---|
| NP15043 | RB-03-06490-007-DRPK<br>Study Plan No. PB-02-06490-007-DRPK | 4.2.2.2.1 | Rat Single Dose Pharmacokinetics Report PERa |
| NP15052 | RB-03-06490-002-DDPK<br>Study Code S 9490-6 BP.D.5010.PO.PH.REG | 4.2.2.2.2 | Dog Multiple Dose Pharmacokinetics Report PERa |
| NP15064 | Study No. 5124 | 4.2.3.1.1 | Mouse Single Dose Toxicity Report PERa |
| NP15065 | Study No. 5123 | 4.2.3.1.2 | Rat Single Dose Toxicity Report PERa |
| NP08100 | Study No. 4642<br>TO.R.4642.PO.4.REG | 4.2.3.2.1 | Rat Multiple Dose Toxicity Report PERa and PERe |



Reference ID: 3020370

| | | | |
|---|---|---|---|
| NP15015 | RB-03-09490-013-DRPK<br>IRIS Study Code No. PKA-5492-002-DEU<br>Servier Plan No. S 09490-6.TO.R.4642.PO.4.REG<br>AAI Study Code LA211 | 4.2.3.2.2 | Rat Multiple Dose Toxicokinetics Report<br>PERa and PERe |
| NP15081 | Study No. 5003<br>TO.D.5003.PO.4.REG | 4.2.3.2.3 | Dog Multiple Dose Toxicity Report<br>PERa and PERe |
| NP07996 | Study No. 4643<br>TGE.SE.4643.HIS.REG | 4.2.3.3.1.1 | *in vitro* Genotoxicity Report<br>(Ames) PERa |
| NP08568 | Covance Study No. 303/278<br>Covance Report No. 303/278-D6173 | 4.2.3.3.1.2 | *in vitro* Genotoxicity Report<br>(Mouse Lymphoma) PERa |
| NP08569 | Covance Study No. 303/276<br>Covance Report No. 303/276-D6172<br>TOX-9460-014-GBR/001 | 4.2.3.3.1.3 | *in vitro* Genotoxicity Report<br>(Chromosome Aberrations) PERa |
| NP08649 | Study No. 5011<br>S9490-6 TGE.S.5011.PO.MNU.REG | 4.2.3.3.2.1 | *in vivo* Genotoxicity Report<br>(Mouse Micronucleus) PERa |
| 6620 | TGE.SE.6620.HIS.REG | 4.2.3.7.6.1 | *in vitro* Genotoxicity Report<br>(Ames) AMLb with Impurities Y 2051-1 and Y 1766-1 |
| 8224400 | TOX-05895-003 | 4.2.3.7.6.2 | *in vitro* Genotoxicity Report<br>(Chromosome Aberration) AMLb with Impurities Y 2051-1 and Y 1766-1 |
| 6579 | TO.R.6579.PO.4.REG | 4.2.3.7.6.3 | Rat Multiple Dose Toxicity<br>AMLb with Impurities Y 2051-1 and Y 1766-1 |

## 3.2 Studies Not Reviewed

| | | | |
|---|---|---|---|
| NP08575 | RB-02-06490-004-DSVA | 4.2.2.1.1 | Method Validation,<br>Mouse Plasma Perindoprilat |
| NP08576 | PB-01-09490-014-DRVA<br>AAI Study Code LA179 | 4.2.2.1.2 | Method Validation,<br>Rat Plasma Perindoprilat |
| NP15014 | RB-03-06490-005-DDVA | 4.2.2.1.3 | Method Validation,<br>Dog Plasma Perindoprilat |
| NP16030 | RB-04-06490-012-DSST | 4.2.2.1.4 | Bioanalytical Stability Report,<br>Mouse Perindoprilat |
| NP15047 | Study Plan No. CHM-06490-007-DEU<br>AAI Study Code NA027 | 4.2.2.1.5 | Bioanalytical Stability Report,<br>Rat Perindoprilat |
| NP16031 | RB-04-06490-011-DDST | 4.2.2.1.6 | Bioanalytical Stability Report,<br>Dog Perindoprilat |

## 3.3 Previous Reviews Referenced

NONE

### 3.4 Disclaimer

Tables and figures in this document are created by the reviewer unless identified.

## 4 Pharmacology

N/A



Reference ID: 3020370

# 5 Pharmacokinetics/ADME/Toxicokinetics

## 5.1 PK/ADME

### 5.1.1 Rat single PO/IV dose PERa pharmacokinetics report (NP15043)

This study (NP15043) was performed in [redacted] (b) (4) during Dec 2002 – Jan 2003, sponsored by the [redacted] (b) (4) .

Male Wistar rats received a single dose of perindopril arginine (PERa, S 9490-6) at the dose equivalent of 0.8 mg/kg free acid by oral (PO) gavage or intravenous (IV) infusion (over 10 minutes). Blood samples (3 rats per time point, 3 times per rat) were collected at 0.167, 0.5, 1, 1.5, 3, 4, 6, 8, and 24 h after IV dosing and 0.25, 0.5, 1, 1.5, 3, 4, 6, 8, and 24 h after PO dosing. Urine was collected before dosing and 24 hours after dosing. Since perindopril and its glucuronide Y 1303 are rapidly hydrolyzed to their corresponding acid perindoprilat and its glucuronide Y 1304, perindoprilat (S 9780) and its glucuronide Y 1304 were measured in plasma and urine using liquid scintillation chromatography [redacted] (b) (4) mass spectrometric detection (LC-MS/MS) after solid-phase extraction.

Plasma concentrations of perindoprilat and Y 1304 are shown in Figure 1 and PK parameters are summarized in Table 1. After oral administration of PERa, Tmax for perindoprilat and perindoprilat glucuronide Y 1304 were 1 h and 30 min, respectively. This delayed Tmax of perindoprilat compared to its glucuronide Y 1304 suggests that Y 1304 is directly formed from perindopril via its glucuronide Y 1303 rather than from perindoprilat itself. The plasma AUC ratio of Y 1304 to perindoprilat after IV dosing was about 15 times lower than after PO dosing. This was possibly related to a pre-systemic formation of perindopril glucuronide from perindopril, and subsequent hydrolysis to Y 1304.

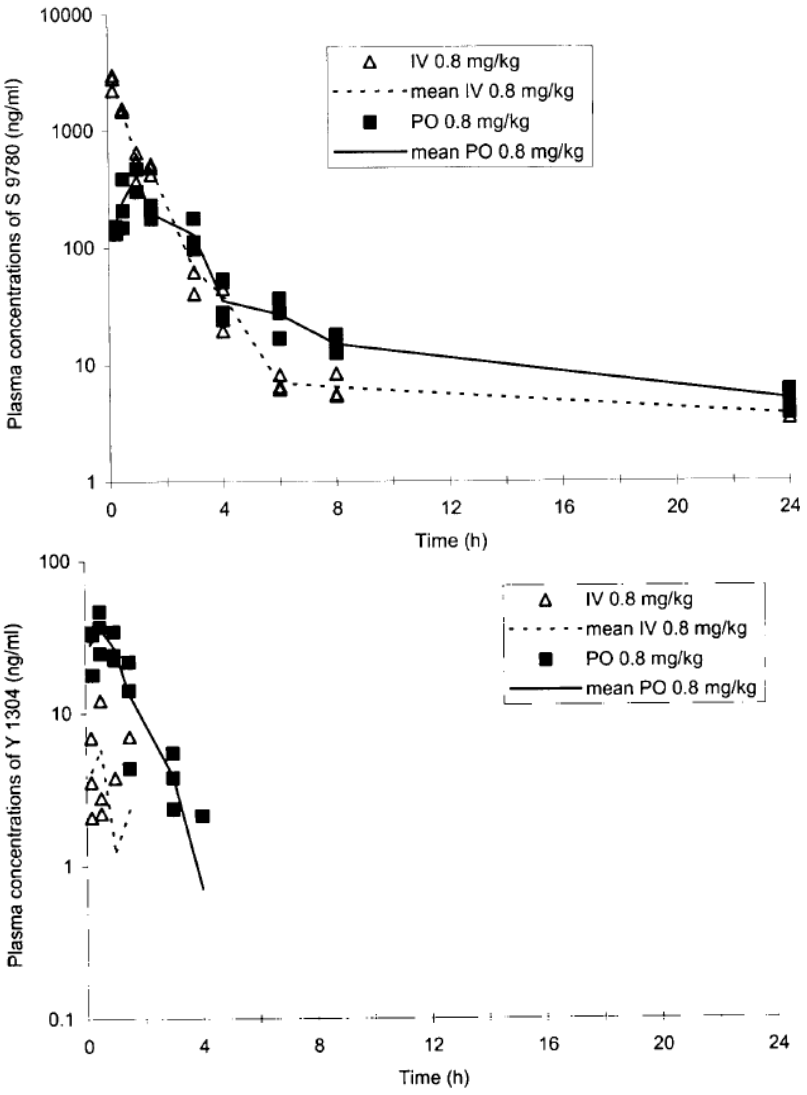
After oral administration of PERa, perindoprilat and Y 1304 AUCs were 1017 and 50.9 ng•h/ml, respectively, indicating direct hydrolysis of PERa into perindoprilat as being the main transformation route in rats. Bioavailability was ~44% for the PERa oral dose.

Perindoprilat was rapidly eliminated with a short principal half-life of about 0.6 h representing about 88 % of the AUC. The urinary excretion of perindoprilat was 46.3 % after IV and 39.8 % after PO administration of PERa, whilst the urinary excretion of Y 1304 was less than 4 % of the dose after IV and PO administration.



Reference ID: 3020370

Figure 1. Plasma concentrations of perindoprilat (S9780, upper) and Y 1304 (bottom) after a single dose of PERa in rats (Sponsor's Figure).






Reference ID: 3020370

Table 1. PK parameters of perindoprilat (S9780) and Y 1304 after a single dose of PERa in rats (Sponsor's Table)

| Pharmacokinetic Parameters | | S 9780 | | | | Y 1304 | |
|---|---|---|---|---|---|---|---|
| | | PO | | IV | | PO | IV |
| **Plasma** | | | | | | | |
| $C_{max}$ | (ng/ml) | 419 | | 2677 | | 36.0 | 5.71 |
| $t_{max}$ | (h) | 1.00 | | 0.167 | | 0.500 | 0.500 |
| $C_{last}$ | (ng/ml) | 5.07 | | 3.71 | | BLQ (0.713) | 2.34 |
| $t_{last}$ | (h) | 24.0 | | 24.0 | | 4.00 | 1.50 |
| $AUC_t$ | (ng.h/ml) | 943 | | 2151 | | 50.2 | 4.37 |
| $AUC_{24}$ | (ng.h/ml) | 943 | | 2151 | | 50.9 | 7.53 |
| AUC | (ng.h/ml) | 1017 | | 2292 | | 50.9 | 7.53 |
| $t_{½,1}$ | (h) | 0.658 | 89.7 [1] | 0.583 | 87.6 [1] | - | - |
| $t_{½,z}$ | (h) | 10.1 | 10.3 [1] | 26.3 | 12.4 [1] | 0.634 | 0.939 |
| $\lambda_z$ | (1/h) | 0.0688 | | 0.0264 | | 1.09 | 0.738 |
| $R_{ac,D1}$ | | 1.08 | | 1.07 | | 1.00 | 1.00 |
| AUC ratio$_{Y\ 1304/S\ 9780}$** | | 0.0330 | | 0.00217 | | - | - |
| **Urine** | | | | | | | |
| Ae | (µg) | 74.8 | | 85.3 | | 9.72 | 0.427 |
| fe* | (%) | 39.8 | | 46.3 | | 3.39 | 0.152 |
| $CL_R$ | (ml/min/kg) | 5.11 | | 2.56 | | 12.3 | 3.65 |
| fe ratio$_{Y\ 1304/S\ 9780}$ | | 0.0852 | | 0.00329 | | - | - |

[1] % AUC in which the corresponding half-life is involved
Ae = cumulative amount of compound excreted into urine during post-dose 24 hours
AUC = area under the plasma drug concentration-time curve from time 0 to infinity
$CL_R$ = renal clearance of the compound
fe* = excretion of S 9780 or Y 1304 expressed as % of the administered dose of PERa, corrected by molar mass
** = AUC ratio corrected by molar mass

### 5.1.2 A comparative repeat oral dose PK study for PERa (S 9490-6) and PERe (S 9490-3) in dogs (NP15052)

This study (NP15052) was performed in (b) (4) during Sept 2002 – Dec 2002, sponsored by the (b) (4)

The study was performed in 4 male beagle dogs in 2 treatment phases using a cross over design with a 7-day wash out period. Each animal received an oral dose of 0.834 mg/kg/day (as free acid) of PERa or PERe once daily for 7 days. Blood samples were collected before dosing and 0.25, 0.5, 1, 2, 4, 6, 8, 10, and 24 h after dosing on days 1 and 7. Urine samples were collected during predose period and during post-dose 24 h on days 1 and 7. Perindopril (S 9490), perindoprilat (S 9780), and their respective glucuronides Y 1303 and Y 1304 in plasma and urine samples were determined using LC-MS/MS after solid-phase extraction. The limit of quantitation is 0.5 ng/ml for perindopril, 1 ng/ml for perindoprilat, and ~ 2 ng/ml for both glucuronides.

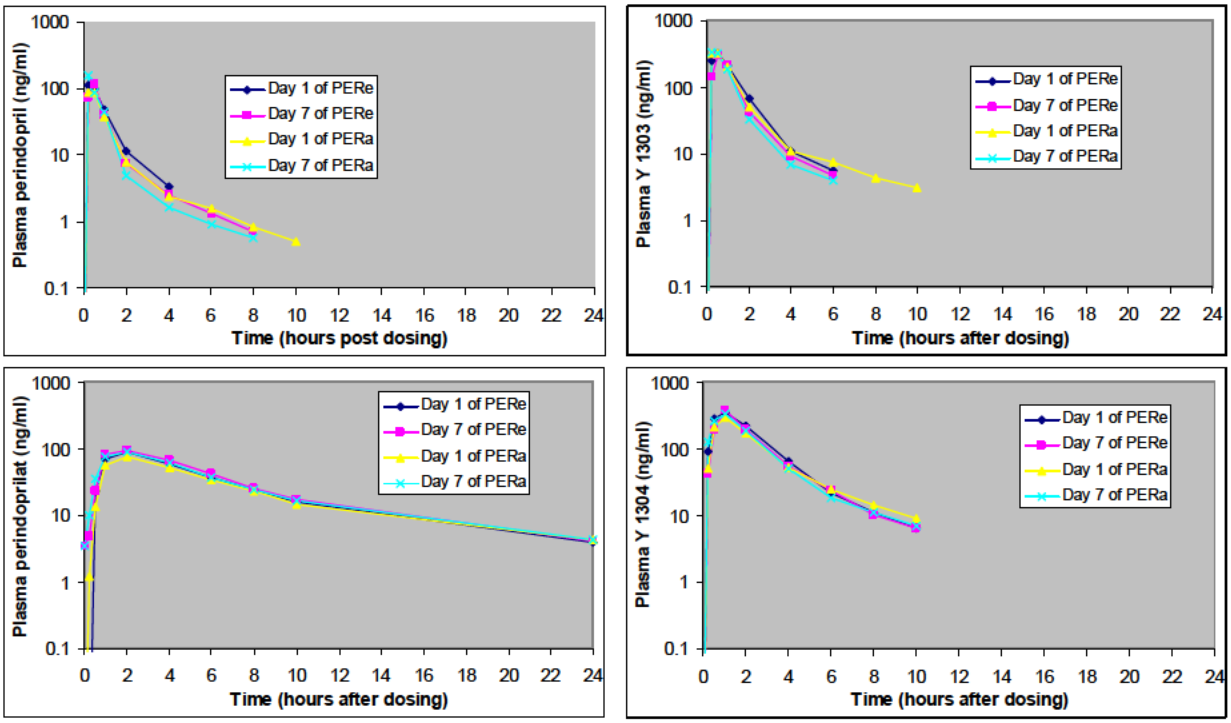
After oral administration of each perindopril salt, no differences were observed in the PK parameters of perindopril, perindoprilat, or their respective glucuronides (Table 2 & Figure 2). After repeated once daily oral administration, both PERe and PERa were rapidly absorbed, with perindopril Tmax 0.5 h. Tmax of perindoprilat (2 h) follows its glucuronide Y 1304 (1 h), indicating the formation of this glucuronide directly from

Perindopril rather than from perindoprilat itself. Perindopril was rapidly and extensively metabolized into perindoprilat and perindoprilat Glucuronide (Y 1304), which are the main metabolites determined in plasma and in urine (mean percentage of dose excreted were ~12-14 % and ~9-12 % respectively on day 1).

Perindopril glucuronide (Y1303) was formed to a less extent, and perindopril was the minor circulating component. The urinary excretion of these two compounds was negligible (at most 3% of the oral dose). These data indicate that both perindopril hydrolysis into perindoprilat and glucuronidation pathway occur at the same extent in dogs.

After oral administration of PERe or PERa, the mean terminal half-life was ~3 h for perindopril and Y 1303, 6-7 hours for perindoprilat, and ~5 h for Y 1304. No accumulation was observed after repeated oral dosing for 7 days for both compounds.

Figure 2. Plasma concentrations of perindopril & it metabolites after PO dosing in dogs






Reference ID: 3020370

Table 2. PK parameters after oral administration of PERe and PERa in dogs (Sponsor's Table)

| Day 1 | Administration of PERe | | | | Administration of PERa | | | |
|---|---|---|---|---|---|---|---|---|
| Plasma Parameters | Perindopril | Perindoprilat | Y 1303 | Y 1304 | Perindopril | Perindoprilat | Y 1303 | Y 1304 |
| $C_{max}$ (ng/mL) | 149 | 90.9 | 402 | 379 | 109 | 76.5 | 368 | 295 |
| $T_{max}$* (h) | 0.375 (0.25–1) | 2 (2-2) | 0.5 (0.25-1) | 0.75 (0.5-2) | 0.375 (0.25–1) | 2 (2-2) | 0.5 (0.5-1) | 1 (0.5-1) |
| AUC (ng·h/mL) | 119 | 613 | 451 | 951 | 96.7 | 554 | 429 | 784 |
| $t_{½,z}$ (h) | 1.30 | 6.38 | 1.90 | 4.94 | 2.84 | 7.15 | 2.67 | 3.06 |
| **Excretion Parameters** | | | | | | | | |
| fe (%) | 2.28 | 11.8 | 3.12 | 11.6 | 2.12 | 14.3 | 2.02 | 8.97 |
| $CL_R$ (mL/min/kg) | 2.84 | 2.75 | 1.72 | 2.50 | 3.17 | 3.61 | 0.98 | 2.39 |

| Day 7 | Administration of PERe | | | | Administration of PERa | | | |
|---|---|---|---|---|---|---|---|---|
| Plasma Parameters | Perindopril | Perindoprilat | Y 1303 | Y 1304 | Perindopril | Perindoprilat | Y 1303 | Y 1304 |
| $C_{max}$ (ng/mL) | 134 | 95.1 | 313 | 375 | 171 | 91.1 | 402 | 366 |
| $T_{max}$ (h) | 0.5 (0.25-0.5) | 2 (2-2) | 0.5 (0.5-1) | 1 (1-1) | 0.375 (0.25-0.5) | 2 (1-2) | 0.375 (0.25-0.5) | 1 (0.5-1) |
| AUC (ng·h/mL) | 105 | 663 | 367 | 809 | 108 | 634 | 396 | 842 |
| $t_{½,z}$ (h) | 2.25 | 6.14 | 1.99 | 1.75 | 2.00 | 6.75 | 2.92 | 4.02 |
| **Excretion Parameters** | | | | | | | | |
| fe (%) | 2.19 | 17.0 | 2.02 | 8.88 | 1.90 | 14.9 | 2.28 | 8.46 |
| $CL_R$ (mL/min/kg) | 2.90 | 3.49 | 1.24 | 2.16 | 2.73 | 3.31 | 1.22 | 2.00 |

* $T_{max}$ is median (range)
AUC = area under the plasma drug concentration-time curve from time 0 to infinity; $CL_R$ = renal clearance of the compound; $C_{max}$ = maximal plasma concentration; fe = excretion of the compound expressed as a fraction (%) of the administered dose of perindopril; PERa = perindopril arginine; PERe = perindopril erbumine; $t_{½,z}$ = terminal half-life; $T_{max}$ = time to maximal plasma concentration.

## 5.2 Toxicokinetics

Included in toxicity studies

# 6 General Toxicology

## 6.1 Single-Dose Toxicity

### 6.1.2 Single dose toxicity study with oral PERa in Swiss OF1 mice

This study (NP15064) was performed in [redacted] (b) (4) according to GLP regulations, during Mar – Jun, 2003. The sponsor is [redacted] (b) (4)

Swiss OF1 mice (6/sex/group, approximately 6 weeks old at dosing) were orally gavaged with a single dose of PERa (S 9490-6) at 0 (control, purified water) or 2000 mg free acid/kg (dosing volume 10 ml/kg). Animals were then examined daily for changes in appearance and behaviour and for death. Body weights were periodically measured. All mice were killed and necropsied at the end of a 2-week observation period.

No death occurred throughout the study. No changes in appearance, behavior, or body weight were observed. One male mouse received PERa had a whitish area (5 mm) on the left liver lobe, which was considered a spontaneous finding in laboratory mice of this strain at this age. No target organs were identified at macroscopic examination.

### 6.1.3 Single dose toxicity study with oral PERa in rats

This study (NP15065) was performed in [redacted] (b) (4) according to GLP regulations, during Mar – Jun, 2003. The sponsor is [redacted] (b) (4).

Wistar rats (6/sex/group, approximately 6 weeks old at dosing) were orally gavaged with a single dose of PERa (S 9490-6) at 0 (control, purified water) or 2000 mg free acid/kg (dosing volume 10 ml/kg). Animals were then examined daily for changes in appearance and behaviour and for death. Body weights were periodically measured. All rats were killed and necropsied at the end of a 2-week observation period.

No death occurred throughout the study. Excessive salivation was observed for all rats within 30 minutes after dosing. No other abnormalities or target organs were identified.

## 6.2 Repeat-Dose Toxicity

### 6.2.1 In rats

**Study title:** 4-week repeat dose toxicity study with PERa & PERe in Wistar rats

| | |
|---|---|
| Study no.: | NP08100, NP15015 |
| Study report location: | [redacted] (b) (4) |
| Conducting laboratory and location: | |
| Date of study initiation: | Dec 28, 2000 |
| GLP compliance: | Yes |
| QA statement: | Yes |
| Drug, lot #, and % purity*: | PERe (S 9490-3), Lot #55 132, 99.7%<br>PERa (S 9490-6), Lot # FF 826, 99.7% |

*PERa batch FF 826 contained various impurities including [redacted] (b) (4)

**Key Study Findings**

- No death occurred during the study.
- Transiently decreased spontaneous motor activity and/or extensive salvation in 2/10 males after first dose of PERe at the high dose 33 mg free acid/kg
- Slight increases in water intake, urinary volume, urinary chloride and sodium elimination, associated with minimal decreases in serum sodium and chloride concentrations, in all the treated (0.8-33 mg/kg) groups (similar magnitude for both salt)
- Slightly lower heart weight in treated animals (0.8-33 mg/kg), without histological changes or a dose-dependent relationship.
- Dose-related higher incidence of erosions and/or ulcerations in the glandular stomach mucosa in treated groups, slightly higher in PERe groups than in PERa groups



Reference ID: 3020370

- The salient findings observed with both PERa and PERe at the same dose levels were similar, and are class-effects of ACE inhibitors.
- Under the conditions of this study, PERa (S 9490-6, batch with various impurities) and PERe had a similar safety profile.

Methods

| | |
|---|---|
| Doses: | 8 and 33 mg free acid/kg of PERe<br>0.8, 8, and 33 mg free acid/kg of PERa<br>Dosage selection was based on the results of a previous 6-week toxicity study in Wistar rats with daily PO PERe at doses 0.83, 8.3 and 33.4 mg free acid/kg. The dose 0.83 mg free acid/kg was well tolerated, with minimal changes in body weight, water intake and serum sodium concentration. Similar findings were noted at 8.3 and 33.4 mg free acid/kg, with a dose-related severity, as well as changes in red blood cell parameters, serum lipids, serum and urinary creatinine or potassium concentrations. |
| Frequency of dosing: | Once daily for 4 weeks |
| Route of administration: | Oral |
| Dose volume: | 10 ml/kg |
| Formulation/Vehicle: | Solution / water |
| Species/Strain: | Wistar rats |
| Number/Sex/Group: | 10 in main study, 6 in TK study |
| Age: | 6 weeks old |
| Weight: | 128-146 g for males; 124-145 g for females |
| Satellite groups: | 6/sex/group for TK |
| Unique study design: | This study was designed to compare the safety profile of PERa (S 9490-6) with that of PERe (S 9490-3) & to evaluate the potential effect of batch FF 826 of PERa containing various impurities including (b) (4) |
| Deviation from study protocol: | No impact to the results |

**Observations and Results**

**Mortality**

All animals were checked daily for mortality or signs of morbidity. No death occurred during the study.

**Clinical Signs**

Animals were checked for clinical signs 2-3 times per day. Transiently decreased spontaneous motor activity and/or excessive salivation were observed in 2/10 males in the PERe 33 mg free acid/kg group on day 1, 2-15 min after dosing.

**Body Weights**

Body weight was recorded once during the acclimation phase and twice a week until week 4. The body weight was similar among treated and control groups.

**Feed Consumption**

Food and water intakes were measured once a week (quantities over approximately 7 days). Food intake was similar among treated and control groups.

Water intake during the 4-week study period was higher in all treated groups than in controls without dose-response relationship (Table 3), indicating the maximum effects at the low dose 0.8 mg free acid/kg/day. There was no evidence of a difference in severity between PERe- and PERa-treated groups.

Table 3. Group mean water intake (g/animal/day) in rats (Sponsor's Table)

| Test Substance | Dose Level (mg free acid/kg) | Week 1 | | Week 2 | | Week 3 | | Week 4 | | Weeks 1 to 4 | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | M | F | M | F | M | F | M | F | M | F |
| Vehicle | 0 | 24 | 22 | 27 | 23 | 30 | 23 | 29 | 25 | 735 | 629 |
| S 9490-3 (PERe) | 8 | 28** *+17%* | 25 | 33** *+22%* | 27** *+17%* | 35 | 27 | 33 | 28 | 878* *+19%* | 718 |
| | 33 | 26 | 24 | 30 | 24 | 32 | 24 | 29 | 27 | 794 | 665 |
| S 9490-6 (PERa) | 0.8 | 29* *+21%* | 28** *+27%* | 32 | 30** *+30%* | 33 | 29* *+26%* | 32 | 30 | 856 | 787* *+25%* |
| | 8 | 29 | 25 | 34* *+26%* | 27 | 37 | 26 | 35 | 27 | 910* *+24%* | 708 |
| | 33 | 28 | 23 | 31 | 23 | 34 | 24 | 32 | 26 | 848 | 651 |

* = $p < 0.05$; ** = $p < 0.01$; % *versus* control mean values

**Ophthalmoscopy**

Ophthalmologic examinations of the appendages, optic media and fundus by indirect ophthalmoscopy were performed once during the acclimation phase and once in the last week of the dosing phase. There were no treatment-related findings.

**ECG**

None

**Hematology**

Blood samples for hematology and clinical chemistry were collected on day 29 after an overnight feed deprivation of approximately 16 hours. Hematology parameters included –

- Erythrocyte count
- Hematocrit (Ht)
- Mean corpuscular volume (MCV)
- Hemoglobin (Hb)
- Mean corpuscular hemoglobin (MCH)
- Mean corpuscular hemoglobin concentration (MCHC)
- Reticulocyte count
- Total leukocyte count
- Differential leukocyte count
- Platelet count (Plts)
- Prothrombin time (PT)
- Activated partial thromboplastin time (APTT)

Leukocyte counts were lower in PERa-treated males than in control males at doses ≥ 0.8 mg/kg, due to decreases in eosinophil, lymphocyte and/or monocyte counts, which were also of statistical significance at doses ≥ 0.8 mg/kg (Table 4). Mean corpuscular volume was slightly lower in females at PERa 0.8 mg free acid/kg (Table 4). However, all these changes were slight, not in both sexes, and not dose-dependent. The sponsor stated that these values remained within the 5-95% confidence limits established in the testing laboratory. Moreover, the difference in these values between the PERa- and PERe- treated animals was not statistically significant. Therefore, the slightly lower values in neutrophil, eosinophil, lymphocyte, and/or monocyte counts are not of toxicological significance.

Table 4. Differences in rat hematological parameters at the end of dosing

| | | PERe | | PERa | | |
|---|---|---|---|---|---|---|
| | Control | 8 mg/kg | 33 mg/kg | 0.8 mg/kg | 8 mg/kg | 33 mg/kg |
| Males | | | | | | |
| Leucocyte count (G/L) | 7.8±1.6 | 6.8±1.3 | 7.1±1.9 | 6.3±1.0* | 5.8±1.1** | 6.4±1.31* |
| Neutrophils (G/L) | 1.08±0.31 | 1.19±0.31 | 1.13±0.48 | 0.96±0.21 | 0.81±0.13 | 0.90±0.21 |
| Eosinophils (G/L) | .094±.056 | .087±.044 | .074±.028 | .046±.012** | .063±.026 | .075±.025 |
| Basophils (G/L) | .018±.01 | .015±.044 | .019±.01 | .010±.007 | .012±.004 | .011±.007 |
| Lymphocytes (G/L) | 6.28±1.49 | 5.12±1.00 | 5.56±1.38 | 4.95±0.89* | 4.65±0.94** | 5.05±1.16 |
| Monocytes (G/L) | .326±.087 | .294±.07 | .319±.101 | .29±.057 | .226±0.93* | .319±.068 |
| Females | | | | | | |
| MCV (fl) | 57.9±1.4 | 56.8±1.8 | 56.7±1.3 | 55.6±1.3* | 57.5±2.2 | 56.2±2.6 |

* p<0.05 vs control; ** p<0.01 vs control

**Clinical Chemistry**

Parameters of clinical chemistry included –

- Alanine aminotransferase (ALT)
- Albumin

| | |
|---|---|
| Aspartate aminotransferase (AST) | Proteinogram* |
| Glutamate dehydrogenase (GLDH) | Urea |
| Alkaline phosphatase (ALP) | Creatinine |
| Total Bilirubin | Sodium |
| Glucose | Potassium |
| Total cholesterol | Chloride |
| Triglycerides | Calcium |
| Total protein | Inorganic phosphorus |

* Electrophoresis & Quantitative densitometry performed on the 5 last animals/sex/ group, for albumin/globulins ratio

Serum sodium and chloride concentrations were minimally lower in all treated groups than in controls (Table 5). There was no evidence of a difference in severity between the PERe- and PERa- treated groups. Serum glutamate dehydrogenase activity (GLDH), total protein or calcium concentrations in males given PERe were minimally lower than in controls, which were not dose-related and remained within the 5-95% confidence limits established in testing facility. Therefore, they were not of any toxicological significance.

Table 5. Differences in rat serum chemistry at the end of dosing

| | | PERe | | PERa | | |
|---|---|---|---|---|---|---|
| | Control | 8 mg/kg | 33 mg/kg | 0.8 mg/kg | 8 mg/kg | 33 mg/kg |
| Males | | | | | | |
| GLDH (U/L) | 9.1±1.4 | 7.5±0.8** | 8.1±0.9 | 8.8±2.0 | 7.7±0.9 | 8.9±1.6 |
| Total protein (g/L) | 66.6±1.8 | 65.3±2.5 | 64.4±1.3* | 65.9±2.1 | 64.0±2.8 | 65.7±2.3 |
| Sodium (mmol/L) | 143.2±1.0 | 140.2±1.9** | 142.3±0.9 | 140.5±2.0** | 140.4±2.6** | 141.1±1.3* |
| Chloride (mmol/L) | 104.0±1.1 | 101.5±2.0** | 103.8±0.6 | 101.1±1.2** | 102.1±2.3* | 102.5±1.2 |
| Calcium (mmol/L) | 2.73±0.10 | 2.56±0.23* | 2.71±0.07 | 2.67±0.13 | 2.61±0.11 | 2.67±0.09 |
| Females | | | | | | |
| Sodium (mmol/L) | 142.9±0.8 | 140.5±1.7** | 140.8±1.7** | 138.9±2.0** | 139.5±1.8** | 140.4±1.6** |
| Chloride (mmol/L) | 105.6±1.1 | 104.1±1.5* | 105.4±1.3 | 102.3±1.2** | 102.8±2.0** | 103.3±1.8** |

* p<0.05 vs control; ** p<0.01 vs control

**Urinalysis**

Urine was collected overnight on day 28 under conditions of feed deprivation and water availability for approximately 16 hours. The volume of collected urine was measured. Parameters included –

| | |
|---|---|
| Creatinine | Protein |
| Sodium | Specific gravity |
| Potassium | Semi-quantitative estimations |
| Chloride | Urinary sediment (Performed on the *5* last animals/sex/group) |

The 24-hour urinary eliminations were calculated and expressed as mmol/24h for sodium, potassium, and chloride or as μmol/24h for creatinine.

Urinary volumes collected overnight, chloride and sodium urinary eliminations were slightly higher in PERe 8 mg/kg group and in PERa 0.8 and 8 mg/kg groups than in



Reference ID: 3020370

controls (Table 6). Potassium urinary elimination tended to be elevated, but with individual values within the 5-95% confidence limits established in testing facility. All these changes were not dose-related, minimal, and not of toxicological relevance. There were no salient changes in urinary semi-quantitative estimation, nor in specific gravity, among all the groups.

Table 6. Differences in rat urinalysis at the end of dosing

| | | PERe | | PERa | | |
|---|---|---|---|---|---|---|
| | Control | 8 mg/kg | 33 mg/kg | 0.8 mg/kg | 8 mg/kg | 33 mg/kg |
| Males | | | | | | |
| Urinary volume (ml/24 h) | 26.8±11.5 | 43.0±19.4* | 23.6±15.8 | 54.8±32.2* | 42.1±22.2 | 34.1±12.2 |
| Sodium urinary elimination (mmol/24 h) | *0.66±0.33* | 0.68±0.28 | 0.50±0.23 | *0.95±0.35* | *0.70±0.30* | *0.52±0.18* |
| Chloride urinary elimination (mmol/24 h) | 0.92±0.34 | 1.15±0.40 | 0.83±0.27 | 1.41±0.45** | 1.21±0.32 | 0.94±0.23 |
| Potassium urinary elimination (mmol/24 h) | 1.89±0.44 | 2.20±0.39 | 1.90±0.44 | 2.21±0.40 | 2.36±0.42 | 2.05±0.32 |
| | | PERe | | PERa | | |
| | Control | 8 mg/kg | 33 mg/kg | 0.8 mg/kg | 8 mg/kg | 33 mg/kg |
| Females | | | | | | |
| Urinary volume (ml/24 h) | *17.5±15.1* | 21.5±16.8 | 23.7±10.1 | *32.6±21.2* | *25.3±14.6* | *11.8±10.4* |
| Sodium urinary elimination (mmol/24 h) | 0.46±0.27 | 0.36±0.16 | 0.39±0.12 | 0.51±0.15 | 0.47±0.15 | 0.33±0.14 |
| Chloride urinary elimination (mmol/24 h) | *0.56±0.29* | 0.56±0.15 | 0.56±0.19 | *0.71±0.18* | *0.69±0.12* | *0.47±0.18* |
| Potassium urinary elimination (mmol/24 h) | 0.79±0.28 | 1.00±0.25 | 0.95±0.18 | 1.00±0.32 | 1.04±0.21 | 0.78±0.18 |

The group means were compared by a one-way analysis of variance. When the analysis is significant, pairwise comparisons were performed between dosed and control groups using Dunnett's test. * $p<0.05$ vs control; ** $p<0.01$ vs control. Italic numbers: $p<0.05$ by a one-way analysis of variance but $>0.05$ in pairwise comparisons.

## Gross Pathology

At the end of the dosing period, after a minimum of 16 hours feed deprivation, a detailed autopsy (listed below) was performed for all animals. Bone marrow smears were prepared. As no relevant hematologic and or histological changes were noted during the study, examination of smears was not performed.

**Cardiovascular System:**
- Heart*
- Aorta

**Respiratory System:**
- Trachea
- Lungs + main stem bronchi*

**Digestive System:**
- Tongue
- Esophagus
- Stomach
- Duodenum
- Jejunum
- Ileum + Peyer's patches
- Caecum
- Colon
- Liver*
- Pancreas
- Submaxillary and sublingual glands (left)*

**Urogenital System:**
- Kidneys*
- Urinary bladder
- Testes*
- Epididymides*
- Prostate*
- Seminal vesicles*
- Ovaries*
- Uterus (and oviducts)*
- Vagina

**Hematopoietic System:**
- Spleen*
- Thymus*
- Colic mesenteric lymph nodes
- Bone marrow (sternum)

**Endocrine System:**
- Pituitary Gland*
- Thyroid and Parathyroid Glands*
- Adrenal Glands*

**Nervous System and Sense Organs:**
- Brain*
- Spinal cord (cervical)
- Sciatic nerve (right)
- Eyes
- Optic nerves
- Harderian gland (left)

**Musculoskeletal System**
- Femur (right)
- Muscle (right quadrutus fernoris)

**Skin and Mammary Gland**

* weighed organs

There was a higher incidence of reddish or whitish foci/areas, often depressed, in the glandular stomach mucosa of PERe- and PERa-treated groups (Table 7). The incidence and severity of these changes were dose-dependent, with no evidence of a difference between PERe and PERa treatment.

Table 7. Incidence and severity of foci/areas in the glandular stomach mucosa (Sponsor's Table)

| Test Substance | Dose Level (mg free acid/kg) | Reddish or Whitish Foci/Areas | | | |
|---|---|---|---|---|---|
| | | Male | | Female | |
| Vehicle | 0 | 1/10 | one | 0/10 | |
| S 9490-3 (PERe) | 8 | 5/10 | one to multiple | 3/10 | one or two |
| | 33 | 7/10 | one to several | 6/10 | one to multiple |
| S 9490-6 (PERa) | 0.8 | 2/10 | one or several | 0/10 | |
| | 8 | 2/10 | one | 4/10 | one or two |
| | 33 | 3/10 | one or two | 5/10 | one or several |

## Organ Weights

The organs listed under "Gross Pathology" with asterix * were weighed. Absolute and/or relative heart weights were slightly lower than controls for all treated groups, without a dose-response relationship (Table 8).



Reference ID: 3020370

Table 8. Difference from controls in mean absolute and relative heart weights (Sponsor's Table)

| Test Substance | Dose Level (mg free acid/kg) | Absolute Weight (g) | | Relative Weight (% body weight) | |
|---|---|---|---|---|---|
| | | Male | Female | Male | Female |
| S 9490-3 (PERe) | 8 | -10% | -17% ** | -8% * | -16% ** |
| | 33 | - 6% | -18% ** | -7% | -20% ** |
| | 0.8 | - 5% | -15% ** | -3% | -15% ** |
| S 9490-6 (PERa) | 8 | -11% | -14% ** | -9% ** | -13% ** |
| | 33 | - 7% | -18% ** | -7% * | -17% ** |

* = $p < 0.05$; ** = $p < 0.01$; % *versus* control mean values

**Histopathology**

*Adequate Battery*

All tissues selected for microscopic examination were histologically processed. Microscopic examination was performed on all above listed organs of all animals from control and high dose groups, all macroscopic anomalies, and the kidneys of all animals from the low and middle dose groups. As a correlation was noted between the gross and microscopic changes in the glandular stomach mucosa, no examination of this tissue was performed for the animals of the low and middle dose groups.

*Peer Review*

Undersigned pathologist Dr Hiline Bertheux performed the histological examinations. No information about peer review is available in the application.

*Histological Findings*

There were minimal to mild erosions and/or ulcerations in the glandular stomach mucosa for most of the treated animals that had shown gross gastric observations, *versus* none in controls (Table 9). The incidence of these findings was dose-dependent, and was slightly higher in PERe groups than in PERa groups. The severity was broadly similar among treated groups.



Reference ID: 3020370

Table 9. Incidence and severity of erosions and ulcerations in the stomach mucosa (Sponsor's Table)

| Test Substance | Dose Level (mg free acid/kg) | Erosions | | Ulcerations | |
|---|---|---|---|---|---|
| | | Male | Female | Male | Female |
| Vehicle | 0 | 0/10 | 0/10 | 0/10 | 0/10 |
| S 9490-3 (PERe) | 8① | 5/5 +/++ | 2/3 + | 0/5 | 0/3 |
| | 33 | 5/10 +/++ | 4/10 +/++ | 0/10 | 4/10 +/++ |
| S 9490-6 (PERa) | 0.8① | 2/2 +/++ | 0/0 | 0/2 | 0/0 |
| | 8① | 1/2 + | 3/4 + | 0/2 | 1/4 ++ |
| | 33 | 2/10 +/++ | 2/10/ + | 1/10 + | 1/10 + |

①: Only animals with gross changes in the glandular stomach mucosa were examined.
Severity: +: minimal; ++: mild.

Minimal renal tubular basophilia (one to few foci; unilateral and sometimes bilateral) was noted in animals of all groups, including controls (Table 10). The incidence of this change was slightly higher in treated males than in control males (up to 6/10 *vs* 2/10). Females did not show this difference. No histomorphologic findings were noted in the heart. No other relevant findings were noted in the other organs of treated animals

Table 10. Incidence and severity of tubular basophilia in the kidney (Modified from a sponsor's table)

| Test Substance | Vehicle | | PERe | | | | PERa | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dose Group (mg free acid/kg/day) | 0 | | 8 | | 33 | | 0.8 | | 8 | | 33 | |
| Sex | M | F | M | F | M | F | M | F | M | F | M | F |
| Basophilic tubules Minimal | 2 | 2 | 2 | 4 | 6 | 2 | 3 | 1 | 4 | 1 | 4 | 2 |

**Special Evaluation**

NONE

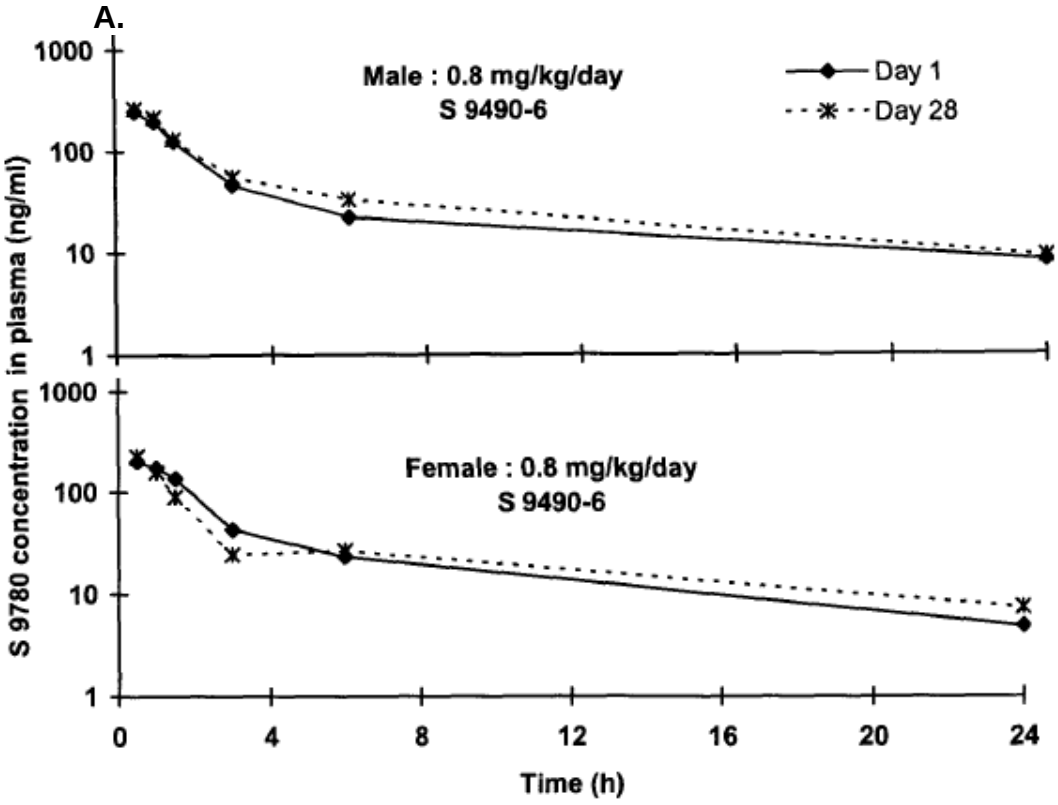
**Toxicokinetics**

Blood samples for TK were collected from the animals in treated satellite groups on days 1 and 28 at 0.5, 1.5, 3, 6, and 24 hours post-dose (n=3/sex/goup/time point). The concentration of perindoprilat (S 9780) in plasma samples were determined by liquid-chromatography (b) (4) mass spectrometry detection.

Irrespective of the salt S 9490-6 (PERa) and S 9490-3 (PERe) administered, the toxicokinetic parameters of S 9780 (perindoprilat) were similar (Figure 3 and Table 11). Tmax of S 9789 was 0.5 h (the first sampling time). Systemic exposure (Cmax and AUC24) of S 9780 increased dose proportionally in the dose range tested from 0.8 to 33

mg free acid/kg/day, was similar between days 1 and 28, and was slightly higher in males (AUC male/female ratio 1.1-1.8).

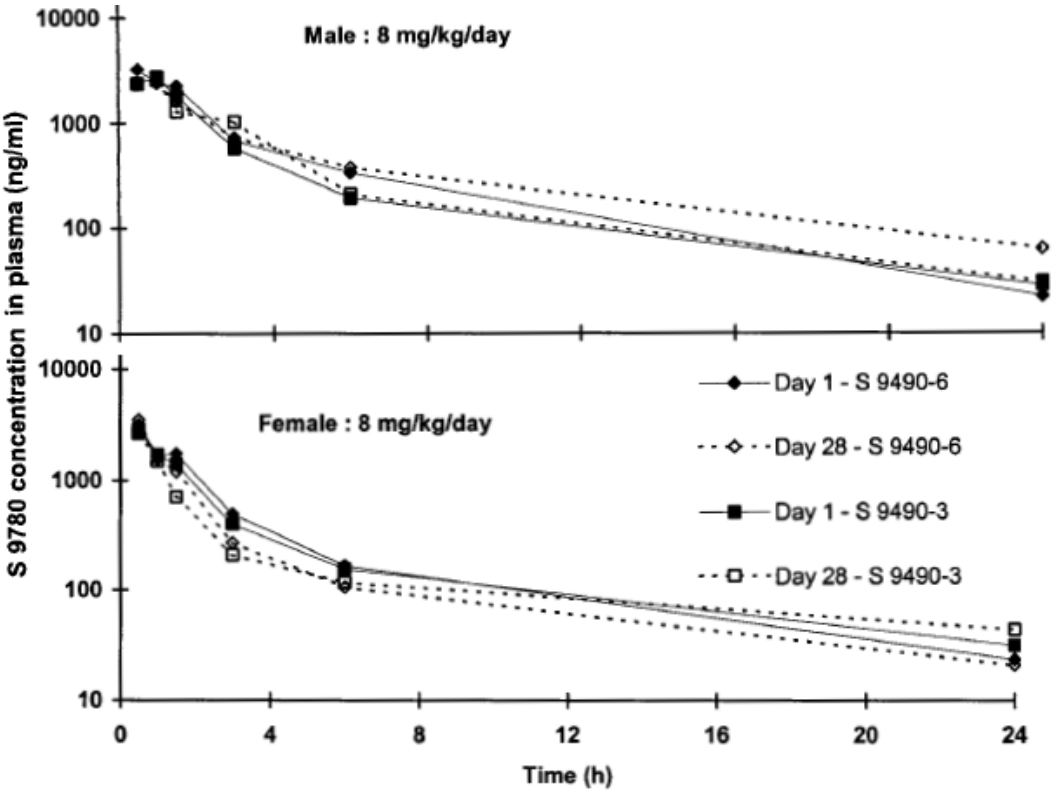
Figure 3. Plasma concentrations (free acid form) of S 9780 (perindoprilat) following repeat oral S 9490-6 (PERa) and S 9490-3 (PERe) administration in rats (Modified from sponsor's figures)






Reference ID: 3020370

B.


Male : 8 mg/kg/day
Female : 8 mg/kg/day
S 9780 concentration in plasma (ng/ml)
10000
1000
100
10
0
4
8
12
16
20
24
Time (h)
Day 1 - S 9490-6
Day 28 - S 9490-6
Day 1 - S 9490-3
Day 28 - S 9490-3

C.

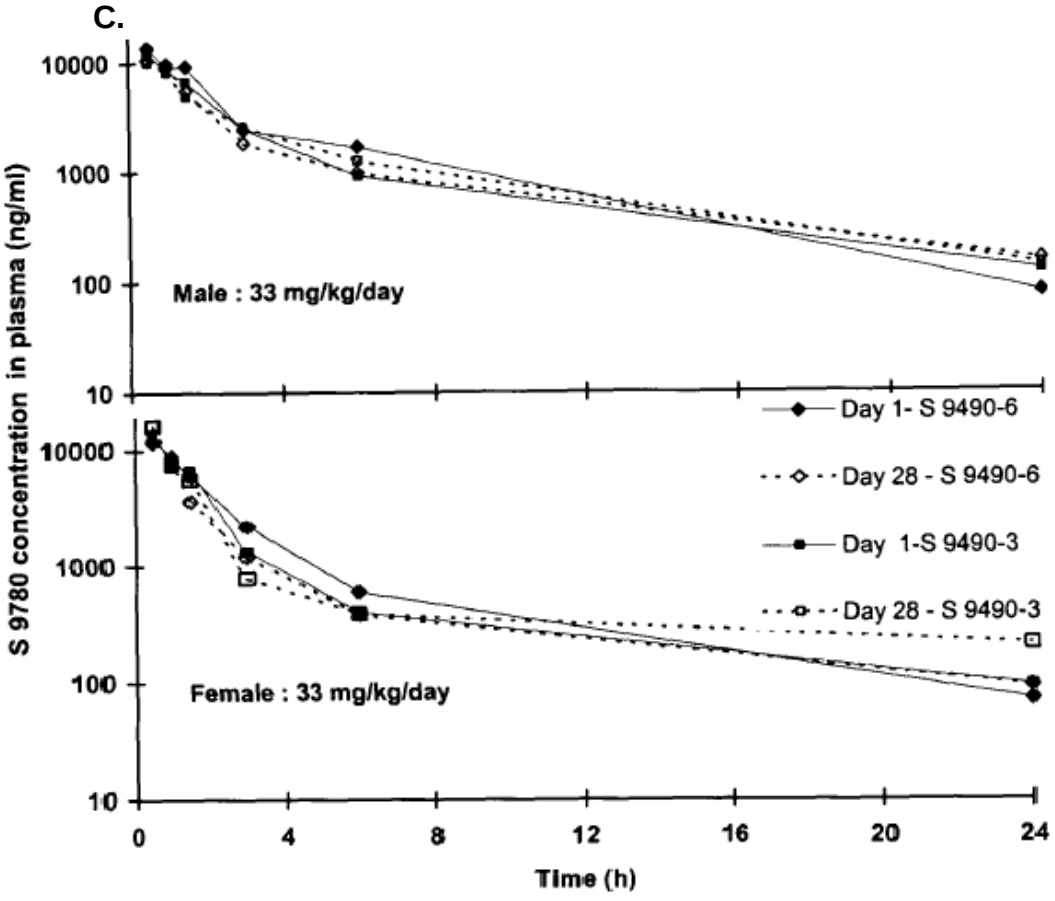

Male : 33 mg/kg/day
Female : 33 mg/kg/day
S 9780 concentration in plasma (ng/ml)
10000
1000
100
10
0
4
8
12
16
20
24
Time (h)
Day 1- S 9490-6
Day 28 - S 9490-6
Day 1-S 9490-3
Day 28 - S 9490-3

Table 11. Perindoprilat system exposure in rats given repeat oral S-9490-6 (PERa) or S 9490-3 (PERe) (Sponsor's table)

| Salt | | S 9490-6 | | | | | | S 9490-3 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Dose (mg free acid/kg) | | 0.8 | | 8 | | 33 | | 8 | | 33 | |
| Sexes | | M | F | M | F | M | F | M | F | M | F |
| $C_{max}$ (ng/ml) | Day 1 | 248 | 200 | 3253 | 3200 | 13700 | 12043 | 2763 | 2787 | 12200 | 14000 |
| | Day 28 | 265 | 225 | 2433 | 3540 | 10677 | 11667 | 2573 | 2653 | 10297 | 16133 |
| $AUC_{24}$ (ng.h/ml) | Day 1 | 720 | 646 | 8871 | 6490 | 36888 | 25748 | 7202 | 5910 | 30356 | 23506 |
| | Day 28 | 869 | 633 | 9208 | 5167 | 28748 | 20048 | 7707 | 4635 | 30706 | 23624 |

**Dosing Solution Analysis**

S 9490-3 (PERe) and S 9490-6 (PERa) solutions (in purified water), at concentrations 0.05, 1.0 and 10.0 mg free acid/ml, were stable for 12 days when stored in a refrigerator (Table 12).

Table 12. Stability of dosing solution (Sponsor's Table)

| PERe in purified water | Results (% / Theory) | | |
|---|---|---|---|
| Sampling Time | T0 | T0 + 5 days | T0 + 12 days |
| Preparation at 0.05 mg free acid/ml | 105.2 | 100.6 | 100.0 |
| Preparation at 1.00 mg free acid/ml | 106.1 | 98.9 | 100.5 |
| Preparation at 10.0 mg free acid/ml | 104.8 | 98.7 | 99.4 |

| PERa in purified water | Results (% / Theory) | | |
|---|---|---|---|
| Sampling Time | T0 | T0 + 5 days | T0 + 12 days |
| Preparation at 0.05 mg free acid/ml | 100.2 | 102.0 | 98.8 |
| Preparation at 1.00 mg free acid/ml | 100.3 | 103.2 | 97.8 |
| Preparation at 10.0 mg free acid/ml | 104.7 | 102.9 | 98.4 |

### 6.2.2 In dogs

**Study title:** 4-week repeat dose toxicity study in dogs – A comparative study of oral PERe and PERa

| | |
|---|---|
| Study no.: | NP15081 |
| Study report location: | (b) (4) |
| Conducting laboratory and location: | |
| Date of study initiation: | Sept 24, 2002 |
| GLP compliance: | Yes |
| QA statement: | Yes |
| Drug, lot #, and % purity: | PERe (S 9490-3), Lot #56 940, 99%<br>PERa (S 9490-6), Lot # FH 815, 99% |

**Key Study Findings**

- A decrease (~20% *vs* pre-test) in red blood cell count and Hb was seen in 1/3 females at PERa 4.17 mg/kg and 1/3 females at PERa 20.87 mg/kg.
- Medulla and/or papilla mineralization was observed in kidneys of the PERa-treated dogs at all dose levels with similar incidence and severity among doses.
- Three PERa-treated and four PERe-treated animals showed minimal to mild bronchopneumonia or chronic peri-bronchiolitis, with similar incidence and severity between the two salts.
- TK profiles of perindopril and perindoprilat were similar after repeat oral administration of both perindopril salts

Methods

| | |
|---|---|
| Doses: | 4.17 and 20.87 mg free acid/kg of PERe; 0.83, 4.17, and 20.87 mg free acid/kg of PERa. PERe doses were based on a previous 26-week oral toxicity study with PERe (0.83, 4.17 or 20.87 mg free acid/kg/day) in the beagle dog. PERe-related changes in this study were slight decreases in body weight gain and in arterial blood pressure at 20.87 mg free acid/kg. PERa doses were consequently selected as 0.83,4.17 and 20.87 mg free acid/kg. |
| Frequency of dosing: | Once a day (for 28 days to 30 days) |
| Route of administration: | Oral by gavage |
| Dose volume: | 2 ml/kg |
| Formulation/Vehicle: | Solution / water |
| Species/Strain: | Beagle dogs |
| Number/Sex/Group: | 3 |
| Age: | 8 and 9 months |
| Weight: | 7.9 - 10.3 kg for males & 7.2 - 9.3 kg for females |
| Satellite groups: | None |
| Unique study design: | This study was designed to compare the safety and toxicokinetic profile of PERa (S 9490-6) with that of PERe (S 9490-3) |
| Deviation from study protocol: | No impact to the results |

**Observations and Results**

**Mortality**

All animals were checked daily for mortality or signs of morbidity. No death occurred during the study.

**Clinical and Vital Signs**

Animals were checked for clinical signs 2-3 times per day. Rectal temperature was measured in week -2 and -1, then before dosing and 1 hour after dosing, on days 13 and 27. Occult blood in faces was checked in weeks -2 and 4.

The incidence of isolated, transient liquid stools was slightly higher in treated groups than in controls but without dose-related effect nor clear differences between both salts. In males, the incidence of soft stools was higher at the dose 20.87 mg free acid/kg of PERe or PERa than in other groups. No clear differences were seen between both salts. These incidences may not be drug-related, thus are not of toxicological significance.

There were no effects on rectal temperature. There were no traces of blood in feces of any animals.

**Body Weights**

Body weight was recorded once a week throughout the study. There were no drug-related effects on body weight.

**Feed Consumption**

Feed intakes were recorded daily. There were no drug-related effects on feed intakes.

**Ophthalmoscopy**

Ophthalmologic examinations were performed for all animals in weeks -2 and 4, including pupillary light reflexes, examination of the appendages, anterior segment and lens using a slit-lamp and a fundus examination using an indirect ophthalmoscope. Pupils were dilated using tropicamide before examination.

One female at the PERa dose 4.17 mg free acid/kg showed unilateral posterior focal subcapsular cataract on the left eye on Day 27. The sponsor stated that this cataract was not drug-related, since this change was isolated, not dose-related, and this type of cataract (unilateral, posterior, subcapsular) is known to occur in the untreated Marshall beagle dogs. The reviewer agrees with the sponsor's interpretation.

**ECG**

ECGs were recorded for a minimum of 10 seconds for all conscious animals in weeks -2 and -1, and then before dosing and 1 hour after dosing on days 13 and 27. Heart rate and rhythm were examined. PR and QT intervals and QRS complex duration were calculated preferentially on lead 11.

Quantitatively, there were no PERe or PERa-effects on heart rate, PR and QT intervals or QRS complex duration (Table 13).

Occasional atrioventricular (A-V) blocks and/or sinus arrests in a few animals before and/or after dosing on days 13 and/or 27 were reported:

* First degree A-V blocks: female #151 at the PERa dose 20.87 mg free acid/kg
* Second degree A-V blocks: male #13 and female #63 at the PERe dose 4.17 mg free acid/kg, and female # 92 at the PERa dose 4.17 mg free acid/kg
* Sinus arrest: male #41 at the PERa dose 4.17 mg free acid/kg

The sponsor considered these A-V blocks not to be related to the administration of PERe or PERa, since they were isolated, not dose-dependent, and are known to occur in untreated beagle dogs. The reviewer agrees with the sponsor's interpretation.



Reference ID: 3020370

Table 13. Individual ECG values

| Group | Dog # | Time | Heart rate (bpm) | PR (ms) | QRS (ms) | QT (ms) | Dog # | Heart rate (bpm) | PR (ms) | QRS (ms) | QT (ms) | Group | Dog # | Time | Heart rate (bpm) | PR (ms) | QRS (ms) | QT (ms) | Dog # | Heart rate (bpm) | PR (ms) | QRS (ms) | QT (ms) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Day -12 | 79 | 83 | 58 | 200 | | 130 | 82 | 43 | 180 | | | Day -12 | 96 | 90 | 35 | 185 | | 102 | 92 | 58 | 172 |
| | | Day -2 | 69 | 93 | 60 | 208 | | 125 | 88 | 43 | 200 | | | Day -2 | 85 | 90 | 50 | 180 | | 122 | 98 | 55 | 190 |
| | | Day 13-0h | 88 | 90 | 53 | 202 | | 117 | 85 | 47 | 203 | | | Day 13-0h | 105 | 92 | 40 | 177 | | 97 | 98 | 57 | 210 |
| | | Day 13-1h | 80 | 95 | 55 | 210 | | 102 | 98 | 40 | 213 | | | Day 13-1h | 104 | 93 | 42 | 178 | | 88 | 100 | 58 | 210 |
| | | Day 27-0 | 67 | 95 | 57 | 216 | | 98 | 100 | 40 | 202 | | | Day 27-0 | 101 | 87 | 40 | 192 | | 131 | 103 | 52 | 195 |
| | 1M | Day 27-1h | 76 | 80 | 57 | 205 | 51F | 108 | 102 | 40 | 202 | | 31M | Day 27-1h | 82 | 80 | 43 | 187 | 81F | 116 | 102 | 58 | 200 |
| | | Day -12 | 109 | 80 | 60 | 187 | | 114 | 95 | 42 | 182 | | | Day -12 | 84 | 85 | 50 | 213 | | 93 | 90 | 58 | 203 |
| | | Day -2 | 118 | 90 | 48 | 202 | | 106 | 98 | 38 | 183 | | | Day -2 | 76 | 100 | 40 | 218 | | 111 | 87 | 55 | 187 |
| | | Day 13-0h | 137 | 85 | 58 | 180 | | 94 | 107 | 50 | 202 | | | Day 13-0h | 69 | 98 | 47 | 218 | | 97 | 93 | 57 | 205 |
| | | Day 13-1h | 135 | 83 | 55 | 182 | | 92 | 115 | 42 | 210 | | | Day 13-1h | 74 | 102 | 43 | 223 | | 92 | 88 | 53 | 203 |
| | | Day 27-0h | 101 | 90 | 55 | 205 | | 108 | 105 | 48 | 198 | | | Day 27-0h | 64 | 98 | 43 | 222 | | 104 | 97 | 50 | 200 |
| | 2M | Day 27-1h | 120 | 83 | 60 | 195 | 52F | 97 | 112 | 45 | 207 | | 32M | Day 27-1h | 73 | 100 | 45 | 210 | 82F | 93 | 87 | 53 | 202 |
| | | Day -12 | 102 | 80 | 55 | 185 | | 90 | 87 | 60 | 200 | | | Day -12 | 105 | 98 | 43 | 180 | | 102 | 103 | 42 | 200 |
| | | Day -2 | 126 | 82 | 55 | 200 | | 91 | 97 | 60 | 213 | | | Day -2 | 101 | 92 | 42 | 195 | | 128 | 98 | 42 | 188 |
| | | Day 13-0h | 102 | 82 | 58 | 205 | | 96 | 98 | 60 | 210 | | | Day 13-0h | 110 | 98 | 47 | 185 | | 93 | 100 | 48 | 208 |
| | | Day 13-1h | 87 | 88 | 55 | 198 | | 100 | 103 | 60 | 218 | PERa | | Day 13-1h | 95 | 93 | 48 | 198 | | 93 | 92 | 45 | 208 |
| | | Day 27-0 | 103 | 90 | 53 | 207 | | 99 | 97 | 67 | 210 | 0 83 | | Day 27-0 | 93 | 100 | 42 | 185 | | 98 | 95 | 55 | 203 |
| Control | 3M | Day 27-1h | 90 | 88 | 57 | 217 | 53F | 107 | 100 | 58 | 212 | mg/kg | 33M | Day 27-1h | 85 | 97 | 42 | 197 | 83F | 93 | 98 | 50 | 210 |
| | | Day -12 | 114 | 82 | 45 | 183 | | 82 | 83 | 60 | 203 | | | Day -12 | 94 | 102 | 43 | 172 | | 97 | 85 | 60 | 205 |
| | | Day -2 | 117 | 85 | 40 | 197 | | 94 | 85 | 58 | 198 | | | Day -2 | 82 | 10 | 53 | 197 | | 107 | 100 | 42 | 197 |
| | | Day 13-0h | 124 | 90 | 40 | 203 | | 69 | 83 | 58 | 203 | | | Day 13-0h | 63 | 115 | 47 | 190 | | 106 | 100 | 50 | 188 |
| | | Day 13-1h | 107 | 90 | 40 | 205 | | 66 | 87 | 55 | 200 | | | Day 13-1h | 80 | 113 | 45 | 195 | | 103 | 93 | 50 | 200 |
| | | Day 27-0h | 104 | 93 | 40 | 200 | | 71 | 90 | 58 | 200 | | | Day 27-0h | 75 | 110 | 52 | 197 | | 125 | 92 | 58 | 193 |
| | 11M | Day 27-1h | 112 | 93 | 40 | 197 | 61F | 66 | 92 | 55 | 202 | | 41M | Day 27-1h | 70 | 105 | 53 | 210 | 91F | 134 | 93 | 53 | 200 |
| | | Day -12 | 98 | 83 | 43 | 175 | | 100 | 98 | 40 | 205 | | | Day -12 | 98 | 107 | 43 | 205 | | 66 | 97 | 57 | 210 |
| | | Day -2 | 98 | 88 | 50 | 180 | | 106 | 102 | 43 | 185 | | | Day -2 | 80 | 88 | 50 | 192 | | 82 | 107 | 55 | 217 |
| | | Day 13-0h | 80 | 87 | 53 | 200 | | 116 | 100 | 55 | 195 | | | Day 13-0h | 53 | 103 | 57 | 217 | | 64 | 137 | 58 | 220 |
| | | Day 13-1h | 84 | 85 | 52 | 198 | | 113 | 100 | 50 | 195 | | | Day 13-1h | 76 | 102 | 47 | 190 | | 84 | 133 | 48 | 215 |
| | | Day 27-0 | 94 | 98 | 48 | 192 | | 108 | 112 | 47 | 183 | | | Day 27-0 | 67 | 100 | 57 | 220 | | 79 | 110 | 53 | 210 |
| | 12M | Day 27-1h | 129 | 88 | 53 | 183 | 62F | 101 | 105 | 42 | 202 | | 42M | Day 27-1h | 77 | 93 | 53 | 208 | 92F | 82 | 110 | 45 | 200 |
| | | Day -12 | 88 | 102 | 45 | 178 | | 113 | 98 | 57 | 210 | | | Day -12 | 88 | 85 | 50 | 185 | | 138 | 100 | 60 | 173 |
| | | Day -2 | 89 | 105 | 56 | 190 | | 92 | 107 | 45 | 205 | | | Day -2 | 99 | 78 | 60 | 193 | | 140 | 105 | 60 | 180 |
| | | Day 13-0h | 82 | 107 | 55 | 188 | | 82 | 137 | 50 | 208 | | | Day 13-0h | 73 | 88 | 57 | 195 | | 141 | 102 | 62 | 180 |
| PERe | | Day 13-1h | 80 | 125 | 53 | 195 | | 92 | 102 | 55 | 212 | PERa | | Day 13-1h | 85 | 87 | 58 | 193 | | 134 | 100 | 60 | 182 |
| 4.17 | | Day 27-0h | 80 | 102 | 50 | 190 | | 100 | 107 | 53 | 202 | 4.17 | | Day 27-0h | 75 | 97 | 58 | 202 | | 142 | 112 | 53 | 172 |
| mg/kg | 13M | Day 27-1h | 86 | 98 | 47 | 193 | 63F | 88 | 110 | 43 | 203 | mg/kg | 43M | Day 27-1h | 95 | 93 | 55 | 195 | 93F | 143 | 98 | 57 | 190 |
| | | Day -12 | 99 | 85 | 50 | 200 | | 98 | 83 | 57 | 193 | | | Day -12 | 101 | 97 | 72 | 203 | | 102 | 98 | 58 | 198 |
| | | Day -2 | 107 | 88 | 40 | 197 | | 114 | 88 | 57 | 187 | | | Day -2 | 112 | 103 | 37 | 182 | | 127 | 100 | 48 | 180 |
| | | Day 13-0h | 77 | 97 | 50 | 200 | | 116 | 88 | 60 | 203 | | | Day 13-0h | 89 | 105 | 40 | 198 | | 88 | 105 | 55 | 210 |
| | | Day 13-1h | 88 | 102 | 42 | 210 | | 116 | 87 | 57 | 192 | | | Day 13-1h | 119 | 105 | 40 | 163 | | 76 | 100 | 55 | 223 |
| | | Day 27-0 | 83 | 100 | 45 | 202 | | 107 | 95 | 52 | 200 | | | Day 27-0 | 100 | 102 | 37 | 187 | | 101 | 100 | 57 | 200 |
| | 21M | Day 27-1h | 86 | 97 | 53 | 200 | 71F | 100 | 93 | 58 | 200 | | 101M | Day 27-1h | 99 | 100 | 40 | 198 | 151F | 85 | 107 | 55 | 223 |
| | | Day -12 | 85 | 93 | 52 | 200 | | 70 | 97 | 60 | 210 | | | Day -12 | 111 | 73 | 42 | 178 | | 69 | 83 | 45 | 185 |
| | | Day -2 | 95 | 95 | 43 | 203 | | 92 | 98 | 52 | 207 | | | Day -2 | 89 | 77 | 50 | 180 | | 116 | 85 | 40 | 175 |
| | | Day 13-0h | 91 | 97 | 50 | 205 | | 67 | 100 | 57 | 203 | | | Day 13-0h | 91 | 77 | 45 | 187 | | 91 | 83 | 55 | 198 |
| | | Day 13-1h | 78 | 93 | 47 | 218 | | 83 | 93 | 45 | 188 | | | Day 13-1h | 99 | 80 | 48 | 198 | | 93 | 82 | 52 | 197 |
| | | Day 27-0h | 96 | 98 | 42 | 203 | | 68 | 103 | 53 | 205 | | | Day 27-0h | 93 | 75 | 48 | 198 | | 111 | 83 | 40 | 180 |
| | 22M | Day 27-1h | 87 | 97 | 43 | 212 | 72F | 79 | 97 | 45 | 208 | | 102M | Day 27-1h | 103 | 82 | 45 | 190 | 152F | 106 | 82 | 43 | 182 |
| | | Day -12 | 82 | 90 | 40 | 198 | | 97 | 97 | 53 | 178 | | | Day -12 | 79 | 85 | 58 | 210 | | 128 | 67 | 52 | 180 |
| | | Day -2 | 113 | 97 | 48 | 185 | | 129 | 95 | 47 | 185 | | | Day -2 | 85 | 92 | 55 | 210 | | 137 | 67 | 55 | 182 |
| | | Day 13-0h | 129 | 93 | 40 | 185 | | 133 | 97 | 48 | 198 | | | Day 13-0h | 75 | 97 | 42 | 200 | | 123 | 78 | 53 | 180 |
| PERe | | Day 13-1h | 122 | 93 | 40 | 178 | | 139 | 100 | 50 | 180 | PERa | | Day 13-1h | 82 | 93 | 42 | 202 | | 100 | 80 | 47 | 195 |
| 20.87 | | Day 27-0 | 91 | 97 | 45 | 197 | | 145 | 102 | 48 | 178 | 20.87 | | Day 27-0 | 100 | 95 | 42 | 190 | | 110 | 80 | 57 | 185 |
| mg/kg | 23M | Day 27-1h | 103 | 97 | 42 | 183 | 73F | 136 | 98 | 52 | 175 | mg/kg | 103M | Day 27-1h | 87 | 87 | 40 | 202 | 153F | 104 | 80 | 40 | 180 |

## Hematology

Blood samples for hematology and clinical chemistry were collected from all animals in week -2, and before dosing during the dosing period on days 14 and 28, after an overnight fast. Hematological parameters were:

- Red Blood Cell Count
- Packed Cell Volume
- Mean Corpuscular Volume
- Hemoglobin Concentration
- Platelet Count
- Mean Corpuscular Hemoglobin
- Mean Corpuscular Hemoglobin Concentration
- Total and Differential Leukocyte Counts
- Prothrombin time
- Activated partial thromboplastin time

Bone marrow smears were prepared from the sternum and stained for all animals at necropsy. Myelograms were not warranted, based upon results of the study.

Red blood cell counts (RBC) and hemoglobin (Hb) concentrations of some animals in both control and treated groups decreased during the study. The incidence was higher in females than in males. The severity increased in females at PERa doses ≥4.17 mg/kg (Table 14). The decreases in animals #92 and #151 exceed the 5-95% confidence interval of historical data, being of toxicity concern (3).

Table 14. Individual RBC and Hb values

| Group | Dog # | Time (day) | RBC (T/L) | Hb mmol/L | RBC % change | Hb % change | Dog # | RBC (T/L) | Hb mmol/L | RBC % change | Hb % change |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | D-13 | 7.04 | 10.2 | | | | 6.74 | 9.7 | | |
| | | D14 | 7.36 | 10.6 | 4.55 | 3.92 | | 6.96 | 10.2 | 3.26 | 5.15 |
| | 1M | D28 | 7.64 | 10.9 | 8.52 | 6.86 | 51F | 6.72 | 9.9 | -0.30 | 2.06 |
| | | D-13 | 7.79 | 11.4 | | | | 7.38 | 10.6 | | |
| | | D14 | 8.22 | 11.7 | 5.52 | 2.63 | | 6.88 | 9.9 | -6.78 | -6.60 |
| | 2M | D28 | 7.66 | 11.1 | -1.67 | -2.63 | 52F | 6.53 | 9.6 | -11.52 | -9.43 |
| | | D-13 | 6.93 | 10.1 | | | | 7.93 | 11 | | |
| | | D14 | 6.3 | 9.1 | -9.09 | -9.90 | | 7.03 | 9.9 | -11.35 | -10.00 |
| Control | 3M | D28 | 6.25 | 8.9 | -9.81 | -11.88 | 53F | 6.7 | 9.4 | -15.51 | -14.55 |
| | | D-13 | 7.85 | 10.9 | | | | 7.06 | 10.4 | | |
| | | D14 | 7.79 | 11.1 | -0.76 | 1.83 | | 6.85 | 10 | -2.97 | -3.85 |
| | 11M | D28 | 7.47 | 10.8 | -4.84 | -0.92 | 61F | 6.75 | 10 | -4.39 | -3.85 |
| | | D-13 | 6.55 | 9.3 | | | | 7.21 | 10.2 | | |
| | | D14 | 6.09 | 8.7 | -7.02 | -6.45 | | 6.54 | 9.3 | -9.29 | -8.82 |
| | 12M | D28 | 6.49 | 9.3 | -0.92 | 0.00 | 62F | 6.71 | 9.7 | -6.93 | -4.90 |
| PERe | | D-13 | 7.09 | 9.9 | | | | 7.8 | 10.6 | | |
| 4.17 | | D14 | 6.84 | 9.4 | -3.53 | -5.05 | | 6.62 | 8.9 | -15.13 | -16.04 |
| mg/kg | 13M | D28 | 7.02 | 9.9 | -0.99 | 0.00 | 63F | 6.92 | 9.5 | -11.28 | -10.38 |
| | | D-13 | 7.31 | 10.1 | | | | 7.27 | 10.7 | | |
| | | D14 | 7.23 | 9.7 | -1.09 | -3.96 | | 7.2 | 10.6 | -0.96 | -0.93 |
| | 21M | D28 | 7.19 | 9.9 | -1.64 | -1.98 | 71F | 7.08 | 10.4 | -2.61 | -2.80 |
| | | D-13 | 7 | 9.3 | | | | 7.41 | 10.3 | | |
| | | D14 | 6.74 | 8.9 | -3.71 | -4.30 | | 6.6 | 9.4 | -10.93 | -8.74 |
| | 22M | D28 | 6.48 | 8.5 | -7.43 | -8.60 | 72F | 6.95 | 9.9 | -6.21 | -3.88 |
| PERe | | D-13 | 8.16 | 10.9 | | | | 7.26 | 10.9 | | |
| 20.87 | | D14 | 7.83 | 10.7 | -4.04 | -1.83 | | 7.24 | 10.5 | -0.28 | -3.67 |
| mg/kg | 23M | D28 | 7.8 | 10.7 | -4.41 | -1.83 | 73F | 6.94 | 10.4 | -4.41 | -4.59 |
| | | D-13 | 7.66 | 10.7 | | | | 7.37 | 10.9 | | |
| | | D14 | 7.57 | 11 | -1.17 | 2.80 | | 7.19 | 10.6 | -2.44 | -2.75 |
| | 31M | D28 | 7.58 | 11 | -1.04 | 2.80 | 81F | 6.7 | 10 | -9.09 | -8.26 |
| | | D-13 | 7.32 | 10.4 | | | | 7.89 | 11.2 | | |
| | | D14 | 7.31 | 10.5 | -0.14 | 0.96 | | 6.59 | 9.5 | -16.48 | -15.18 |
| | 32M | D28 | 7.59 | 11.2 | 3.69 | 7.69 | 82F | 6.63 | 9.6 | -15.97 | -14.29 |
| PERa | | D-13 | 7.4 | 10.1 | | | | 7.14 | 10.4 | | |
| 0.83 | | D14 | 6.63 | 9.4 | -10.41 | -6.93 | | 6.08 | 8.9 | -14.85 | -14.42 |
| mg/kg | 33M | D28 | 6.72 | 9.7 | -9.19 | -3.96 | 83F | 6.56 | 9.6 | -8.12 | -7.69 |
| | | D-13 | 7.14 | 9.8 | | | | 7.46 | 10.4 | | |
| | | D14 | 6.6 | 9.3 | -7.56 | -5.10 | | 6.93 | 9.6 | -7.10 | -7.69 |
| | 41M | D28 | 6.71 | 9.6 | -6.02 | -2.04 | 91F | 6.81 | 9.6 | -8.71 | -7.69 |
| | | D-13 | 7.5 | 10.7 | | | | 6.07 | 8.5 | | |
| | | D14 | 7.13 | 10.2 | -4.93 | -4.67 | | 4.97 | 7.1 | -18.12 | -16.47 |
| | 42M | D28 | 7.09 | 10.2 | -5.47 | -4.67 | 92F | 4.93 | 7.1 | -18.78 | -16.47 |
| PERa | | D-13 | 7.7 | 10.9 | | | | 7.69 | 10.8 | | |
| 4.17 | | D14 | 7.64 | 10.9 | -0.78 | 0.00 | | 7.95 | 11.2 | 3.38 | 3.70 |
| mg/kg | 43M | D28 | 7.71 | 11 | 0.13 | 0.92 | 93F | 7.87 | 11.2 | 2.34 | 3.70 |
| | | D-13 | 7.24 | 9.7 | | | | 7.01 | 9.9 | | |
| | | D14 | 7.03 | 9.7 | -2.90 | 0.00 | | 5.58 | 7.9 | -20.40 | -20.20 |
| | 101M | D28 | 6.83 | 9.4 | -5.66 | -3.09 | 151F | 5.58 | 7.9 | -20.40 | -20.20 |
| | | D-13 | 7.1 | 10.1 | | | | 7.7 | 10.9 | | |
| | | D14 | 6.76 | 9.9 | -4.79 | -1.98 | | 7.17 | 10.3 | -6.88 | -5.50 |
| | 102M | D28 | 6.53 | 9.4 | -8.03 | -6.93 | 152F | 6.63 | 9.5 | -13.90 | -12.84 |
| PERa | | D-13 | 6.19 | 8.7 | | | | 7 | 9.9 | | |
| 20.87 | | D14 | 5.83 | 8.4 | -5.82 | -3.45 | | 6.92 | 9.9 | -1.14 | 0.00 |
| mg/kg | 103M | D28 | 5.83 | 8.4 | -5.82 | -3.45 | 153F | 6.9 | 10 | -1.43 | 1.01 |



Reference ID: 3020370

**Clinical Chemistry**

Serum chemistry parameters were:

| | |
|---|---|
| Electrolytes ($Na^+$, $K^+$, $Cl^-$) | Total cholesterol |
| Calcium | Triglycerides |
| Inorganic phosphorus | Total bilirubin |
| Glucose | Alkaline phosphatase |
| Urea | Alanine aminotransferase |
| Creatinine | Aspartate aminotransferase |
| Albumin | Glutamate dehydrogenase |
| Total proteins | Creatine kinase |

Protein fractions by electrophoresis included the following parameters:

| | |
|---|---|
| Albumin | Beta- 1 and beta-2 globulins |
| Alpha-1 globulins | Gamma globulins |
| Alpha-2 globulins | Albumin to globulin ratio |

On day 14, dog #13 at the PERe dose 4.17 mg/kg and #43 at the PERa dose 4.17 mg/kg had high alanine aminotransferase (192 vs 65 and 98 vs 28 U/L, respectively) and glutamate dehydrogenase values (11 vs 6 and 11 vs 3 U/L, respectively). On Day 28, these enzyme activities returned to normal values (5 and 6 U/L, respectively). These changes were not related to the tested compounds, since these enzyme changes did not persist at the end of the dosing period, were not dose-dependent, and were not associated with other changes.

**Urinalysis**

Urine samples were collected overnight for at least 16 hours under conditions of food deprivation in week -1 and on day 29 or 30. Urinary quantitative parameters included volume, proteins, creatinine, and specific gravity. Urinary semi-quantitative and microscopic evaluations included appearance, color, glucose, bilirubin, ketones, pH, occult blood, urobilinogen, nitrites, leukocytes, and sediments.

There were no drug-related effects on urinary parameters.

**Gross Pathology**

At the end of the dosing period, a detailed necropsy was performed for all animals after overnight fast. There were no drug-related macroscopic observations.

**Organ Weights**

For all animals, the following organs were weighed after careful trimming (paired organs were weighed together) -



Reference ID: 3020370

| | | | |
|---|---|---|---|
| Adrenal glands | Liver | Salivary glands | Thyroid glands (+ parathyroids) |
| Brain | Lungs | (submaxillary) | Uterus |
| Epididymides | Ovaries | Spleen | Pituitary gland |
| Heart | Testes | Thymus | Prostate |
| Kidneys | | | |

There were no drug-related effects on organ weights.

**Histopathology**

*Adequate Battery*

Any macroscopic anomalies and representative samples of the organs and tissues listed below from all animals were histologically processed. Microscopic examination was performed on all list tissues for all animals.

| | | |
|---|---|---|
| Adrenal glands | Larynx | Spleen |
| Aorta | Liver | Sternum (with bone |
| Brain | Lungs | marrow) |
| Caecum | Lymph nodes (mandibular, mesenteric) | Stomach |
| Colon | Mammary gland | Testes |
| Duodenum | Optic nerves | Thymus |
| Epididymides | Ovaries | Thyroid glands |
| Oesophagus | Oviducts | Tongue |
| Eyes | Pancreas | Tonsils |
| Femur | Parathyroid glands | Trachea |
| Gall bladder | Pituitary gland | Ureters |
| Heart | Prostate | Urinary bladder |
| Ileum (and Peyer patch) | Salivary glands (parotid and submaxillary) | Uterus |
| Jejunum | Sciatic nerve | Vagina |
| Kidneys | Skeletal muscle | |
| Knee (articular capsule) | Skin | |
| Lacrimal gland | Spinal cord | |

*Peer Review*

Pathologist Dr Dominique Henri Douvin performed the histological examination. There is no information about peer review.

*Histological Findings*

Three PERa-treated and four PERe-treated animals showed minimal to mild bronchopneumonia or chronic peri-bronchiolitis, with similar incidence and severity between the two salts. Medulla and/or papilla mineralization was observed in kidneys of the PERa-treated dogs at all dose levels with similar incidence and severity among doses (Table 15).

Table 15. Summary of histological findings

| Organs | Findings | Degree | Vehicle | | PERe | | | | PERa | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 4.17 mg/kg | | 20.87 mg/kg | | 0.83 mg/kg | | 4.17 mg/kg | | 20.87 mg/kg | |
| | | | M | F | M | F | M | F | M | F | M | F | M | F |
| Lung | Broncho-pneumonia | Mild | | | 1/3 | | | | | | | | | |
| | Granuloma | Mild | 1/3 | | | | | | | | | | | |
| | Foreign body granuloma | Mild | | 1/3 | | | | 1/3 | | | | | | |
| | Chronic peri-bronchiolitis | Minimal | | | | | | | | | | | 1/3 | |
| | | Mild | | | | 1/3 | 1/3 | 1/3 | | | 1/3 | | | 1/3 |
| | Alveolar histiocytosis ("foam-cells") | Minimal | | | | 1/3 | | | | | | | | |
| Kidney | Chronic interstitial nephritis, unilateral, focal | Minimal | | | | | | | | | | | | 1/3 |
| | Mineralization medulla and/or papilla | Minimal | | | | | | | | 1/3 | | 1/3 | 1/3 | |
| | | Mild | | | | | | | 1/3 | | | | | |

## Special Evaluation

## Toxicokinetics

Blood samples for TK were collected from all animals before dosing and 30 min, 1, 2, 4, 6, 10, and 24 h after dosing on days 1 and 28. The derived plasma samples were dispatched to (b) (4) for determination of perindopril and perindoprilat concentrations, using liquid chromatography (b) (4) mass spectrometric detection after solid phase extraction.

After once daily oral administration of S 9490-3 (PERe) or S 9490-6 (PERa) in male and female beagle dogs for 4 weeks, all sampled dogs were exposed to perindopril (Table 16 & Table 18) and its active metabolite perindoprilat (Table 17 & Table 19). Whatever the dose administered, Tmax occurred at 0.5 h for perindopril and between 1 and 2 h for perindoprilat after administration of both PERe or PERa. The exposures (AUC24) to perindopril and perindoprilat were dose proportional after administration of both PERe and PERa, and were similar on days 1 and 28 and between sexes (Table 18 & Table 19). TK profiles of the active metabolite perindoprilat were similar after repeat oral dosing of PERe or PERa in dogs (Figure 4).

Table 16. Plasma perindopril concentration following repeat oral doses of PERe or PERa in dogs

| Sampling time (h) | | PERe 4.17 mg/kg | | | | PERe 20.87 mg/kg | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Day 1 | | Day 28 | | Day 1 | | Day 28 | |
| | | Male | Female | Male | Female | Male | Female | Male | Female |
| 0 | **Plasma perindopril concentration, ng/ml.** BLQ: Below limit of quantitation (0.5 ng/ml) | BLQ | BLQ | BLQ | BLQ | BLQ | BLQ | BLQ | 0.733 |
| 0.5 | | 1001 | 1362 | 869 | 857 | 5058 | 6884 | 8200 | 8478 |
| 1 | | 236 | 240 | 160 | 206 | 879 | 1314 | 2013 | 1652 |
| 2 | | 51.1 | 16.3 | 35.4 | 39.7 | 435 | 166 | 261 | 164 |
| 4 | | 17.2 | 5.43 | 23.5 | 9.97 | 58.5 | 44.5 | 46.5 | 16.9 |
| 6 | | 8.24 | 3.65 | 9.04 | 4.77 | 37.2 | 26.2 | 19.9 | 9.37 |
| 10 | | 3.44 | 3.39 | 3.61 | 0.85 | 12.6 | 9.01 | 8.1 | 2.83 |
| 24 | | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |

| Sampling time (h) | PERa 0.83 mg/kg | | | | PERa 4.17 mg/kg | | | | PERe 20.87 mg/kg | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Day 1 | | Day 28 | | Day 1 | | Day 28 | | Day 1 | | Day 28 | |
| | Male | Female | Male | Female | Male | Female | Male | Female | Male | Female | Male | Female |
| 0 | BLQ | BLQ | BLQ | BLQ | BLQ | BLQ | BLQ | BLQ | BLQ | BLQ | BLQ | 0.5 |
| 0.5 | 91.7 | 158 | 90.1 | 121 | 657 | 649 | 791 | 782 | 4275 | 6558 | 6051 | 6203 |
| 1 | 16.7 | 30.8 | 26.4 | 31.8 | 159 | 104 | 250 | 167 | 1425 | 1168 | 1638 | 1567 |
| 2 | 7.53 | 9.46 | 9.06 | 20.2 | 55.2 | 17.4 | 88.4 | 50.4 | 291 | 99.4 | 383 | 180 |
| 4 | 2.61 | 2.6 | 3.43 | 3.06 | 19.7 | 9.91 | 28.3 | 12 | 58.8 | 44 | 63 | 44.3 |
| 6 | 1.31 | 0.903 | BLQ | 1.04 | 8.36 | 4.69 | 11.8 | 6.16 | 24.9 | 27.3 | 20.9 | 24.2 |
| 10 | BLQ | BLQ | BLQ | BLQ | 3.99 | 2.51 | 3.92 | 4.9 | 22.3 | 11.8 | 13.1 | 4.85 |
| 24 | BLQ | BLQ | BLQ | BLQ | BLQ | BLQ | 0.758 | BLQ | 2.01 | 1.2 | BLQ | BLQ |

Table 17. Plasma perindoprilat concentration following repeat oral doses of PERe or PERa in dogs

| Sampling time (h) | | PERe 4.17 mg/kg | | | | PERe 20.87 mg/kg | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Day 1 | | Day 28 | | Day 1 | | Day 28 | |
| | | Male | Female | Male | Female | Male | Female | Male | Female |
| 0 | **Plasma perindoprilat concentration, ng/ml**. BLQ: Below limit of quantitation (0.5 ng/ml) | BLQ | BLQ | 8.26 | 12.3 | BLQ | BLQ | 40.7 | 75.1 |
| 0.5 | | 234 | 461 | 382 | 542 | 1587 | 2923 | 1465 | 3694 |
| 1 | | 749 | 978 | 703 | 897 | 3474 | 5236 | 4069 | 6563 |
| 2 | | 839 | 986 | 700 | 860 | 4048 | 5530 | 4638 | 7059 |
| 4 | | 392 | 417 | 392 | 343 | 2095 | 2593 | 2382 | 3314 |
| 6 | | 152 | 179 | 174 | 137 | 1050 | 1225 | 1063 | 1594 |
| 10 | | 54 | 41.1 | 64.4 | 27.1 | 265 | 292 | 264 | 383 |
| 24 | | 7.8 | 9.71 | 11 | 6.35 | 48.3 | 16.8 | 42.2 | 43.7 |

| Sampling time (h) | PERa 0.83 mg/kg | | | | PERa 4.17 mg/kg | | | | PERe 20.87 mg/kg | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Day 1 | | Day 28 | | Day 1 | | Day 28 | | Day 1 | | Day 28 | |
| | Male | Female | Male | Female | Male | Female | Male | Female | Male | Female | Male | Female |
| 0 | BLQ | BLQ | 2.82 | 3.35 | BLQ | BLQ | 16 | 11.5 | BLQ | BLQ | 41.2 | 54.5 |
| 0.5 | 40.6 | 39.6 | 68.3 | 43.5 | 358 | 412 | 255 | 386 | 1454 | 2321 | 1623 | 2692 |
| 1 | 89.7 | 114 | 110 | 103 | 720 | 718 | 591 | 672 | 3332 | 4296 | 3600 | 4928 |
| 2 | 87.9 | 116 | 106 | 113 | 757 | 700 | 709 | 688 | 3954 | 4591 | 4374 | 4404 |
| 4 | 46.1 | 58 | 58.7 | 74.4 | 366 | 311 | 418 | 647 | 2029 | 2619 | 2635 | 2280 |
| 6 | 26.4 | 24.5 | 28.2 | 37.5 | 145 | 145 | 227 | 161 | 843 | 1417 | 1204 | 1086 |
| 10 | 9.1 | 6.47 | 8.24 | 10.9 | 41.4 | 36.8 | 58 | 55.7 | 238 | 385 | 318 | 386 |
| 24 | 1.97 | 1.66 | 2.94 | 2.06 | 8.48 | 15.5 | 14.1 | 7.32 | 45.1 | 46 | 40.5 | 44.1 |

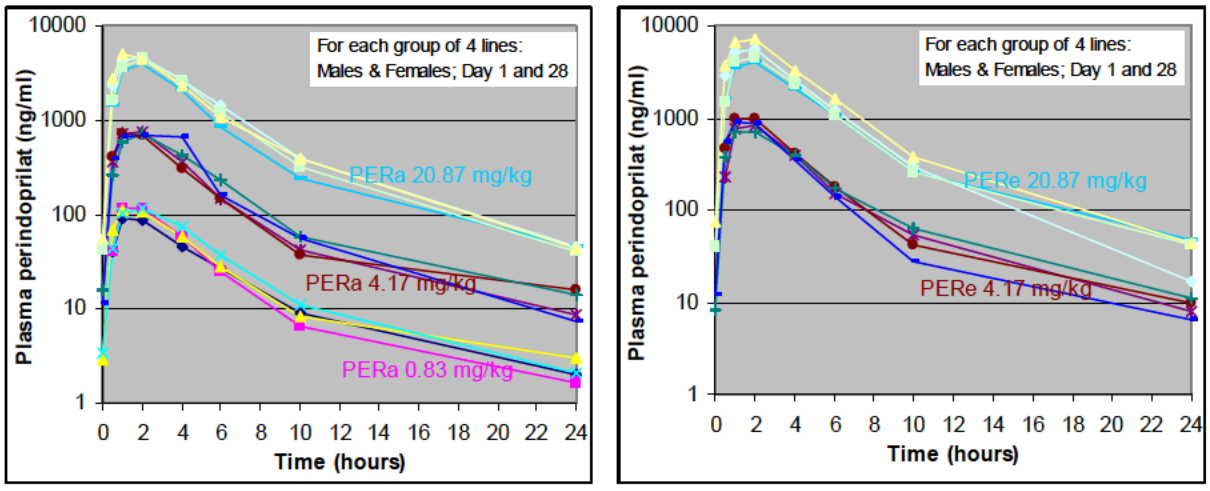
Table 18. Systemic perindopril exposure following repeat oral dosing of S 9490-3 (PERe) or S 9490-6 (PERa) in dogs (Sponsor's Table)

| Dose level (mg free acid/kg) | $AUC_{24}$ (ng.h/ml) | | | | $C_{max}$ (ng/ml) | | | |
|---|---|---|---|---|---|---|---|---|
| | Males | | Females | | Males | | Females | |
| | Day 1 | Day 28 | Day 1 | Day 28 | Day 1 | Day 28 | Day 1 | Day 28 |
| S 9490-3 | | | | | | | | |
| 4.17 | 748 | 631 | 779 | 611 | 1001 | 869 | 1302 | 857 |
| 20.87 | 3687 | 5457 | 4301 | 5034 | 5058 | 8200 | 6884 | 8478 |
| S 9490-6 | | | | | | | | |
| 0.83 | 74.3 | 82.5 | 110 | 110 | 91.7 | 90.1 | 158 | 121 |
| 4.17 | 570 | 765 | 424 | 595 | 657 | 791 | 649 | 782 |
| 20.87 | 3584 | 4534 | 3972 | 4188 | 4275 | 6051 | 6558 | 6203 |

Table 19. Systemic perindoprilat exposure following repeat oral dosing of S 9490-3 (PERe) or S 9490-6 (PERa) in dogs (Sponsor's Table)

| Dose level (mg free acid/kg) | $AUC_{24}$ (ng.h/ml) | | | | $C_{max}$ (ng/ml) | | | |
|---|---|---|---|---|---|---|---|---|
| | Males | | Females | | Males | | Females | |
| | Day 1 | Day 28 | Day 1 | Day 28 | Day 1 | Day 28 | Day 1 | Day 28 |
| S 9490-3 | | | | | | | | |
| 4.17 | 3487 | 3529 | 4014 | 3408 | 839 | 711 | 1007 | 906 |
| 20.87 | 18340 | 20137 | 23494 | 30487 | 4048 | 4638 | 5544 | 7296 |
| S 9490-6 | | | | | | | | |
| 0.83 | 461 | 550 | 511 | 605 | 90.3 | 117 | 123 | 125 |
| 4.17 | 3270 | 3576 | 3221 | 3245 | 760 | 709 | 788 | 705 |
| 20.87 | 17148 | 20751 | 23009 | 21672 | 3954 | 4374 | 4591 | 5280 |

Figure 4. Plasma perindoprilat concentrations following repeat oral doses of PERe or PERa in dogs




**Dosing Solution Analysis**



Reference ID: 3020370

Dosing preparations containing 0.05, 1.00 and 10.0 mg free acid/ml of PERe or PERa in purified water were stable for up to 12 days in a refrigerator. PERe and PERa concentrations in the dosing preparations during the 4-week period were 95.9 to 102.6% of the intended values.

### 6.2.3 A toxicity study in rats with amlodipine containing impurities

**Study title:** A 4-weeks repeated oral dose toxicity study in rats with (b) (4) spiked amlodipine besylate

| | |
|---|---|
| Study no.: | 6579 |
| Study report location: | (b) (4) |
| Conducting laboratory and location: | |
| Date of study initiation: | Mar 22, 2010 |
| GLP compliance: | Yes |
| QA statement: | Yes |
| Drug, lot #, and % purity: | (1) Amlodipine Besylate (AMLb), Lot # L0020227, Purity 99.3%<br>(2) Amlodipine Besylate spiked with two impurities (b) (4) each (AMLb SPIKED), Lot # L0031791 |

**Key Study Findings**

1/10 females of the AMLb and 1/10 females of the AMLb SPIKED at the dose 14 mg base/kg were prematurely dead or killed in week 3-4. The two premature death females and two surviving females in the 14 mg/kg AMLb group had distended abdomen (dilation of the small intestine), less or absence of stools, dyspnea, hunched posture, decreased motor activity, partial blepharoptosis and piloerection, low feed intake, and /or major changes in various clinical pathology parameters. Histopathological observation revealed moderate ulcerative enteritis of the ileum in some of these animals. Dilation of the small intestine, with or without any microscopic changes, was considered to be the probable cause of morbidity and to be the cause of the poor clinical status at the dose 14 mg base/kg of AMLb or AMLb SPIKED. No relevant differences of gastro-intestinal tract effects were noted between AMLb and AMLb SPIKED.

AMLb and AMLb SPIKED induced minimal and dose-related decreases in serum sodium and chloride concentrations (both sexes), increases in serum urea concentration (males only) and increases in urinary volume (high-dosed females only). Those changes were associated with a diffuse hypertrophy of the zona glomerulosa of the adrenal cortex with similar intensity whatever the batch.

Higher relative heart weight was observed for both sexes with dose-related incidence and severity. Severity of these findings was not enhanced when the AMLb was spiked with the two impurities (b) (4)

Under the condition of this study, toxicological profiles are similar between AMLb and AMLb SPIKED.

Methods

| | |
|---|---|
| Doses: | 7 and 14 mg free base/kg of AMLb or AMLb SPIKED* |
| Frequency of dosing: | Once daily for 4 weeks |
| Route of administration: | Oral |
| Dose volume: | 10 ml/kg |
| Formulation/Vehicle: | Solution / 1% (w/v) hydroxyethylcellulose in purified water |
| Species/Strain: | Wistar rats |
| Number/Sex/Group: | 10 |
| Age: | 6 weeks old |
| Weight: | 134 - 164 g for males & 116 - 147 g for females |
| Satellite groups: | None |
| Unique study design: | This study was to compare the safety profile of AMLb SPIKED with that of AMLb, & to evaluate the potential effect of AMLb impurities (b) (4) each. |
| Deviation from study protocol: | No impact to the results |

*Dosage selection was based on: (1) The results of a 4-week toxicity study in Wistar rats - oral AMLb 21 mg base/kg/day was lethal. There were marked clinical signs, decreases in body weight and feed intake, several clinical chemistry changes, increases in urinary volume and relative kidney weights, and macroscopic and/or microscopic evidences of changes in the liver and thyroid glands, adrenal glands, intestines and mesenteric lymph nodes, bone and bone marrow. (2) The results from Sprague-Dawley rats study where AMLb was lethal in a 4-week toxicity study at a dose of 28 mg base/kg/day and non-lethal in a 3-month toxicity study at a dose of 21 mg base/kg/day. (3) The toxicological data of Amlodipine Besylate + Benazepril Hydrochloride in Sprague-Dawley rats where AMLb (alone or combined with Benazepril) was lethal in a 6-month toxicity study at a dose of 18 mg base/kg and non-lethal in a 3-month toxicity study at the same dose. (4) No adverse effects were observed in other study with AMLb (alone or combined with Benazepril) at a dose of 18 mg base/kg for 3 weeks.

**Observations and Results**

**Mortality**

All animals were checked 2-3 times daily for mortality or signs of morbidity.

One (#359) of 10 females at the AMLb dose 14 mg/kg was found dead before dosing on day 21. Clinical signs during 0-3 days before death included hunched posture, decreased motor activity, distended abdomen, partial blepharoptosis, piloerection, dyspnea, reddish nasal discharge, and sporadic excessive salivation. Necropsy

revealed a small thymus and spleen together with enlarged (dark red) adrenals. Microscopic findings included multifocal pulmonary microthrombi (slight) suggesting intravascular coagulation, marked decreased cortico-medullary ratio in thymus and moderate lymphoid atrophy in spleen. These latter findings were correlated with gross findings and were indicative of stress and/or in line with a poor clinical condition. The underlying cause of death for this animal was not determined.

One (#460) of 10 females at the AMLb SPIKED dose 14 mg/kg was killed on day 23 because of poor clinical condition. Clinical signs 0-3 days preceding its premature kill included decreased motor activity and piloerection, partial blepharoptosis, dyspnea and hunched posture. Necropsy revealed a small and pale spleen, a little liquid yellowish content in an otherwise empty stomach and jejunum, and dilated ileum, caecum and colon. Microscopic findings included moderate ulcerative ileitis and slight enteritis of the jejunum, and slight decreased cortico-medullary ratio in thymus. Ulcerative ileitis and enteritis of the jejunum were considered to be the probable cause of morbidity.

**Clinical Signs**

Animals were checked for clinical signs 2-3 times per day.

Among surviving animals received AMLb 14 mg base/kg or AMLb SPIKED 14mg base/kg, several animals showed partial or total blepharoptosis, decreased motor activity, dyspnea, distended abdomen (#356 and 351) and piloerection, and sporadic sialism, mostly in week 4 (Table 20). Decreased motor activity and sialism observed in the groups of AMLb 14 mg base/kg or AMLb SPIKED 14mg base/kg were mostly transient and sporadic. The incidence, severity, and duration of the clinical signs were similar between the AMLb groups and AMLb SPIKED groups.

Table 20. Number of animals showed clinical signs

| | | | AMLO | | | | AMLO SPIKED | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 mg/kg | | 7 mg/kg | | 14 mg/kg | | 7 mg/kg | | 14 mg/kg | |
| Clinical signs | Male | Female | Male | Female | Male | Female | Male | Female | Male | Female |
| Partial blepharoptosis | | | | | | 3 | | | 2 | 1 |
| Blepharoptosis | | | | | | 1 | | | | |
| Decreased motor activity | | | | | | 2 | 3 | | 4 | 1 |
| Hunched posture | | | | | | 1 | | | | 1 |
| Localized Alopecia | | 1 | | | | | 1 | | | 1 |
| Crust | | | | 1 | 1 | | 1 | | | |
| Piloerection | | | | | 1 | 3 | | | | 2 |
| Dyspnea | | | | | 1 | 2 | | | | 1 |
| Absent stools | | | | | | | | | | 1 |
| Excessive salivation | | | 2 | | 8 | 8 | 2 | | 8 | 7 |
| Nasal discharge | | | | | | 1 | | | | |
| Distended abdomen | | | | | | 3 | | | | |

AMLO = AMLb, amlodipine besylate

**Body Weights**

Body weight was recorded once during the acclimation phase and twice a week until week 4. The body weight trended lower (~5%), and body weight gain was lower, in both males and females received AMLb 14 mg/kg/day (Table 21). Compared with controls, rats received AMLb SPIKED did not shown any differences in body weight or body weight gain.

Table 21. Body weight gain in AMLb-treated animals

| | | | Male | | Female | |
|---|---|---|---|---|---|---|
| | Base Weight Day | From: | Abs Gain -1 | % Gain -1 | Abs Gain -1 | % Gain -1 |
| Group | -1 | To: | 28 | 28 | 28 | 28 |
| Vehicle | 148.0 | Mean | 145.4 | 98.40 | 59.1 | 45.01 |
| | 8.4 | S.D. | 14.1 | 9.84 | 8.3 | 7.60 |
| | 10 | N | 10 | 10 | 10 | 10 |
| AMLb 7 mg/kg | 148.1 | Mean | 148.1 | 100.19 | 56.7 | 43.05 |
| | 8.1 | S.D. | 11.1 | 8.48 | 5.3 | 4.29 |
| | 10 | N | 10 | 10 | 10 | 10 |
| AMLb 14 mg/kg | 147.8 | Mean | 128.4* | 87.01* | 51.3* | 38.91 |
| | 9.2 | S.D. | 18.1 | 11.87 | 6.4 | 4.88 |
| | 10 | N | 10 | 10 | 9 | 9 |

* p<0.05 vs vehicle

**Food Consumption**

Food and water intakes were measured once a week (quantities over approximately 7 days). Females treated with AMLb 7 and 14 mg/kg showed dose-related lower food intake ($p<0.05$ with controls, Table 22). The lower food intake for females treated with AMLb SPIKED 14 mg/kg was transient. Males from the treated and control groups showed similar food intake.



Reference ID: 3020370

Table 22. Food intake during the study

| | | Males | | | | | Females | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | From Day | 1 | 7 | 14 | 21 | Total 1 | 1 | 7 | 14 | 21 | Total 1 |
| Group | To Day | 7 | 14 | 21 | 28 | 28 | 7 | 14 | 21 | 28 | 28 |
| Control | Mean | 20.1 | 22.4 | 24.6 | 23.7 | 615.4 | 15.9 | 17.2 | 17.8 | 18.5 | 469.4 |
| | S.D. | 2.0 | 2.0 | 1.9 | 1.7 | 48.7 | 1.2 | 1.3 | 1.7 | 2.2 | 38.8 |
| AMLb 7 mg/kg | Mean | 20.4 | 22.8 | 25.4 | 24.3 | 629.2 | 15.3 | 16.4 | 17.0 | 16.8* | 443.6 |
| | S.D. | 1.2 | 1.3 | 1.2 | 1.2 | 30.3 | 1.0 | 1.1 | 0.9 | 1.1 | 25.1 |
| AMLb 14 mg/kg | Mean | 19.4 | 21.9 | 23.1 | 22.5 | 588.5 | 14.8* | 16.5 | 16.8 | 16.0** | 433.9 |
| | S.D. | 1.7 | 2.1 | 1.8 | 2.5 | 49.7 | 0.9 | 0.8 | 1.0 | 1.3 | 18.8 |
| AMLb SPIKED 7 mg/kg | Mean | 19.5 | 21.7 | 24.3 | 23.6 | 603.7 | 15.1 | 16.0 | 16.6 | 16.6 | 435.3 |
| | S.D. | 1.3 | 1.7 | 1.6 | 2.1 | 42.8 | 1.0 | 1.3 | 1.5 | 1.4 | 34.6 |
| AMLb SPIKED 14 mg/kg | Mean | 20.0 | 22.9 | 24.6 | 23.9 | 620.0 | 14.8* | 16.5 | 17.0 | 17.1 | 446.0 |
| | S.D. | 0.9 | 1.5 | 1.6 | 1.6 | 33.0 | 1.2 | 1.3 | 1.3 | 1.4 | 33.8 |

Unit: g/animal/day. Total = Total consumption for the whole period (g/animal)

**Ophthalmoscopy**

Ophthalmologic examinations of the appendages, optic media and fundus by indirect ophthalmoscopy were performed once during the acclimation phase and once in the last week of the dosing phase. There were no treatment-related findings.

**ECG**

None

**Hematology**

Blood samples for hematology and clinical chemistry were collected on day 29 after an overnight feed deprivation of approximately 16 hours. Hematology parameters included –

- Red Blood Cell Count
- Packed Cell Volume
- Mean Corpuscular Volume
- Hemoglobin Concentration
- Mean Corpuscular Hemoglobin
- Mean Corpuscular Hemoglobin Concentration
- Total and Differential Leucocyte Counts
- Prothrombin time
- Activated partial thromboplastin time

One female (# 351) at AMLb 14 mg base/kg had higher values for erythrocytes (9.6 T/L *versus* 8.3 T/L in controls) and neutrophils (2.9 G/L *versus* 0.64 G/L in controls), while lower values for lymphocytes (1.6 G/l *versus* 3.7 G/L in controls), on day 30. These changes may be related to the poor clinical status of this animal and not a direct effect of the test compound. When compared to controls, there were higher values (of statistical significance) in 14 mg/kg AMLb and/or AMLb SPIKED groups (*i.e.* red blood



Reference ID: 3020370

cell mass parameters, platelet counts, coagulation times, neutrophils and/or monocytes). The differences were of very low magnitude, without any biological relevance, mostly within the 5-95% confidence interval of historical control data. Thus, these differences between treated and control groups were not toxicologically relevant.

**Clinical Chemistry**

Parameters of clinical chemistry include –

| | |
|---|---|
| Electrolytes ($Na^+$, $K^+$, $Cl^-$) | Total Cholesterol |
| Calcium | Triglycerides |
| Inorganic Phosphorus | Total Bilirubin |
| Glucose | Alkaline Phosphatase |
| Urea | Alanine Aminotransferase |
| Creatinine | Aspartate Aminotransferase |
| Albumin | Glutamate Dehydrogenase |
| Total Proteins | |

Protein fractions by electrophoresis (on the 5 last surviving animals per sex and group) included the following parameters:

| | |
|---|---|
| Albumin | Beta globulins |
| Alpha-1 globulins | Gamma globulins |
| Alpha-2 globulins | Albumin to globulin ratio |

As shown in Table 23, serum sodium and chloride concentrations were minimally, dose-dependently, and generally statistically significantly lower in AMLb- or AMLb SPIKED-treated animals than controls. AMLb- or AMLb SPIKED-treatment also resulted in higher serum urea concentration (up to 1.5 fold, dose-dependent manner, statistically significant in males). There were no differences between AMLb and AMLb SPIKED groups.

When compared to controls, serum total protein, albumin, triglyceride and/or total cholesterol concentrations, alkaline phosphatase and/or alanine aminotransferase activities were lower or higher (statistical significance) in AMLb and/or AMLb SPIKED groups. These differences were minor, and group mean values were within the 5-95% confidence interval of historical control data. These differences were attributed to a few individual animals: females #351 and #355 at 14 mg base/kg AMLb had lower serum albumin concentration; females #451 and #459 at 14 mg base/kg of AMLb SPIKED had higher alanine aminotransferase values (still below a 2-fold change and not associated with any changes in glutamate dehydrogase activity). Therefore, these differences in the treated groups were not of toxicological significance.

Table 23. Serum chemistry findings of statistically significant

| Group | Sex | | Na mmol/l | Cl mmol/l | Urea mmol/l | Alb g/l | Prot g/l | T_Chol mmol/l | Trig mmol/l | ALP U/l | ALT U/l |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | m | Mean | 144.60 | 101.82 | 4.62 | 44.06 | 61.79 | 1.46 | 0.62 | 116.5 | 17.7 |
| | | S.D. | 0.79 | 0.84 | 0.70 | 1.64 | 2.31 | 0.20 | 0.19 | 17.5 | 1.8 |
| | | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| AMLO 7 mg/kg | m | Mean | 143.72 | 98.87** | 4.95 | 44.28 | 62.44 | 1.52 | 0.61 | 132.1 | 19.1 |
| | | S.D. | 1.08 | 1.43 | 0.82 | 1.28 | 0.88 | 0.24 | 0.14 | 23.9 | 2.7 |
| | | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| AMLO 14 mg/kg | m | Mean | 142.31** | 95.69** | 7.05** | 45.78** | 62.78 | 1.48 | 0.55 | 156.1* | 21.5** |
| | | S.D. | 1.67 | 2.19 | 1.06 | 1.10 | 1.79 | 0.15 | 0.11 | 47.1 | 2.5 |
| | | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| AMLO SPIKED 7mg/kg | m | Mean | 144.14 | 99.34** | 5.54* | 44.47 | 62.29 | 1.54 | 0.61 | 132.5 | 20.6* |
| | | S.D. | 0.89 | 0.91 | 0.92 | 1.22 | 1.75 | 0.11 | 0.21 | 26.3 | 3.4 |
| | | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| AMLO SPIKED 14 mg/kg | m | Mean | 140.48** | 93.74** | 6.82** | 45.42 | 61.22 | 1.52 | 0.47 | 139.2 | 22.9** |
| | | S.D. | 2.65 | 2.05 | 1.55 | 1.61 | 2.44 | 0.19 | 0.11 | 27.9 | 3.1 |
| | | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Control | f | Mean | 144.12 | 103.54 | 6.51 | 48.06 | 63.32 | 1.46 | 0.33 | 83.8 | 14.8 |
| | | S.D. | 1.13 | 1.07 | 0.74 | 2.00 | 2.22 | 0.26 | 0.09 | 33.0 | 2.8 |
| | | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| AMLO 7 mg/kg | f | Mean | 142.62* | 100.70** | 7.14 | 47.18 | 63.14 | 1.78 | 0.38 | 70.0 | 13.9 |
| | | S.D. | 2.34 | 1.69 | 1.54 | 1.49 | 2.16 | 0.28 | 0.06 | 29.1 | 2.6 |
| | | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| AMLO 14 mg/kg | f | Mean | 140.79** | 95.93** | 6.83 | 46.34* | 60.50* | 1.67 | 0.42* | 71.4 | 16.2 |
| | | S.D. | 1.79 | 1.76 | 0.95 | 1.47 | 2.31 | 0.34 | 0.07 | 15.7 | 4.4 |
| | | N | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 |
| AMLO SPIKED 7mg/kg | f | Mean | 143.35 | 99.67** | 6.99 | 45.95 | 61.94 | 1.67* | 0.35 | 70.2 | 13.3 |
| | | S.D. | 1.10 | 1.74 | 0.51 | 2.16 | 2.25 | 0.31 | 0.11 | 10.3 | 0.9 |
| | | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| AMLO SPIKED 14 mg/kg | f | Mean | 141.52** | 97.84** | 7.30 | 47.28 | 61.98 | 1.94** | 0.38 | 73.4 | 20.2 |
| | | S.D. | 1.50 | 1.66 | 1.25 | 1.37 | 1.99 | 0.17 | 0.07 | 15.7 | 10.0 |
| | | N | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 |

* p<0.05 vs control; ** p<0.01 vs controls. AMLO = AMLb, amlodipine besylate

**Urinalysis**

Urine samples were collected overnight for at least 16 hours under conditions of food deprivation in week -1 and on day 29 or 30. Urinary quantitative parameters included volume, proteins, creatinine, and specific gravity. Urinary semi-quantitative and microscopic evaluations included appearance, color, glucose, bilirubin, ketones, pH, occult blood, urobilinogen, nitrites, leukocytes, and sediments.

When compared to controls, urinary volumes were higher for females at AMLb 14 mg base/kg (p<0.05) and trended higher for females at AMLb SPIKED 14 mg base/kg. The higher urine volumes were associated with a decrease in mean urinary protein (p<0.05) for females at 14 mg base/kg AMLb or AMLb SPIKED (Table 24).

Table 24. Urinary findings of statistically significance

| Group | | Male Vol/24h ml | Male U_Prot g/l | Female Vol/24h ml | Female U_Prot g/l |
|---|---|---|---|---|---|
| Control | Mean | 19.909 | 0.820 | 7.397 | 0.148 |
| | S.D. | 13.629 | 0.299 | 2.602 | 0.036 |
| | N | 10 | 10 | 10 | 10 |
| AMLO 7 mg/kg | Mean | 18.572 | 1.053 | 5.902 | 0.149 |
| | S.D. | 15.457 | 0.853 | 3.752 | 0.046 |
| | N | 10 | 10 | 10 | 10 |
| AMLO 14 mg/kg | Mean | 19.201 | 0.696 | 18.099* | 0.127* |
| | S.D. | 13.499 | 0.283 | 13.897 | 0.136 |
| | N | 10 | 10 | 9 | 9 |
| AMLO SPIKED 7 mg/kg | Mean | 11.535 | 0.870 | 6.688 | 0.133 |
| | S.D. | 8.724 | 0.319 | 4.424 | 0.048 |
| | N | 10 | 10 | 10 | 10 |
| AMLO SPIKED 14 mg/kg | Mean | 26.284 | 0.634 | 14.513 | 0.104* |
| | S.D. | 13.190 | 0.300 | 11.928 | 0.039 |
| | N | 10 | 10 | 9 | 9 |

* p<0.05 vs controls. AMLO = AMLb, amlodipine besylate

**Gross Pathology**

At the end of the dosing period, a detailed necropsy was performed for all surviving animals after overnight fast. Detailed necropsy was performed for animals of premature death.

Female #351 given 14 mg base/kg AMLb had dilated ileum with brownish liquid content and a dark red Peyer's patch. In addition, this female had a small thymus, a small spleen and enlarged adrenals. Female #356 of the same dose group had a small thymus. These latter changes were considered as stress-related and not direct toxic effects of AMLb.

The other macroscopic changes noted in this study were few. Those observed among AMLb- or AMLb SPIKED-dosed rats at 7 or 14 mg base/kg were either sporadic and/or without any dose related trend. None of these changes were of any toxicological significance.

**Organ Weights**

For all animals killed at scheduled necropsies, the following organs were weighed after careful trimming (paired organs were weighed together) -

| | | | |
|---|---|---|---|
| Adrenal glands | Liver | Salivary glands | Thymus |
| Brain | Lungs | (mandibular/sublingual) | Thyroid glands |
| Epididymides | Ovaries | Seminal vesicles | Uterus |
| Heart | Pituitary gland | Spleen | |
| Kidneys | Prostate | Testes | |

In males, both AMLb and AMLb SPIKED resulted in dose-dependent elevations in heart weight (being statistically significant at ≥ 7 mg/kg) and liver weight (being statistically significant at 14 mg/kg). Epididymide weights were lower in a dose-dependent manner in AMLb- (at 14 mg/kg, $p<0.05$ vs control) and AMLb SPIKED- (at ≥ 7 mg/kg, $p<0.05$ vs control) groups . At 14 mg/kg, both AMLb and AMLb SPIKED groups showed lower thymus weight, being statistically significant only in AMLb SPIKED group. In females, AMLb resulted in higher kidney weight at 14 mg/kg, and lower mandibular/sublingual gland weight at ≥ 7 mg/kg. The effects of AMLb SPIKED on organ weight are similar to those of AMLb (Table 25).

Table 25. Organ weight findings of statistically significant

| MALES | | 0 mg/kg | 7 mg/kg AMLO | 14 mg/kg AMLO | 7 mg/kg SPIKED | 14 mg/kg SPIKED |
|---|---|---|---|---|---|---|
| Terminal Bodyweight ..... (n=10) | Mean (g) | 264.2 | 264.1 | 240.4 | 258.4 | 250.4 |
| | S.D. | 16.2 | 15.2 | 21.6 | 19.3 | 14.0 |
| HEART ..... (n=10) | Mean (g) | 0.8346 | 0.8915** | 0.8652** | 0.8702* | 0.8851** |
| | S.D. | 0.0575 | 0.0603 | 0.0986 | 0.0727 | 0.0625 |
| | Mean (%) | 0.316 | 0.338** | 0.360** | 0.337* | 0.354** |
| | S.D. | 0.019 | 0.014 | 0.022 | 0.015 | 0.025 |
| LIVER ..... (n=10) | Mean (%) | 2.863 | 2.950 | 3.024* | 2.932 | 3.013** |
| | S.D. | 0.107 | 0.124 | 0.149 | 0.171 | 0.138 |
| EPIDIDYMIDES ..... (n=10) | Mean (g) | 0.8726 | 0.8323 | 0.7670* | 0.7997* | 0.7721** |
| | S.D. | 0.1003 | 0.0963 | 0.0542 | 0.0582 | 0.0617 |
| | Mean (%) | 0.332 | 0.316 | 0.321* | 0.311* | 0.309** |
| | S.D. | 0.047 | 0.037 | 0.032 | 0.034 | 0.030 |
| THYMUS ..... (n=10) | Mean (g) | 0.6032 | 0.6422 | 0.4667 | 0.5511 | 0.4828* |
| | S.D. | 0.1261 | 0.1428 | 0.0901 | 0.0627 | 0.0775 |
| | Mean (%) | 0.228 | 0.244 | 0.193 | 0.215 | 0.192* |
| | S.D. | 0.043 | 0.056 | 0.024 | 0.032 | 0.025 |
| FEMALES (n=9-10) | | | | | | |
| Terminal Bodyweight ..... | Mean (g) | 172.5 | 167.8 | 160.7 | 164.7 | 166.3 |
| | S.D. | 10.4 | 11.0 | 12.1 | 12.8 | 6.9 |
| MANDIBULAR / SUBLINGUAL GLANDS | Mean (g) | 0.3703 | 0.3400* | 0.3150** | 0.3487 | 0.3467 |
| | S.D. | 0.0290 | 0.0319 | 0.0345 | 0.0446 | 0.0262 |
| | Mean (%) | 0.215 | 0.203* | 0.197** | 0.212 | 0.208 |
| | S.D. | 0.017 | 0.021 | 0.024 | 0.020 | 0.014 |
| KIDNEYS ..... | Mean (g) | 1.255 | 1.281 | 1.289* | 1.248 | 1.293 |
| | S.D. | 0.079 | 0.083 | 0.090 | 0.066 | 0.093 |
| | Mean (%) | 0.728 | 0.764 | 0.805* | 0.761 | 0.777 |
| | S.D. | 0.041 | 0.038 | 0.068 | 0.061 | 0.042 |

AMLO = AMLb, amlodipine besylate

**Histopathology**

*Adequate Battery*

For all animals, including those found dead or prematurely killed, any macroscopic anomalies and representative samples of the organs and tissues listed below were histologically processed.

| | | | |
|---|---|---|---|
| Adrenal glands | Heart | Ovaries | Spinal cord |
| Aorta | Ileum | Oviducts | Spleen |
| Brain | Jejunum | Pancreas | Sternum |
| Caecum | Kidneys | Parathyroid glands | Testes |
| Colon | Larynx | Peyer's patch | Thymus |
| Duodenum | Liver | Pituitary gland | Thyroid glands |
| Epididymides | Lungs | Prostate | Tongue |
| Eyes | Lymph nodes | Salivary glands (parotid and | Trachea |
| Femur ① and stifle joint | (mandibular, mesenteric) | mandibular /sublingual) | Ureters |
| Forestomach | Mammary gland | Sciatic nerve | Urinary bladder |
| Glandular stomach | Oesophagus | Seminal vesicles | Uterus |
| Harderian gland | Optic nerves | Skeletal muscle | Vagina |
| | | Skin | |

① Bone marrow smears were performed and stained with May-Grünwald Giemsa for all animals, except those found dead.

Microscopic examination was performed for animals on all listed organs and macroscopic anomalies of animals from the control and high dose groups (AMLb and AMLb SPIKED), and all macroscopic anomalies and adrenal glands of all animals from the low dose groups (AMLb and AMLb SPIKED).

*Peer Review*

Microscopic examination was performed by (b) (4)
No information about peer review is available in the application.

*Histological Findings*

Female #351 given 14 mg base/kg AMLb had moderate ulcerative enteritis of the ileum, and slight decreased cortico-medullary ratio in thymus that correlated with gross finding. The ulcerative enteritis of the ileum was treatment-related.

Minimal to moderate diffuse hypertrophy/vacuolation of the zona glomerulosa in the adrenal glands was present in 1/10 control females, and most of the treated males and females (Table 26). In affected animals, the cells of the zona glomerulosa were enlarged with clear or foamy cytoplasmic vacuolation. Females in the 14 mg/kg AMLb and 14 mg/kg AMLb SPIKED groups had a higher incidence of tubular basophilic foci and/or focal cortical inflammatory cell infiltration in the kidney (Table 26).

Table 26. Treatment-related histological findings in rats

| | | | AMLO, mg/kg | | AMLO SPIKED, mg/kg | |
|---|---|---|---|---|---|---|
| Male | | Control | 7 | 14 | 7 | 14 |
| **ADRENAL CORTEX** | | | | | | |
| number examined | | 10 | 10 | 10 | 10 | 10 |
| **Diffuse hypertrophy/vacuolation - zona glomerulosa** | (minimal) | 0 | 6 | 0 | 8 | 4 |
| | (slight) | 0 | 0 | 10 | 0 | 6 |
| | Total | 0 | 6 | 10 | 8 | 10 |
| Female | | | | | | |
| **ADRENAL CORTEX** | | | | | | |
| number examined | | 10 | 10 | 10 | 10 | 10 |
| **Diffuse hypertrophy/vacuolation - zona glomerulosa** | (minimal) | 1 | 7 | 2 | 8 | 4 |
| | (slight) | 0 | 2 | 5 | 1 | 6 |
| | (moderate) | 0 | 0 | 2 | 0 | 0 |
| | Total | 1 | 9 | 9 | 9 | 10 |
| **KIDNEYS** | | | | | | |
| number examined | | 10 | - | 10 | - | 10 |
| **Focus(i) of basophilic (regenerating) tubules** | (minimal) | 1 | - | 4 | - | 2 |
| **Focal cortical inflammatory cell infiltration** | (minimal) | 0 | - | 2 | - | 0 |

AMLO = AMLb, amlodipine besylate

**Special Evaluation**

NONE

**Toxicokinetics**

NONE

**Dosing Solution Analysis**

The concentrations of the AMLb and AMLb SPIKED in the dosing solutions were 95.3-103.6% of the intended concentrations, during the experiment.

## 7 Genetic Toxicology

## 7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

### 7.1.1 Effect of Perindopril Arginine

**Study title:** Detection of reverse mutation in histidine-requiring Salmonella typhzmurium and tryptophan-requiring Escherichia coli

| | |
|---|---|
| Study no.: | NP07996 |
| Study report location: | (b) (4) |
| Conducting laboratory and location: | |
| Date of study initiation: | Jan 17, 2001 |
| GLP compliance: | Yes |
| QA statement: | Yes |
| Drug, lot #, and % purity: | Perindopril arginine (PERa), FF826, 99% (containing (b) (4) % of impurity (b) (4) ) |

**Key Study Findings**

Negative.

Methods

| | |
|---|---|
| Strains: | *S.* typhimurium TA100, TA1535, TA1537 and TA98, E. coli *WP2* (pKMI 01) and *WP2* uvrA (pKMI 01) |
| Concentrations in definitive study: | 50, 150, 500, 1500 and 5000 ug free acid/plate |
| Basis of concentration selection: | The highest concentration 5000 ug free acid/plate was the recommended maximum concentration, and based on previous studies showing no precipitate nor cytotoxicity at concentrations < 30000 ug salt/plate. |
| Negative control: | Water |
| Positive control: | See Table 27 |
| Formulation/Vehicle: | Solution / Water |
| Incubation & sampling time: | Incubation at 37°C for 3 days, count revertant colonies at end of the incubation |

Table 27. positive controls and concentrations (Sponsor's Table)

| Strains | Without metabolic activation | With metabolic activation |
|---|---|---|
| *TA100* | Sodium azide 8 μg/plate in 0.9 % (w/v) NaCl | 2-anthramine 2.5 μg/plate in dimethyl sulfoxide |
| *TA1535* | | |
| *TA1537* | 9-aminoacridine 100 μg/plate in dimethyl sulfoxide | |
| *TA98* | 4-nitroquinoline N-oxide 0.5 μg/plate in dimethyl sulfoxide | |
| *WP2 uvrA (pKM101)* | | |
| *WP2 (pKM101)* | Mitomycin C 0.1 μg/plate in 0.9 % (w/v) NaCl | 2-anthramine 15 μg/plate in dimethyl sulfoxide |

**Study Validity**

The genotypic integrity of each tester bacterial strain was demonstrated by confirming tests. No precipitate was observed whatever the assay. The revertant colony number of negative control remained within the historical data of the negative solvent control. Each positive control group showed an increase in the number of revertant colonies, demonstrating the sensitivity of the test system.

**Results**

As shown in Table 28, the numbers of revertant colonies increased at certain concentrations in strains TA98, TA 100, and WP2. All these increases did not reach the 2-fold increase over the solvent control or remained within the historical data of the negative solvent control, were not reproducible and not concentration-related. Thus, under the conditions of this study, PERa (batch FF 826) was not mutagenic at tested concentrations ranging from 50 to 5000 ug free acid/plate, with or without metabolic activation.

Table 28. Numbers of revertant colonies

| CONC. (µg free acid/ plate) | ASSAY | without metabolic activation | | | | | | | with metabolic activation | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 0 | 50 | 150 | 500 | 1500 | 5000 | positive control | 0 | 50 | 150 | 500 | 1500 | 5000 | positive control |
| | 2 | 0 | 50 | 150 | 500 | 1500 | 5000 | | 0 | 50 | 150 | 500 | 1500 | 5000 | |
| | 3 | 0 | 50 | 150 | 500 | 1500 | 5000 | | | | | | | | |
| STRAIN | ASSAY | | | | | | | | | | | | | | |
| TA 100 | 1 | 151.3 | 138.0 | 155.7 | 158.3 | 190.7 | 180.7 | 609.0 | 164.0 | 177.7 | 184.7 | 194.7 | 192.7 | 236.7 | 1170.7 |
| | 2 | 137.7 | 148.7 | 159.7 | 177.3 | 193.0 | 208.0 | 521.3 | 201.3 | 187.3 | 182.7 | 192.3 | 220.7 | 257.3 | 1474.3 |
| | 3 | 172.7 | 159.3 | 162.7 | 179.0 | 198.0 | 211.0 | 688.3 | | | | | | | |
| TA 1535 | 1 | 18.0 | 17.7 | 15.0 | 20.7 | 9.7 | 9.0 | 603.3 | 14.0 | 11.3 | 14.7 | 15.7 | 15.7 | 12.3 | 163.7 |
| | 2 | 19.7 | 19.0 | 21.7 | 18.7 | 13.0 | 11.7 | 771.0 | 14.7 | 14.0 | 12.7 | 15.3 | 18.0 | 15.3 | 230.0 |
| TA 1537 | 1 | 8.7 | 8.0 | 7.0 | 8.3 | 11.7 | 7.0 | 372.3 | 14.0 | 8.7 | 13.3 | 6.3 | 10.0 | 6.7 | 155.0 |
| | 2 | 11.7 | 14.7 | 12.7 | 12.3 | 9.3 | 10.7 | 526.0 | 6.7 | 9.3 | 8.7 | 10.7 | 8.3 | 6.7 | 212.3 |
| TA 98 | 1 | 29.7 | 29.0 | 24.7 | 27.0 | 30.0 | 30.7 | 391.3 | 38.3 | 36.7 | 43.3 | 38.0 | 35.0 | 48.7 | 934.3 |
| | 2 | 31.7 | 31.7 | 27.7 | 31.3 | 32.7 | 39.3 | 330.3 | 30.0 | 29.3 | 30.7 | 35.0 | 33.3 | 49.7 | 1011.7 |
| WP2 uvrA (pKM 101) | 1 | 48.7 | 42.0 | 50.3 | 38.7 | 40.0 | 38.7 | 560.0 | 66.7 | 67.3 | 60.0 | 59.0 | 56.7 | 60.0 | 402.3 |
| | 2 | 42.3 | 42.0 | 43.3 | 43.0 | 34.3 | 45.7 | 619.3 | 53.7 | 52.3 | 46.0 | 46.7 | 57.3 | 62.0 | 617.0 |
| WP2 (pKM 101) | 1 | 55.7 | 52.7 | 48.3 | 58.7 | 59.7 | 53.7 | 243.7 | 55.7 | 55.7 | 56.3 | 65.3 | 66.7 | 69.0 | 119.3 |
| | 2 | 62.7 | 73.0 | 65.3 | 66.7 | 91.7 | 62.7 | 264.3 | 55.3 | 55.0 | 55.7 | 59.7 | 63.0 | 59.0 | 119.7 |

### 7.1.2 Effect of Amlodipine Spiked with Impurities

**Study title:** Ames assay with amlodipine spiked with (b) (4)

| | |
|---|---|
| Study no.: | 6620 |
| Study report location: | (b) (4) |
| Conducting laboratory and location: | |
| Date of study initiation: | May 3, 2010 |
| GLP compliance: | Yes |
| QA statement: | Yes |
| Drug, lot #, and % purity: | Amlodipine besylate spiked with impurities (b) (4) each, Lot# L0031791, Purity |

**Key Study Findings**

Negative

Methods

| | |
|---|---|
| Strains: | *S.* typhimurium TA100, TA1535, TA1537 and TA98, E. coli *WP2* (pKMI 01) and *WP2* uvrA (pKMI 01) |
| Concentrations in definitive study: | 1.5, 5, 15, 50, 150, and 300 µg base/plate |
| Basis of concentration selection: | A preliminary toxicity carried out on strains *TA100* and *WP2 uvrA* at the concentrations of 0 (DMSO), 50, 150, 500, 1500 and 5000 µg base/plate with or without S9 showed precipitation at 5000 µg base/plate and cytotoxicity at ≥ 150 or 500 µg base/plate |
| Negative control: | DMSO |
| Positive control: | Sodium azide, Mitomycin C, 2-anthramine, 9-aminoacridine, and 4-NQO (Table 29) |
| Formulation/Vehicle: | Solution / saline or DMSO |
| Incubation & sampling time: | Incubation at 37°C for 3 days, count revertant colonies at end of the incubation |

Table 29. Positive control substances and concentrations (Sponsor's Table)

| Strains | Without Metabolic Activation | With Metabolic Activation |
|---|---|---|
| *TA100* | sodium azide (Sigma)<br>8 µg/plate in 0.9% (w/v) sodium chloride injection | 2-anthramine<br>(Aldrich)<br>2.5 µg/plate in sterile dimethyl sulphoxide |
| *TA1535* | | |
| *TA1537* | 9-aminoacridine, hydrochloride, hydrate (Sigma)<br>100 µg/plate in sterile dimethyl sulphoxide | |
| *TA98* | 4-nitroquinoline N-oxide<br>(Sigma)<br>0.5 µg/plate in sterile dimethyl sulphoxide | |
| *WP2 uvrA (pKM101)* | | |
| *WP2 (pKM101)* | mitomycin C (Améticine®) (Sanofi Synthelabo)<br>0.1 µg/plate in 0.9% (w/v) sodium chloride injection | 2-anthramine<br>(Aldrich)<br>15 µg/plate in sterile dimethyl sulphoxide |

**Study Validity**

The genotypic integrity of each tester bacterial strain was demonstrated by confirming tests. No precipitate was observed whatever the assay. The revertant colony number of negative control remained within the historical data of the negative solvent control. Each positive control group showed an increase in the number of revertant colonies, demonstrating the sensitivity of the test system.

**Results**

As shown in Table 30 and Table 31, the numbers of revertant colonies increased at 150 ug/ml without S9 in stain TA98. This increase was not reproducible, not concentration-related, and secondary to cytotoxicity of the test compound. Thus, under the conditions of this study, amlodipine spiked was not mutagenic at tested concentrations ranging from 1.5 to 300 ug free acid/plate, with or without metabolic activation.

Table 30. Numbers of revertant colonies, without S9

| Test Article | Concentration (µg/plate) Exp 1 | Concentration (µg/plate) EXP 2 | *TA98* Exp 1 | *TA98* EXP 2 | *TA100* Exp 1 | *TA100* EXP 2 | *TA1535* Exp 1 | *TA1535* EXP 2 | *TA1537* Exp 1 | *TA1537* EXP 2 | *WP2 (pKM101)* Exp 1 | *WP2 (pKM101)* EXP 2 | *WP2 uvrA (pKM101)* Exp 1 | *WP2 uvrA (pKM101)* EXP 2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DMSO | | | 28 | 29 | 113 | 106 | 15 | 20 | 11 | 11 | 58 | 71 | 119 | 111 |
| Amlodipine spiked with (b) (4) | 1.5 | | 39 | | 116 | | 18 | | 15 | | 59 | | 139 | |
| | 5 | 5 | 25 | 27 | 137 | 106 | 19 | 16 | 10 | 9 | 61 | 89 | 134 | 113 |
| | 15 | 15 | 26 | 45 | 154 | 108 | 15 | 25 | 9 | 13 | 65 | 70 | 128 | 104 |
| | 50 | 50 | 17 | 32 | 94 | 113 | 11 | 22 | 3 | 11 | 63 | 73 | 137 | 117 |
| | 150 | 150 | 7 | 84 ** | 1 | 88 | 0 | 16 | 0 | 23 | 58 | 79 | 105 | 110 |
| | | 300 | | 0 | | 14 | | 1 | | 0 | | 84 | | 71 |
| 4-nitroquinoline N-oxid | 0.5 | 0.5 | 139 ** | 220 ** | NA | NA | NA | NA | NA | NA | NA | NA | 1612 ** | 370 ** |
| sodium azide | 8 | 8 | NA | NA | 1254 ** | 1078 ** | 1784 ** | 1878 ** | NA | NA | NA | NA | NA | NA |
| 9-aminoacridine | 100 | 100 | NA | NA | NA | NA | NA | NA | 1308 ** | 3895 ** | NA | NA | NA | NA |
| mitomycin C | 0.1 | 0.1 | NA | NA | NA | NA | NA | NA | NA | NA | 336 ** | 284 ** | NA | NA |

** p<0.01 vs DMSO and at least 2-fold DMSO

Table 31. Numbers of revertant colonies, with S9

| Test Article | Concentration (µg/plate) Exp 1 | Concentration (µg/plate) Exp 2 | *TA98* Exp 1 | *TA98* Exp 2 | *TA100* Exp 1 | *TA100* Exp 2 | *TA1535* Exp 1 | *TA1535* Exp 2 | *TA1537* Exp 1 | *TA1537* Exp 2 | *WP2 (pKM101)* Exp 1 | *WP2 (pKM101)* Exp 2 | *WP2 uvrA (pKM101)* Exp 1 | *WP2 uvrA (pKM101)* Exp 2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DMSO | 0 | 0 | 32 | 37 | 204 | 141 | 17 | 15 | 16 | 12 | 106 | 106 | 146 | 111 |
| Amlodipine spiked with (b) (4) | 1.5 | | 35 | | 153 | | 8 | | 9 | | 82 | | 147 | |
| | 5 | 5 | 29 | 37 | 181 | 123 | 14 | 15 | 12 | 11 | 90 | 126 | 149 | 110 |
| | 15 | 15 | 37 | 48 | 142 | 134 | 11 | 14 | 10 | 15 | 100 | 126 | 155 | 119 |
| | 50 | 50 | 37 | 35 | 198 | 138 | 11 | 24 | 14 | 15 | 83 | 105 | 147 | 116 |
| | 150 | 150 | 26 | 34 | 76 | 148 | 8 | 15 | 79 | 12 | 76 | 116 | 146 | 115 |
| | | 300 | | 10 | | 215 | | 12 | | 4 | | 106 | | 107 |
| 2-anthramine | 2.5 | 2.5 | 3461 ** | 3343 ** | 4759 ** | 4795 ** | 171 ** | 215 ** | 259 ** | 223 ** | NA | NA | 1025 ** | 763 ** |
| | 15 | 15 | NA | NA | NA | NA | NA | NA | NA | NA | 241 ** | 270 ** | NA | NA |

** p<0.01 vs DMSO and at least 2-fold DMSO

## 7.2 *In Vitro* Assays in Mammalian Cells

### 7.2.1 Mouse lymphoma L5178Y cell assay (MLA)



Reference ID: 3020370

**Study title:** Effect of perindopril arginine on L5178Y TK$^{+/-}$ mouse lymphoma cell mutation using Microtitire® fluctuation technique

| | |
|---|---|
| Study no.: | NP08568 |
| Study report location: | (b) (4) |
| Conducting laboratory and location: | |
| Date of study initiation: | Dec 3, 2001 |
| GLP compliance: | Yes |
| QA statement: | Yes |
| Drug, lot #, and % purity: | Perindopril arginine, FG301, 99.9% |

**Key Study Findings**

When tested up to 10 mM, perindopril arginine (PERa) did not induce mutation at the *tk* locus of L5178Y mouse lymphoma cells in two independent experiments, in the absence or presence of S-9.

Methods

| | |
|---|---|
| Cell line: | L5 178Y TK$^{+/-}$ mouse lymphoma cells |
| Concentrations in definitive study: | 700, 1400, 2100, 2800, and 3685 ug /ml |
| Basis of concentration selection: | *In the cytotoxicity range-finding experiment for 3 hour treatment, 112.5 to 3685 ug/mL were tested with or without S-9. No marked toxicity was observed (Table 32).<br>* In the cytotoxicity range-finding experiment for 24-hour treatment, 14.06 to 3685 ug/mL were tested without S-9. The highest concentration tested (3685 ug/mL) yielded 20% RTG (Table 32).<br>* Accordingly, concentrations in definitive experiments were chosen, in the absence and presence of S-9, ranging from 700 to 3685 pg acid/mL |
| Negative control: | Water |
| Positive control: | 4-nitroquinoline 1-oxide (NQO, without S9), 0.02-0.1 ug/ml; Benzo(a)pyrene (BP, with S9), 2 and 3 ug/ml |
| Formulation/Vehicle: | Solution / Water |
| Incubation & sampling time: | Treatment: 3 and 24 hours without S9, and 3 hours with S9; at 37$^{o}$C<br>Expression: 2 day at 37$^{o}$C<br>Sampling: 0, 24, and 48 hours during the expression |

Table 32. Cytotoxicity range-finding experiments

| 3 hours incubation | | | | | 24 hours incubation | | |
|---|---|---|---|---|---|---|---|
| Treatment (µg/mL) | -S-9 | | +S-9 | | Treatment (µg/mL) | -S-9 | |
| | %RS | %RTG | %RS | %RTG | | %RS | %RTG |
| 0 | 100.00 | 100 | 100.00 | 100 | 0 | 100.00 | 100 |
| | | | | | 14.06 | 87.59 | 104 |
| | | | | | 28.13 | 40.40 | 62 |
| | | | | | 56.25 | 75.98 | 84 |
| 112.5 | 108.61 | 63 | 99.28 | 86 | 112.5 | 99.67 | 104 |
| 225 | 104.23 | 92 | 120.26 | 63 | 225 | 80.12 | 102 |
| 450 | 126.72 | 66 | 118.07 | 77 | 450 | 77.72 | 138 |
| 900 | 102.31 | 85 | 106.33 | 88 | 900 | 60.01 | 75 |
| 1800 | 103.57 | 89 | 104.94 | 107 | 1800 | 59.73 | 51 |
| 3685 | 90.14 | 70 | 96.32 | 102 | 3685 | 17.66 | 20 |

%RS: Percent relative survival adjusted by post treatment cell counts
%RTG: Percent relative total growth adjusted by post treatment cell counts

**Study Validity - Acceptable**

* No marked toxicity was observed at any concentration tested in the absence or presence of S-9 following 3 hour treatment incubations
* Following 24-hour treatment incubation in the absence of S-9 in experiments 2 and 3, marked toxicity was observed at the highest concentration 3685 ug/m, which yielded 16 and 14% RTG, respectively.
* Negative (solvent) and positive control treatments were included in each mutation experiment in the absence and presence of S-9. Mutant frequencies in negative control cultures fell within acceptable ranges, and clear increases in mutation were induced by the positive control chemicals 4-nitroquinoline 1-oxide (without S-9) and benzo(a)pyrene (with S-9).

**Results**

When tested up to PERa 3685 ug/mL (10 mM), no statistically significant increases in mutant frequency were observed at any concentration tested following 3-hour treatments in the absence or presence of S-9 in experiments 1 and 2. There were small numerical increases in mutant frequency increases at 3685 ug/ml in experiments 2 and 3, 24-hour treatment without S-9. However, the increases were small (1.57, and 1.49 folds value of the solvent control) with marked toxicity (16% and 14% RTG); there was no concentration-related linear trend over the concentration range, and was only statistically significant in experiment 2. Therefore, the increases were of little or no biological significance (Table 33).

Table 33. Summary of cell growth and mutation frequency in the definitive experiments

Experiment 1 **3 hour treatment -/+ S-9**

| Treatment (μg/mL) | -S-9 | | | Treatment (μg/mL) | +S-9 | | |
|---|---|---|---|---|---|---|---|
| | %RS | RTG | MF§ | | %RS | RTG | MF§ |
| 0 | 100.00 | 1.00 | 63.22 | 0 | 100.00 | 1.00 | 64.56 |
| 700 | 97.36 | 0.80 | 67.10 | 700 | 100.35 | 1.06 | 51.88 |
| 1400 | 102.48 | 1.17 | 47.55 | 1400 | 94.24 | 1.04 | 61.32 |
| 2100 | 108.92 | 0.94 | 50.40 | 2100 | 118.59 | 1.08 | 47.13 |
| 2800 | 92.51 | 0.93 | 53.90 | 2800 | 89.51 | 0.97 | 67.34 |
| 3685 | 92.25 | 0.82 | 59.26 | 3685 | 86.78 | 0.95 | 61.54 |
| NQO | | | | BP | | | |
| 0.05 | 106.06 | 0.82 | 115.10 | 2 | 50.04 | 0.78 | 300.53 |
| 0.1 | 76.09 | 0.81 | 216.61 | 3 | 72.70 | 0.55 | 509.50 |

Experiments 2 and 3 **24 hour treatment – S-9, 3 hour treatment + S-9**

| Treatment (μg/mL) | -S-9 | | | Treatment (μg/mL) | +S-9 | | | Treatment (μg/mL) | -S-9 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | %RS | RTG | MF§ | | %RS | RTG | MF§ | | %RS | %RTG | MF§ |
| 0 | 100.00 | 1.00 | 71.99 | 0 | 100.00 | 1.00 | 95.12 | 0 | 100.00 | 1.00 | 112.64 |
| 700 | 88.70 | 0.76 | 71.49 | 700 | 95.81 | 1.05 | 87.09 | 1000 | 82.72 | 0.53 | 129.36 |
| 1400 | 64.31 | 0.62 | 65.41 | 1400 | 96.51 | 0.94 | 74.13 | 2000 | 55.23 | 0.44 | 127.93 |
| 2100 | 56.58 | 0.59 | 62.53 | 2100 | 92.13 | 0.97 | 95.73 | 2400 | 36.97 | 0.43 | 118.49 |
| 2800 | 39.65 | 0.36 | 71.21 | 2800 | 82.73 | 1.04 | 73.84 | 2800 | 29.10 | 0.34 | 135.73 |
| 3685 | 25.66 | 0.16 | 112.68* | 3685 | 79.08 | 0.83 | 88.28 | 3200 | 27.18 | 0.28 | 130.33 |
| NQO | | | | BP | | | | 3685 | 18.23 | 0.14 | 167.55 |
| 0.02 | 66.49 | 0.46 | 199.09 | 2 | 65.25 | 0.62 | 426.79 | NQO 0.02 | 100.82 | 0.91 | 177.83 |
| 0.04 | 56.66 | 0.46 | 255.49 | 3 | 43.69 | 0.40 | 973.13 | 0.04 | 70.56 | 0.77 | 260.73 |

MF: Mutant frequency; §: 5-trifluorothymidine resistant mutants/$10^6$ viable cells 2 days after treatment
%RS: Percent relative survival adjusted by post treatment cell counts
RTG: Relative total growth adjusted by post treatment cell counts
* Comparison with control, Dunnett's test (one-sided), significant at 5% level

### 7.2.2 Chromosome aberrations in cultured human peripheral blood lymphocytes

#### 7.2.2.1 Effect of perindopril arginine

**Study title:** Perindopril arginine on chromosome aberreations in cultured human peripheral blood lymphocytes

| | |
|---|---|
| Study no.: | NP08569 |
| Study report location: | (b) (4) |
| Conducting laboratory and location: | |
| Date of study initiation: | Jan 5, 2001 |
| GLP compliance: | Yes |
| QA statement: | Yes |
| Drug, lot #, and % purity: | Perindopril arginine, Lot #FF826* & FG033, purity 98.9 & 98.3%, respectively |

*Containing impurity S 10807 0.32%

**Key Study Findings**

Not a valid study due to mitotic accumulation

Methods

| | |
|---|---|
| Cell line: | Human peripheral blood lymphocytes |
| Concentrations from chromosome analysis in definitive study: | 1887-3685 ug/ml for 3-hour treatment with and without S9; 1390-2660 ug/ml for 20-hour treatment without S9 |
| Basis of concentration selection: | The highest concentration for chromosome analysis was the highest concentration tested in 3-hour treatment plus 17-hour recovery with or without S9. The concentration selection for chromosome analysis following 20-hour treatment in the absence of S-9 was restricted by the morphology of the chromosomes or evidence of mitotic accumulation. |
| Negative control: | Water |
| Positive control: | 4-Nitroquinoline 1-oxide (NQO) 1.25-5 ug/ml without S9; cyclophosphamide (CPA) 3.125-12.5 ug/ml with S9 |
| Formulation/Vehicle: | Solution /Water |
| Incubation & sampling time: | *Incubation at 37$^{o}$C, 3-hour treatment plus 17-hour recovery with or without S9; or 20-hour treatment without S9<br>*Sampling time: at the end of 20-hour incubation |

**Study Validity**

PERa resulted in mitotic accumulation and shortening of the chromosomes following 20-hour treatment in the absence of S9. These instances may have masked toxicity higher than was apparent from the mitotic index data, and led to inaccurate data interpretation (4). Thus, this study is not interpretable.

**Results**

Compared with solvent, PERa did not increase the incidence of chromosome aberrations in cultured human peripheral blood lymphocytes following 3-hour treatments in both the absence and presence of S-9 (Table 34). In Experiment 2, the highest concentration selected for analyzing chromosome aberrations following 20-hour treatment in the absence of S-9 was restricted to 2662 pg/mL by evidence of shortening of the chromosomes and mitotic accumulation at higher concentration. PERa induced chromosome aberrations following 20-hour treatment in the absence of S-9 (Table 34).

The effects of the test article on chromosome morphology, mitotic accumulation and shortening of the chromosomes, were confirmed in further experiment 3 (Table 35 from the application) following prolonged (20-hour) exposure in the absence of S9. These instances may have masked toxicity higher than was apparent from the mitotic index data, and led to inaccurate data interpretation (4). Thus, the biological significance of these data is uncertain.

Table 34. Summary of the chromosome aberration assay

| Treatment | 3-hour treatment, -S-9, 17 hour recovery | | | | 3-hour treatment, +S-9, 17 hour recovery | | | |
|---|---|---|---|---|---|---|---|---|
| PERa ug/ml | Mitotic index (%) | MIH* (%) | Cells with aberration (+Gaps) | Cells with aberration (-Gaps) | Mitotic index (%) | MIH* (%) | Cells with aberration (+Gaps) | Cells with aberration (-Gaps) |
| Experiment 1 | | | | | | | | |
| Sovent | 11.1 | | 2 | 1 | 9.5 | | 1 | 1 |
| 82.98 | | | | | | | | |
| 103.7 | | | | | | | | |
| 129.7 | | | | | | | | |
| 162.1 | | | | | | | | |
| 202.6 | | | | | | | | |
| 253.2 | | | | | | | | |
| 316.5 | | | | | | | | |
| 395.7 | | | | | | | | |
| 494.6 | Not | | | | | | | |
| 618.2 | scored | | | | | | | |
| 772.8 | 10.8 | 2.7 | | | Not | | | |
| 966 | 9.3 | 16.2 | | | scored | | | |
| 1208 | 7.4 | 33.3 | | | 9.4 | 1.1 | | |
| 1509 | 8.4 | 24.3 | | | 8.8 | 7.4 | | |
| 1887 | 9.3 | 16.2 | 1 | 0 | 9.5 | 0.0 | 1 | 0 |
| 2358 | 6.6 | 40.5 | 0 | 0 | 8.6 | 9.5 | | |
| 2948 | 8.3 | 25.2 | | | 8.1 | 14.7 | 2 | 1 |
| 3685 | 9.1 | 18.0 | 4 | 4 | 7.5 | 21.1 | 3 | 0 |
| Positive | NQO, 5.0 ug/ml | | | | CPA, 6.25 ug/ml | | | |
| | | | 27** | 23** | | | 75** | 67** |
| Experiment 2 | | | | | | | | |
| | 20-hour treatment, -S-9 | | | | 3-hour treatment, +S-9, 17 hour recovery | | | |
| Sovent | 5.4 | | 2 | 1 | 6.5 | | 8 | 7 |
| 321.9 | | | | | | | | |
| 378.7 | | | | | | | | |
| 445.5 | | | | | | | | |
| 524.2 | | | | | | | | |
| 616.7 | | | | | | | | |
| 725.5 | | | | | | | | |
| 853.5 | | | | | | | | |
| 1004 | Not | | | | | | | |
| 1181 | scored | | | | | | | |
| 1390 | 5.6 | -3.7 | 5 | 5 | | | | |
| 1635 | 5.3 | 1.9 | | | Not | | | |
| 1924 | 4.7 | 13.0 | 14 | 13** | scored | | | |
| 2263 | 5.1 | 5.6 | | | 4.9 | 24.6 | 7 | 3 |
| 2662 | 5.2 | 3.7 | 35** | 32** | 4.7 | 27.7 | 4 | 3 |
| 3132 | 7.8 | -44.4 | | | 2.9 | 55.4 | 4 | 1 |
| 3685 | 5.7 | -5.6 | | | 3.5 | 46.2 | | |
| Positive | NQO, 2.5 ug/ml | | | | CPA, 6.25 ug/ml | | | |
| | | | 48** | 42** | | | 75** | 67** |

MIH*: Mitotic inhibition (%) = [1 - (mean mitotic index of treatment/solvent mitotic index)] x 100%
** p<0.01 vs solvent control
Total 200 cells/culture were analyzed

Table 35. Confirming the mitotic accumulation (Sponsor's Table)
Experiment 3, trial 1

| Treatment (µg/mL) | Mitotic index (%) | | | | | |
|---|---|---|---|---|---|---|
| | 20+0, -S-9 Batch FF826 | | | 20+0, -S-9 Batch FG033 | | |
| | A/C | B/D | MIH* | A/C | B/D | MIH* |
| Solvent | 3.8/5.2 | 4.3/6.2 | - | 4.2/4.0 | 3.1/4.6 | - |
| 1285 | 5.2 | 5.7 | 0 | 4.5 | 4.5 | 0 |
| 1428 | 5.6 | 7.1 | 0 | 4.6 | 5.2 | 0 |
| 1586 | 6.6 | 8.2 | 0 | 6.3 | 7.4 | 0 |
| 1763 | 9.3 | 14.0 | 0 | 6.9 | 8.5 | 0 |
| 1958 | 11.3 | 12.1 | 0 | 12.7 | 10.2 | 0 |
| 2176 | 9.0 | 9.4 | 0 | 8.0 | 8.3 | 0 |
| 2418 | 8.1 | 5.9 | 0 | 13.6 | 15.0 | 0 |
| 2686 | 3.7 | 4.6 | 15 | 8.6 | 9.2 | 0 |
| 2985 | 7.6 | 7.1 | 0 | 6.5 | 6.3 | 0 |
| 3317 | 7.8 | 7.3 | 0 | 7.1 | 8.3 | 0 |
| 3685 | 7.9 | 7.3 | 0 | 11.2 | 8.2 | 0 |

Experiment 3, trial 2

| Treatment (µg/mL) | Mitotic index (%) | | | | | |
|---|---|---|---|---|---|---|
| | 20+0, -S-9 Batch FF826 | | | 20+0, -S-9 Batch FG033 | | |
| | A/C | B/D | MIH* | A/C | B/D | MIH* |
| Solvent | 6.7/7.7 | 9.0/8.8 | - | - | - | - |
| 321.9 | 7.4 | 8.8 | 0 | 8.1 | 10.7 | 0 |
| 378.7 | 8.6 | 6.2 | 8 | 6.6 | 6.4 | 22 |
| 445.5 | 6.8 | 5.8 | 22 | 7.9 | 7.3 | 6 |
| 524.2 | 7.7 | 6.4 | 12 | 8.8 | 6.5 | 5 |
| 616.7 | 6.0 | 6.4 | 23 | 6.3 | 5.9 | 24 |
| 725.5 | 8.2 | 7.5 | 2 | 9.8 | 7.4 | 0 |
| 853.5 | 8.4 | 4.2 | 22 | 7.7 | 5.5 | 18 |
| 1004 | 5.7 | 6.9 | 22 | 6.8 | 6.6 | 17 |
| 1181 | 6.2 | 3.9 | 37 | 6.9 | 7.3 | 12 |
| 1390 | 4.8 | 3.8 | 47 | 5.5 | 4.7 | 37 |
| 1635 | 5.8 | 3.6 | 42 | 5.2 | 4.2 | 42 |
| 1924 | 4.9 | 4.6 | 41 | 5.6 | 4.6 | 37 |
| 2263 | 5.1 | 5.9 | 32 | 5.0 | 6.8 | 27 |
| 2662 | 7.8 | 7.0 | 8 | 6.6 | 7.2 | 14 |
| 3132 | 9.6 | 10.4 | 0 | 9.0 | 8.5 | 0 |
| 3685 | 11.9 | 11.7 | 0 | 10.8 | 14.7 | 0 |

MIH*: Mitotic index inhibition. A, B, C, and D: Individual cultures.

### 7.2.2.2 Effect of amlodipine with impurities (b) (4)

**Study title:** Effect of amlodipine spiked with (b) (4) (AMLO SPIKED) on chromosome aberrations in cultured human peripheral blood lymphocytes

| | |
|---|---|
| Study no.: | 8224400 |
| Study report location: | (b) (4) |
| Conducting laboratory and location: | |
| Date of study initiation: | Mar 17, 2010 |
| GLP compliance: | Yes |
| QA statement: | Yes |
| Drug, lot #, and % purity: | Amlodipine Besylate spiked with (b) (4) each, Lot # L0031791, purity for Amlodipine Besylate itself is 99.3% |

**Key Study Findings**

Amlodipine spiked with impurities (b) (4) did not increase the incidence of chromosome aberration at concentration up to 1 ug/ml.

Methods

| | |
|---|---|
| Cell line: | Human peripheral blood lymphocytes |
| Concentrations in definitive study: | 0.01 – 1 ug/ml (Table 36) |
| Basis of concentration selection: | The sponsor claimed that amlodipine besylate concentration higher than 1.0 µg/ml induced mitotic inhibition (referred to SBA Norvasc, NDA 19-787). |
| Negative control: | Water |
| Positive control: | 4-Nitroquinoline 1-oxide (NQO) 2.5-5 ug/ml without S9; Cyclophosphamide (CPA) 6.25-30 ug/ml with S9 |
| Formulation/Vehicle: | Solution / Water |
| Incubation & sampling time: | *Incubation at 37$^{o}$C, 3-hour treatment plus 17-hour recovery with or without S9; or 20-hour treatment without S9<br>*Sampling time: the end of 20-h incubation |

Table 36. Concentration of AMLO SPIKED in all experiments (Sponsor's Table)

| Experiment | Treatment | Concentration range (mg/mL) | | | Final concentration range (μg/mL) | | |
|---|---|---|---|---|---|---|---|
| Range-Finder | 3+17, -S-9 | 0.00003628 | to | 0.01 | 0.003628 | to | 1.00 |
| | 3+17, +S-9 | 0.00003628 | to | 0.01 | 0.003628 | to | 1.00 |
| | 20+0, -S-9 | 0.00003628 | to | 0.01 | 0.003628 | to | 1.00 |
| Experiment 1 | 3+17, -S-9 | 0.0001 | to | 0.01 | 0.01 | to | 1.00 |
| | 3+17, +S-9 | 0.0001 | to | 0.01 | 0.01 | to | 1.00 |
| Experiment 2 | 20+0, -S-9 | 0.0005 | to | 0.01 | 0.05 | to | 1.00 |
| | 3+17, +S-9 | 0.0005 | to | 0.01 | 0.05 | to | 1.00 |

**Study Validity**

- Heterogeneity between replicate cultures is acceptable.
- The proportion of cells with structural aberrations (excluding gaps) in negative control cultures fell within the historical range.
- At least 160 cells out of an intended 200 cells were analyzed at each AMLO SPIKED concentration.
- Positive control chemicals induced statistically significant increases in the proportion of cells with structural aberrations.
- Although the highest concentration tested 1 ug/ml is very low due to mitotic inhibition, 1 ug/ml is still higher than human exposure level at dose 10 mg/day (Cmax <6 ng/ml) (5)

The study results are acceptable.

**Results**

Concentrations selected for chromosome aberration analysis were shown in Table 37. AMLO SPIKED did not increase the incidence of chromosome aberration at concentrations up to 1 ug/ml (Table 38 and Table 39).

Table 37. Mitotic index determinations for AMLO SPIKED (Sponsor's Table)

Range-Finder Experiment, NT = Not tested

| Treatment (µg/mL) | Mitotic index (%) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **3+17 hours, -S-9** | | | **3+17 hours, +S-9** | | | **20+0 hours, -S-9** | | |
| | A | B | MIH* | A | B | MIH* | A | B | MIH* |
| Vehicle | 4.5 | 6.4 | - | 5.6 | 4.8 | - | 4.5 | 4.5 | - |
| 0.003628 | 6.1 | NT | 0 | 5.8 | NT | 0 | 5.4 | NT | 0 |
| 0.006047 | 5.8 | NT | 0 | 4.1 | NT | 21 | 6.0 | NT | 0 |
| 0.01008 | 4.4 | NT | 19 | 5.6 | NT | 0 | 5.7 | NT | 0 |
| 0.01680 | 5.7 | NT | 0 | 4.7 | NT | 10 | 6.4 | NT | 0 |
| 0.02799 | 4.9 | NT | 10 | 5.1 | NT | 2 | 5.9 | NT | 0 |
| 0.04666 | 5.3 | NT | 3 | 5.4 | NT | 0 | 4.7 | NT | 0 |
| 0.07776 | 4.0 | NT | 27 | 6.8 | NT | 0 | 3.2 | NT | 29 |
| 0.1296 | 5.4 | NT | 1 | 4.3 | NT | 17 | 6.6 | NT | 0 |
| 0.216 | 5.2 | NT | 5 | 6.1 | NT | 0 | 4.8 | NT | 0 |
| 0.36 | 5.2 | NT | 5 | 5.0 | NT | 4 | 6.2 | NT | 0 |
| 0.60 | 4.7 | NT | 14 | 5.4 | NT | 0 | 4.8 | NT | 0 |
| 1.00 | 5.0 | NT | 8 | 4.3 | NT | 17 | 3.8 | NT | 16 |

Experiment 1, NS = Not scored

| Treatment (µg/mL) | Mitotic index (%) | | | | | |
|---|---|---|---|---|---|---|
| | **3+17 hours, -S-9** | | | **3+17 hours, +S-9** | | |
| | A/C | B/D | MIH* | A/C | B/D | MIH* |
| Vehicle | 7.2/7.3 | 8.1/7.8 | - | 5.5/6.0 | 5.5/7.3 | - |
| 0.01 | NS | NS | - | NS | NS | - |
| 0.025 | NS | NS | - | NS | NS | - |
| 0.05 | NS | NS | - | NS | NS | - |
| 0.075 | NS | NS | - | NS | NS | - |
| 0.10 | NS | NS | - | NS | NS | - |
| 0.25 | NS | NS | - | 6.2 | 8.3 | 0 |
| 0.50 | **12.7** | **11.3** | **0#** | **7.6** | **7.1** | **0#** |
| 0.75 | **10.9** | **9.9** | **0#** | **7.1** | **6.7** | **0#** |
| 1.00 | **7.9** | **10.7** | **0#** | **6.1** | **5.9** | **1#** |

Experiment 2

| Treatment (µg/mL) | Mitotic index (%) | | | | | |
|---|---|---|---|---|---|---|
| | **20+0 hours, -S-9** | | | **3+17 hours, +S-9** | | |
| | A/C | B/D | MIH* | A/C | B/D | MIH* |
| Vehicle | 9.3/7.9 | 6.6/7.4 | - | 7.8/11.0 | 7.8/7.8 | - |
| 0.050 | 6.6 | 7.1 | 12 | 6.7 | 9.2 | 8 |
| 0.10 | **7.2** | **6.9** | **10#** | **7.3** | **6.0** | **23#** |
| 0.20 | 7.1 | 7.1 | 9 | 6.3 | 6.1 | 28 |
| 0.40 | 6.5 | 8.6 | 3 | **5.7** | **6.0** | **32#** |
| 0.60 | **6.0** | **6.9** | **17#** | 9.4 | 6.0 | 10 |
| 0.80 | 7.2 | 5.4 | 19 | **6.4** | **6.7** | **24#** |
| 1.00 | **7.2** | **6.0** | **15#** | **7.3** | **5.8** | **24#** |

A, B, C, and D were individual replicates
*Mitotic inhibition (%) = [1 - (mean MIT/mean MIC)] x 100%, where T = treatment and C = negative control
# Highlighted concentrations selected for chromosome aberration analysis

Table 38. Summary of chromosome aberration in experiment 1

| AMLO SPIKED (µg/mL) | Replicate | 3+17 hours, Without S9 Cells Scored | Cells with Aberrations +Gaps | Cells with Aberrations -Gaps | MIH (%) | 3+17 hours, with S9 Cells Scored | Cells with Aberrations +Gaps | Cells with Aberrations -Gaps | MIH (%) |
|---|---|---|---|---|---|---|---|---|---|
| Vehicle | A | 100 | 0 | 0 | | 100 | 0 | 0 | |
| | B | 100 | 1 | 1 | | 100 | 1 | 0 | |
| | Totals | 200 | 1 | 1 | | 200 | 1 | 0 | |
| 0.50 | A | 100 | 0 | 0 | | 100 | 0 | 0 | |
| | B | 100 | 0 | 0 | | 100 | 0 | 0 | |
| | Totals | 200 | 0 | 0 | 0 | 200 | 0 | 0 | 0 |
| 0.75 | A | 100 | 0 | 0 | | 100 | 0 | 0 | |
| | B | 100 | 2 | 2 | | 100 | 0 | 0 | |
| | Totals | 200 | 2 | 2 | 0 | 200 | 0 | 0 | 0 |
| 1.00 | A | 100 | 0 | 0 | | 100 | 1 | 1 | |
| | B | 100 | 0 | 0 | | 100 | 2 | 2 | |
| | Totals | 200 | 0 | 0 | 0 | 200 | 3 | 3 | 1 |
| Positive | NQO, 2.50 | | | | | CPA, 12.5 | | | |
| | A | 100 | **15** | **13** | | 43 | **22** | **20** | |
| | B | 77 | **20** | **20** | | 42 | **21** | **20** | |
| | Totals | 177 | 35 | 33[a] | | 85 | 43 | 40[a] | |

a: p<0.001 with vehicle

Table 39. Summary of chromosome aberration in experiment 2

| AMLO SPIKED (µg/mL) | Replicate | 20 hours, Without S9 Cells Scored | Cells with Aberrations +Gaps | Cells with Aberrations -Gaps | MIH (%) | 3+17 hours, with S9 Cells Scored | Cells with Aberrations +Gaps | Cells with Aberrations -Gaps | MIH (%) |
|---|---|---|---|---|---|---|---|---|---|
| Vehicle | A | 100 | 1 | 1 | | 100 | 1 | 0 | |
| | B | 100 | 1 | 1 | | 100 | 1 | 0 | |
| | Totals | 200 | 2 | 2 | - | 200 | 2 | 0 | - |
| 0.10 | A | 100 | 0 | 0 | | 100 | 0 | 0 | |
| | B | 100 | 0 | 0 | | 100 | 0 | 0 | |
| | Totals | 200 | 0 | 0 | 10 | 200 | 0 | 0 | 23 |
| 0.40 | A | | | | | 100 | 0 | 0 | |
| | B | | | | | 100 | 1 | 1 | |
| | Totals | | | | | 200 | 1 | 1 | 32 |
| 0.60 | A | 100 | 0 | 0 | | | | | |
| | B | 100 | 0 | 0 | | | | | |
| | Totals | 200 | 0 | 0 | 17 | | | | |
| 0.80 | A | | | | | 100 | 1 | 1 | |
| | B | | | | | 100 | 0 | 0 | |
| | Totals | | | | | 200 | 1 | 1 | 24 |
| 1.00 | A | 100 | 0 | 0 | | 100 | 2 | 2 | |
| | B | 100 | 0 | 0 | | 100 | 1 | 1 | |
| | Totals | 200 | 0 | 0 | 15 | 200 | 3 | 3 | 24 |
| Positive | NQO, 2.50 | | | | | CPA, 20.0 | | | |
| | A | 74 | **21** | **0** | | 54 | **21** | **20** | |
| | B | 74 | **21** | **9** | | 68 | **15** | **14** | |
| | Totals | 148 | 42 | 9[a] | | 122 | 36 | 34[a] | |

a: p<0.001 with vehicle



Reference ID: 3020370

## 7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

**Study title:** Micronucleus cytogenetic assay in mice bone marrow with oral administration of perindopril arginine (S 9490-6, PERa)

Study no: NP08649
Study report location: (b) (4)
Conducting laboratory and location:
Date of study initiation:
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: Perindopril arginine, Lot #FH815, 98.8%

**Key Study Findings**

No clastogenic potential

Methods

Doses in definitive study: 0, 500, 1000, and 2000 mg acid/kg
Frequency of dosing: Once
Route of administration: Oral
Dose volume: 10 ml/kg
Formulation/Vehicle: Solution / Water
Species/Strain: Swiss mice (OF1, SPF Caw)
Number/Sex/Group: 6, at each sampling point (Table 40)
Satellite groups: None
Basis of dose selection: Not provided
Negative control: Water
Positive control: Cyclophosphamide, 50 mg/kg in 0.9% (w/v) sodium chloride (10 ml/kg), intraperitoneal injection

Table 40. Animal groups and sampling schedule

| | Negative Control Group | | PERa, mg/kg | | | | | Positive Control Group |
|---|---|---|---|---|---|---|---|---|
| Group Identification | 1 | | 2, 500 | 3, 1000 | 4, 2000 | | | 10 |
| Subgroup Identification | 1 | 2 | 1 | 1 | 1 | 2 | ① | 1 |
| Killing Time after dosing (hours) | 24 | 48 | 24 | 24 | 24 | 48 | - | 24 |
| Blood Sampling after dosing (hours) | None | | | | | | 1 h | None |
| Number of Animals per Sex | 12 | | 6 | 6 | 12 | | 6 | 6 |

①Animals exclusively used for determining plasma perindoprilat level.

**Study Validity**

At least 8 males and females combined per group were analysed at each examination time. All the individual numbers of micronucleated polychromatic erythrocytes of the positive and negative controls were within their respective historical range.

**Results**

No mortality or changes in appearance or behaviour were observed among the animals during the study. The relative proportion of polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE) determined on at least 1000 cells (PCE and NCE) was similar between vehicle and PERa-treated animals. One hour after an oral dose of 2000 mg PERa/kg in three additional males and females, plasma levels of the active metabolite perindoprilat were 199 ± 141 and 256 ± 115 ug/ml, in males and females, respectively, demonstrating adequate bone marrow exposure.

The positive control group showed an increase in the number of micronucleated polychromatic erythrocytes, demonstrating the sensitivity of the test system. All the values in the negative control and PERa-treated were within the range of historical negative control values. No statistically and biologically significant or dose-related increase in the number of micronucleated polychromatic erythrocytes *versus* negative controls was seen in the animals dosed with PERa.

Table 41. Summary data of the micronuleus assay

| | | Males | | | Females | | |
|---|---|---|---|---|---|---|---|
| | Time | PCE/NCE per 1000 NCE±PCE | Number of PCEM per 2000 PCE | Number of NCEM per 2000 PCE | PCE/NCE per 1000 NCE±PCE | Number of PCEM per 2000 PCE | Number of NCEM per 2000 PCE |
| Negative control | 24 h | 0.72 ± 0.33 | 1.40 ± 1.14 | 1.60 ± 1.67 | 0.75 ± 0.09 | 1.80 ± 0.84 | 2.80 ± 1.30 |
| | 48 h | 0.62 ± 0.19 | 1.40 ± 2.19 | 3.80 ± 2.17 | 0.80 ± 0.35 | 2.40 ± 1.14 | 1.80 ± 2.17 |
| PERa 500 mg/kg | 24 h | 0.58 ± 0.13 | 2.20 ± 0.45 | 3.40 ± 2.30 | 0.62 ± 0.27 | 2.00 ± 1.58 | 3.80 ± 2.17 |
| PERa 1000 mg/kg | 24 h | 0.55 ± 0.16 | 3.00 ± 3.54 | 4.20 ± 1.64 | 0.61 ± 0.25 | 2.00 ± 1.41 | 4.80 ± 3.49 |
| PERa 2000 mg/kg | 24 h | 0.69 ± 0.24 | 2.00 ± 1.58 | 3.00 ± 1.58 | 0.86 ± 0.22 | 1.40 ± 0.89 | 2.40 ± 1.52 |
| | 48 h | 0.45 ± 0.10 | 2.40 ± 1.52 | 4.60 ± 1.82 | 0.56 ± 0.11 | 1.00 ± 1.22 | 4.40 ± 2.30 |
| Cyclophos-phamid 50 mg/kg | 24 h | 0.73 ± 0.07 | 50.80 ± 2.39 | 3.60 ± 0.89 | 0.80 ± 0.21 | 24.80 ± 13.27 | 2.20 ± 1.92 |

| Histolic data - PCEM counts for 1000 erythrocytes (Study no. 2320 to Study no. 3185) | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Time | Sex | Size | Minimum | Mean | Maximum | |
| Negative control | 24 h | Male | 55 | 0 | 1.08 | 4 | PCEM: Polychromatic erythrocytes, micronucleated |
| | | Female | 35 | 0 | 1.06 | 4 | |
| | 48 h | Male | 55 | 0 | 1.17 | 3 | |
| | | Female | 35 | 0 | 0.84 | 2 | |
| Positive control | 24 h | Male | 55 | 8 | 17.08 | 37 | |
| | | Female | 20 | 4 | 11.2 | 18 | |
| | 48 h | Male | 55 | 5.5 | 13.85 | 32 | |
| | | Female | 20 | 4 | 9.05 | 14 | |

## 7.4 Other Genetic Toxicity Studies

None

## 8 Carcinogenicity

NONE

## 9 Reproductive and Developmental Toxicology

NONE

## 10 Special Toxicology Studies

None

## 11 Integrated Summary and Safety Evaluation

XOMA 985 is a fixed-dose combination of the ACE inhibitor perindopril arginine (PERa 14 mg) and the calcium channel blocker amlodipine besylate (AMLb 10 mg). It is being developed for treatment of hypertension.

The clinical study (PATH, X985400) is a phase 3, multicenter, randomized, double-blind, parallel-group trial in subjects with essential hypertension. Patients with essential hypertension will be randomized in a 1:1:1 ratio to receive XOMA 985 once daily, AMLb 10 mg once daily, or perindopril erbumine (PERe) 16 mg once daily for 6 weeks. The efficacy, safety, and tolerability of XOMA 985, compared to AML and PERe alone, will be evaluated.

Both PERe (ACEON®) and AMLb (NORVASC®) were approved in the US for the treatment of hypertension and coronary artery disease. The PERa/AMLb combination drug product was approved and is currently marketed in the European Union. Since PERa is new to the US, bridging nonclinical studies were performed to demonstrate the similarity in PK profiles and toxicology between PERe and PERa. Impurities in drug substances were also qualified. Results are summarized as follows –

- PK

- In rats, after single dose PERa 0.8 mg/kg, PO, active metabolite perindoprilat PKs were: Tmax 1 h, AUC 1017 ng·h/ml, bioavailability ~44%, principal half-life ~ 0.6 h representing about 88 % of the AUC, and urinary excretion 39.8 %
- In dogs, PERa or PERe 0.834 mg/kg/day were orally dosed once daily for 7 days. PK profiles of perindopril & metabolites perindoprilat, Y1303, & Y1304 were similar.

- General Toxicology

- Single oral dose PERa in mice, 2000 mg/kg - No death, No target organs identified
- Single oral dose PERa in rats, 2000 mg/kg - No death, excessive salivation for all rats within 30 minutes post-dose
- A 4-week repeat dose toxicity study in rats - PERa (contained various impurities including (b) (4) %) 0.8, 8, and 33 mg/kg, PERe 8 and 33 mg/kg, PO, QD x 4 week
  - ✓ No death
  - ✓ Slight increases in water intake, urinary volume, urinary chloride and sodium elimination, associated with minimal decreases in serum sodium and chloride concentrations, in all the treated groups (similar magnitude for both salt)
  - ✓ Dose-dependent higher incidence of erosions and/or ulcerations in the glandular stomach mucosa in treated groups, slightly higher incidence in PERe groups than in PERa groups
  - ✓ TK profiles of perindopril and perindoprilat were similar between PERa and PERe rats.

- A 4-week repeat dose toxicity study in dogs with PERa 0.83, 4.18, and 20.87 mg/kg, PERe 4.17 and 20.87 mg/kg, PO, QD x 4 week
  - ✓ No death
  - ✓ Decreases (~20% *vs* pre-test) in red blood cell count and Hb in 1/3 females at PERa 4.17 mg/kg and 1/3 females at PERa 20.87 mg/kg. Similar severe effects were not seen with PERe.
  - ✓ Medulla and/or papilla mineralization in kidneys of the PERa-treated dogs at all dose levels with similar incidence (1/3) and severity (minimal to mild) among doses
  - ✓ Three PERa-treated and four PERe-treated animals (at doses ≥ 4.17 mg/kg) showed minimal to mild bronchopneumonia or chronic peri-bronchiolitis, with similar incidence and severity between the two salts
  - ✓ TK profiles of perindopril and perindoprilat were similar between PERa- and PERe-treated dogs.
- A 4-week repeat dose toxicity study in rats with amlodipine besylate (AMLb) and AMLb spiked with impurities (b) (4) each % (AMLb SPIKED) – 7 and 14 mg/kg, PO. QD x 4 weeks
  - ✓ Two premature deaths: 1/10 females at 14 mg mg/kg AMLb and 1/10 females at 14 mg/kg AMLb SPIKED, probably due to dilation of the small intestine with or without ulcerative ileitis and enteritis of the jejunum
  - ✓ Gastro-intestinal tract: 1-2 of the surviving females at each AMLb and AMLb SPIKED 14 mg/kg groups had distended abdomen during the fourth dosing week (with or without any microscopic changes at the end of study), low feed intake with or without less body weight gain, major changes in various clinical pathology.
  - ✓ Electrolites: minimal and dose-related decreases in serum sodium and chloride concentrations (both sexes), increases in serum urea concentration (males only) or in urinary volume (high-dosed females only). Those changes were similar between AMLb and AMLb SPLIKED, and associated with a diffuse hypertrophy of the zona glomerulosa of the adrenal cortex.
  - ✓ Higher relative heart weight for both sexes with dose-dependent incidence and severity, Severity of these findings was similar between AMLb and AMLb SPIKED.

o Genetic Toxicology

- PERa was devoid of mutagenic potential in the Ames test, mouse lymphoma L5178Y *tk* cells mutation test, and mouse bone marrow micronucleus cytogenetic assay.
- AMLb spiked with (b) (4) each was devoid of mutagenic potential in the Ames test and chromosome aberration assay in cultured human peripheral blood lymphocytes.

These nonclinical studies were appropriately designed and executed. PERa acts similarly to PERe in terms of metabolism, pharmacokinetics, and toxicity except for moderately lower RBC and Hb and minimal to mild medulla and/or papilla

mineralization. The impurities that may be present in AMLb did not demonstrate toxicity in nonclinical studies.

## 12 Appendix/Attachments

Reference:

1. Giuseppe Mancia G. et al. 2007 ESH-ESC Practice Guidelines for the Management of Arterial Hypertension - ESH-ESC Task Force on the Management of Arterial Hypertension, Journal of Hypertension 25:1751–1762, 2007.

2. Telejko E. Perindopril arginine: benefits of a new salt of the ACE inhibitor perindopril. Curr Med Res Opin. 23:953-60, 2007

3. Cole J et al. Lack of angiotensin II–facilitated erythropoiesis causes anemia in angiotensin-converting enzyme–deficient mice. J Clin Invest 106:1391–1398, 2000

4. Guzman A et al, Assessment of the genotoxic potential of the antipsychotic sigma receptor ligand E-5842. Mutation Research 605: 63–77, 2006

5. Liu Y et al. Pharmacokinetics and bioequivalence evaluation of two formulations of 10-mg amlodipine besylate: An open-label, single-dose, randomized, two-way crossover study in healthy chinese male volunteers. Clinical Therapeutics 31: 777-783, 2009

---

**This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.**

---

/s/

---

BAICHUN YANG
09/26/2011

THOMAS PAPOIAN
09/26/2011
I concur.

**PHARMACOLOGY/TOXICOLOGY REVIEW**

**IND NUMBER**: 108233
**SERIAL NUMBER/DATE/TYPE OF SUBMISSION**:
0001/Dec 16, 2011/Other (SD-5)
**INFORMATION TO SPONSOR:** Yes () No (X)
**SPONSOR AND/OR AGENT**: XOMA (US) LLC

**REVIEWER NAME**: Baichun Yang, PhD, DABT
**DIVISION NAME**: Division of Cardiovascular and Renal Products
**REVIEW COMPLETION DATE**: Jan 6, 2012

**DRUG:**
Code name: XOMA 985

**PHARMACOLOGICAL CLASS:** Fixed-dose Combination of Perindopril Arginine and Amlodipine Besylate
**PROPOSED INVESTIGATIONAL USE:** Treatment of Hypertension

**DECLARATION:** The reviewer created the tables in the review unless otherwise specified.

**BACKGROUND**

Both perindopril erbumine (ACEON®) and amlodipine besylate (NORVASC®) were approved in the US for the treatment of hypertension and coronary artery disease. The perindopril arginine/amlodipine besylate combination drug product is approved and currently marketed in the European Union. Bridging preclinical studies demonstrated the similarity, in general, between the arginine and erbumine salts of perindopril in terms of PK and safety profile. The safety profile of amlodipine besylate with impurities (b) (4) each was similar to that of amlodipine besylate.

**NEWLY SUBMITTED PRECLINICAL STUDIES**

1. AMLODIPINE SPIKED WITH (b) (4): DETECTION OF REVERSE MUTATION IN HISTIDINE-REQUIRING *SALMONELLA TYPHIMURIUM* AND TRYPTOPHAN-REQUIRING *ESCHERICHIA COLI* (AMES TEST) (Study # 6620)

This is a finalized report identical to the report in the original submission except for the completed signature page and quality assurance statement. Under the conditions of this study, amlodipine besylate spiked with (b) (4) each, batch L0031791, did not demonstrate mutagenic potential at tested concentrations ranging from 1.5 to 5000 µg base/plate, either with or without metabolic activation.

2. S 9490-6 (PERINDOPRIL ARGININE): DETECTION OF REVERSE MUTATION IN HISTIDINE-REQUIRING *SALMONELLA TYPHIMURTUM* AND TRYPTOPHANREQUIRING *ESCHERICHIA COLI* (AMES TEST) (Study # NP15054)

This GLP study (NP15054) was conducted at (b) (4) during Feb 17, 2003 – Jun 4, 2003.

Perindopril arginine was spiked with impurities (b) (4) (S 9490-6, batch FI 150). The mutagenic potential of the batch FI 150 of S 9490-6 was tested using four LT2 *Salmonella typhimurium* strains TAI 00, TAI 535, TAI 53 7 and TA98, requiring histidine for growth (his-), and two WP2 *Escherichia coli* strains *WP2* (pKMIOI) and WP2 *uvrA* (PKMIOI), requiring tryptophan for growth (trp-). An assay with preincubation (Assay No. 1) and another with direct plating (Assay No. 2) were carried out on each strain at concentrations of 0, 50, 150, 500, 1500 and 5000 ug free acid/plate both in the presence and the absence of an Aroclor 1254-induced rat liver preparation and cofactors (S9 mix). Concurrently, each strain was treated with a known mutagenic compound (Table 1), with and without metabolic activation, to demonstrate the sensitivity of the test system. The mutagenic activity of the batch F1 150 of S 9490-6 was assessed by counting the number of revertant colonies at the histidine locus (his+) of S. *typhimurium* and at the tryptophan locus (trp+) of E. *coli.*

Table 1. Positive control agents and concentrations (from the submission)

| Strains | Without Metabolic Activation | With Metabolic Activation |
|---|---|---|
| *TA100* | sodium azide 8 µg/plate in 0.9% (w/v) sodium chloride solution | 2-anthramine 2.5 µg/plate in dimethyl sulfoxide |
| *TA1535* | | |
| *TA1537* | 9-aminoacridine 100 µg/plate in dimethyl sulfoxide | |
| *TA98* | 4-nitroquinoline N-oxide 0.5 µg/plate in dimethyl sulfoxide | |
| *WP2 uvrA (pKM101)* | | |
| *WP2 (pKM101)* | mitomycin C 0.1 µg/plate in 0.9% (w/v) sodium chloride solution | 2-anthramine 15 µg/plate in dimethyl sulfoxide |

No precipitate nor cytotoxicity was noted in any of the assays or drug concentrations used. Each positive control group showed an increase in the number of revertant colonies, demonstrating the sensitivity of the test system, and all the criteria for a valid test were met. No significant, reproducible or concentration-related increase in the number of revertant colonies was seen with the batch F1 150 of S 9490-6, with or without metabolic activation, by preincubation or direct plating with any strain (Table 2). Thus, under the conditions of this study, perindopril arginine spiked with impurities (b) (4) did not demonstrate mutagenic potential.

Table 2. Numbers of revertant colonies

| STRAIN | ASSAY | S9490-6 concentration, ug free acid/plate, without S9 | | | | | | | S9490-6 concentration, ug free acid/plate, with S9 | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 0 | 50 | 150 | 500 | 1500 | 5000 | * | 0 | 50 | 150 | 500 | 1500 | 5000 | * |
| | 2 | 0 | 50 | 150 | 500 | 1500 | 5000 | * | 0 | 50 | 150 | 500 | 1500 | 5000 | * |
| TA 100 | 1 | 110.0 | 116.3 | 113.7 | 139.0 | 156.7 | 196.3 | 606.7 | 138.3 | 149.3 | 143.7 | 148.7 | 160.3 | 207.0 | 1193.0 |
| | 2 | 104.0 | 103.0 | 103.0 | 124.0 | 138.0 | 150.3 | 502.3 | 129.7 | 116.0 | 130.7 | 140.0 | 147.0 | 201.3 | 1075.7 |
| TA 1535 | 1 | 38.3 | 40.3 | 40.7 | 43.0 | 43.3 | 32.3 | 914.3 | 40.0 | 39.0 | 51.7 | 47.3 | 44.3 | 44.0 | 173.3 |
| | 2 | 17.7 | 23.0 | 20.7 | 21.7 | 20.3 | 17.0 | 522.7 | 37.3 | 35.0 | 38.7 | 36.3 | 43.7 | 34.3 | 270.0 |
| TA 1537 | 1 | 12.0 | 13.0 | 9.3 | 14.0 | 12.3 | 13.3 | 533.7 | 18.0 | 15.7 | 19.7 | 17.3 | 16.3 | 19.3 | 232.0 |
| | 2 | 10.0 | 13.0 | 9.0 | 7.3 | 8.0 | 6.3 | 653.3 | 10.3 | 9.0 | 14.7 | 13.7 | 9.3 | 6.3 | 164.0 |
| TA 98 | 1 | 20.0 | 24.0 | 23.0 | 25.7 | 29.0 | 37.0 | 343.3 | 31.7 | 34.0 | 28.0 | 35.3 | 29.3 | 40.0 | 1190.0 |
| | 2 | 22.7 | 23.0 | 29.0 | 25.3 | 29.0 | 38.0 | 283.7 | 34.3 | 28.7 | 27.0 | 37.0 | 32.0 | 39.7 | 1023.7 |
| WP2 uvrA (pKM 101) | 1 | 95.3 | 93.7 | 95.0 | 101.7 | 120.7 | 111.0 | 879.3 | 137.0 | 141.3 | 126.3 | 141.0 | 146.0 | 154.3 | 584.0 |
| | 2 | 106.3 | 109.0 | 100.3 | 107.0 | 101.7 | 102.3 | 684.3 | 97.0 | 92.7 | 101.7 | 97.7 | 97.3 | 94.0 | 420.7 |
| WP2 (pKM 101) | 1 | 57.7 | 52.3 | 49.3 | 50.0 | 55.0 | 44.0 | 258.7 | 70.7 | 80.3 | 75.0 | 80.3 | 65.7 | 70.3 | 177.0 |
| | 2 | 58.0 | 64.0 | 65.0 | 64.0 | 65.7 | 64.0 | 243.3 | 56.0 | 65.7 | 57.7 | 63.3 | 63.0 | 61.7 | 149.7 |

*positive control. Detail in table 1.

3. S 9490-6 (Perindopril Arginine): Mutation at the Thymidine Kinase *(tk)* Locus of Mouse Lymphoma L5178Y Cells (MLA) using the microtitre$^{R}$ Fluctuation Technique (Study # NP15013)

This GLP study (NP15013) was conducted at [redacted] (b) (4) during Feb 2003 – May 2003.

Perindopril arginine (S 9490-6) was spiked with the impurities [redacted] (b) (4) (batch F1 150). The batch F1 150 of S 9490-6 ranging from 700 to 3685 ug/mL was tested in the absence and presence of S-9 for mutation potentials at the *tk* locus (5-trifluorothymidine resistance) in mouse lymphoma cells using a fluctuation protocol. A 3-hour treatment incubation period was used for two independent experiments in the presence of S-9. In the absence of S-9, Experiment 1 was performed using a 3-hour treatment incubation and Experiment 2 was performed using a 24-hour treatment incubation. The maximum concentration of the test article (3685 ug/mL, equivalent to 10 mM) was based on data from a previous study. Negative (solvent) and positive controls were included in each experiment in the absence and presence of S-9.

As shown in Table 3, all doses were selected to determine viability and 5-trifluorothymidine (TFT) resistance 2 days after treatment. In the first experiment (3 hour treatment), the highest concentration tested (3685 ug/mL) yielded 112% and 113% adjusted relative total growth in the absence and presence of S-9, respectively. In the second experiment, the highest dose level tested (3685 ug/mL) yielded 26% and 75% relative total growth in the absence and presence of S-9, respectively.

Mutant frequencies (MF) in negative controls fell within acceptable ranges, and clear increases in mutation frequency were induced by the positive control chemicals 4-nitroquinoline 1-oxide (NQO, up to 0.2 ug/ml, without S-9) and benzo(a)pyrene (BP, up to 3 ug/ml, with S-9). No statistically significant increases in mutant frequency were observed following treatment with test article at any dose level tested, in the absence or presence of S-9, in Experiment 1 or 2 (Table 3). Thus, under the conditions employed in this study, perindopril arginine containing [redacted] (b) (4) was not mutagenic in this test system in the absence or presence of S-9.

Table 3. Summary of growth and mutant frequencies under treatments with S 9490-6 containing [redacted] (b) (4) (modified from the submission)

**Experiment 1 (3 hour treatment -/+S-9)**

| Treatment (μg/mL) | -S-9 | | | | Treatment (μg/mL) | +S-9 | | | |
|---|---|---|---|---|---|---|---|---|---|
| | %RS | %RTG | MF§ | | | %RS | %RTG | MF§ | |
| 0 | 100.00 | 100 | 181.97 | | 0 | 100.00 | 100 | 133.80 | |
| 700 | 81.57 | 115 | 128.72 | NS | 700 | 84.04 | 81 | 137.97 | NS |
| 1400 | 59.71 | 92 | 145.73 | NS | 1400 | 88.04 | 92 | 143.04 | NS |
| 2100 | 89.03 | 120 | 143.42 | NS | 2100 | 88.30 | 110 | 146.22 | NS |
| 2800 | 67.65 | 121 | 131.19 | NS | 2800 | 91.34 | 113 | 139.48 | NS |
| 3685 | 65.79 | 112 | 149.96 | NS | 3685 | 81.98 | 113 | 159.67 | NS |
| Linear trend | | | | NS | Linear trend | | | | NS |
| NQO | | | | | BP | | | | |
| 0.15 | 48.47 | 64 | 367.85 | | 2 | 56.48 | 88 | 488.35 | |
| 0.2 | 49.63 | 61 | 678.24 | | 3 | 43.31 | 86 | 704.14 | |

**Experiment 2 (24 hour treatment –S-9, 3 hour treatment +S-9)**

| Treatment (μg/mL) | -S-9 | | | | Treatment (μg/mL) | +S-9 | | | |
|---|---|---|---|---|---|---|---|---|---|
| | %RS | %RTG | MF§ | | | %RS | %RTG | MF§ | |
| 0 | 100.00 | 100 | 90.13 | | 0 | 100.00 | 100 | 116.96 | |
| 700 | 76.76 | 71 | 105.77 | NS | 700 | 88.57 | 85 | 124.01 | NS |
| 1400 | 62.83 | 72 | 77.97 | NS | 1400 | 92.06 | 85 | 119.06 | NS |
| 2100 | 49.79 | 59 | 78.19 | NS | 2100 | 103.64 | 91 | 114.42 | NS |
| 2800 | 31.57 | 32 | 100.88 | NS | 2800 | 97.95 | 80 | 100.27 | NS |
| 3685 | 17.15 | 26 | 79.18 | NS | 3685 | 91.97 | 75 | 109.08 | NS |
| Linear trend | | | | NS | Linear trend | | | | NS |
| NQO | | | | | BP | | | | |
| 0.02 | 63.20 | 75 | 228.27 | | 2 | 60.31 | 55 | 540.19 | |
| 0.04 | 47.62 | 63 | 338.18 | | 3 | 46.08 | 28 | 1024.61 | |

§ 5-TFT resistant mutants/$10^6$ viable cells 2 days after treatment
%RS Percent relative survival adjusted by post treatment cell counts
%RTG Percent relative total growth adjusted by post treatment cell counts
NS Not significant

4. A 4-week toxicity study with S 9490-6 (Batch No. FI 150) in rats (Study # NP15086)

This GLP study (# NP15086) was conducted at [redacted] (b) (4); initiated in Feb 2003.

Four groups of 10 male and 10 female Wistar Han rats received perindopril arginine containing [redacted] (b) (4) (S9490-6, batch No. FI 150), daily by gavage at the dose-level of 0 (purified water), 0.8, 8 or 33 mg free acid /kg/day for 4



Reference ID: 3008317

weeks. The animals were checked daily for mortality and clinical signs. Body weight and food consumption were recorded once a week. Hematology, blood biochemistry and urinalysis were performed at the end of the treatment period. On completion of the treatment period, the animals were killed and submitted to a full macroscopic post-mortem examination. Designated organs were weighed and selected tissues were preserved (Table 4). A microscopic examination was performed on selected tissues (Table 4) of animals in control and high dose groups and on kidneys and macroscopic lesions of all animals.

Table 4. List of tissues/organs for organ weight and histological examination

| Organs | Organ weights | Preservation of tissues | Microscopic examination | Organs | Organ weights | Preservation of tissues | Microscopic examination |
|---|---|---|---|---|---|---|---|
| **Macroscopic lesions** | | X | X | Oviducts | | X | X |
| Adrenals | X | X | X | Pancreas | | X | X |
| Aorta | | X | X | Pituitary gland | | X | X |
| Brain (including medulla/pons cerebellar and cerebral cortex) | X | X | X | Prostate | | X | X |
| | | | | Rectum | | X | X |
| Cecum | | X | X | Salivary glands (sublingual and submandibular) | | X | X |
| Colon | | X | X | Sciatic nerve | | X | X |
| Duodenum | | X | X | Seminal vesicles | | X | X |
| Epididymides | X | X | X | Skeletal muscle | | X | X |
| Esophagus | | X | X | Skin | | X | X |
| Eyes with Harderian glands | | X | X | Spinal cord (cervical, thoracic and lumbar) | | X | X |
| Femoral bone with articulation | | X | X | Spleen | X | X | X |
| Heart | X | X | X | Sternum with bone marrow | | X | X |
| Ileum (with Peyers patches) | | X | X | Stomach with forestomach | | X | X |
| Jejunum | | X | X | Testes | X | X | X |
| Kidneys | X | X | X | Thymus | X | X | X |
| Larynx | | X | X | Thyroids with parathyroids | X | X | X |
| Liver | X | X | X | Tongue | | X | X |
| Lungs with bronchi | | X | X | Trachea | | X | X |
| Lymph nodes (mandibular and mesenteric) | | X | X | Ureters | | X | X |
| Mammary gland area | | X | X | Urinary bladder | | X | X |
| Optic nerves | | X | X | Uterus (horns and cervix) | | X | X |
| Ovaries | X | X | X | Vagina | | X | X |

Minimal but dose-related lower glucose levels were noted in both sexes given 33 mg/kg/day (about -19%); a relationship to treatment with the test item cannot be excluded (Table 5). Higher incidences of kidney tubular basophilia and peritubular fibrosis in the treated females were of no toxicological significance since there was no such findings in the treated males and higher incidences were also seen in control males (Table 5). No other abnormalities were observed in the treated animals. Under the experimental conditions of the study, the No Observed Adverse Effect Level (NOAEL) was at 8 mg/kg/day.

Table 5. Summary of findings after a 4-week treatment with the test article

| | | Male | | | | Female | | | |
|---|---|---|---|---|---|---|---|---|---|
| S 9490-6 Dose (mg/kg/d) | | 0 | 0.8 | 8 | 33 | 0 | 0.8 | 8 | 33 |
| Serum glucose (mmol/L) | Mean | 7.13 | 6.99 | 6.25 | 5.86 * | 6.11 | 5.98 | 5.42 | 4.92 ** |
| | SD | 1.251 | 0.715 | 0.790 | 1.182 | 0.730 | 0.656 | 0.864 | 0.667 |
| KIDNEYS | : | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| - Tubular Basophilia | : | 6 | 6 | 4 | 1 | 1 | - | 5 | 4 |
| - Peritubular Fibrosis | : | 3 | 2 | 3 | 1 | - | - | 1 | 3 |

* p<0.05 vs control; ** p<0.01 vs control



Reference ID: 3008317

5. S 9490-6 (PERINDOPRIL ARGININE) SPIKED WITH [redacted]: DETECTION OF REVERSE MUTATION IN HISTIDINEREQUIRING *SALMONELLA TYPHIMURIUM* AND TRYPTOPHAN-REQUIRING *ESCHERICHIA COLI* (AMES TEST) (Study # 6790)

This GLP study (#6790) was conducted at [redacted] during Mar - Jun, 2011.

Perindopril arginine was spiked with [redacted] impurity [redacted] (S 9490-6, Batch No. L0037679). The mutagenic potential of the batch L0037679 of S 9490-6 was tested in AMES assay, at concentrations of 0, 50, 150, 500, 1500 and 5000 ug free acid/plate both in the presence and the absence of S9. Experimental details were similar to those on page 2.

No precipitate was noted. Cytotoxicity was observed only for the strain *TA1535* in the preincubation method where reductions in the numbers of revertant colonies were seen at 1500 µg free acid/plate without S9 or at 5000 µg free acid/plate with S9 (Table 7). Each positive control group showed an increase in the number of revertant colonies, demonstrating the sensitivity of the test system, and all the criteria for a valid test were met.

No significant, reproducible or concentration-related increase in the number of revertant colonies was seen with perindopril arginine containing [redacted], with or without metabolic activation, by preincubation or direct plating methods with any strain (Tables 6 & 7). Under the conditions of this study, perindopril arginine containing [redacted]% did not demonstrate mutagenic potential at tested concentrations ranging from 50 to 5000 µg free acid/plate, with or without metabolic activation.

Table 6. Mean Revertant Colony Counts in the range finding study

| | | Without S9 | | With S9 | |
|---|---|---|---|---|---|
| Test Article | Concentration (µg/plate) | *TA100* | *WP2 uvrA (pKM101)* | *TA100* | *WP2 uvrA (pKM101)* |
| Sterile Water for Injection | 0 | 97 | 134 | 155 | 135 |
| S 9490-6 spiked with [redacted] | 50 | 110 | 119 | 166 | 131 |
| | 150 | 113 | 127 | 162 | 147 |
| | 500 | 124 * | 132 | 171 | 152 |
| | 1500 | 131 ** | 125 | 210 ** | 145 |
| | 5000 | 121 * | 106 | 220 ** | 149 |
| sodium azide | 8 | 1252 ** | NA | 2-anthramine 2.5 µg/plate | |
| 4-nitroquinoline-N-oxyde | 0.5 | NA | 250 ** | 5647 ** | 957 ** |

* p<0.05 vs negative control; ** p<0.01 vs negative control



Reference ID: 3008317

Table 7. Mean revertant colony counts in the definitive experiments

| Test Article | | Concentration (µg/plate) | TA98 Exp.1 | TA98 Exp.2 | TA100 Exp.1 | TA100 Exp.2 | TA1535 Exp.1 | TA1535 Exp.2 | TA1537 Exp.1 | TA1537 Exp.2 | WP2 (pKM101) Exp.1 | WP2 (pKM101) Exp.2 | WP2 uvrA (pKM101) Exp.1 | WP2 uvrA (pKM101) Exp.2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sterile Water for Injection | Without S9 | 0 | 23 | 31 | 117 | 97 | 13 | 11 | 17 | 14 | 56 | 62 | 107 | 99 |
| S 9490-6 spiked with (b) (4) | | 50 | 23 | 28 | 116 | 110 | 13 | 14 | 15 | 10 | 53 | 60 | 100 | 113 |
| | | 150 | 20 | 27 | 119 | 113 | 10 | 8 | 8 | 10 | 57 | 62 | 104 | 104 |
| | | 500 | 25 | 28 | 134 | 124 * | 13 | 8 | 11 | 20 | 45 | 71 | 114 | 106 |
| | | 1500 | 33 | 29 | 133 | 131 ** | 5 | 8 | 8 | 12 | 53 | 67 | 106 | 122 ** |
| | | 5000 | 30 | 31 | 126 | 121 * | 4* | 8 | 9 | 13 | 47 | 69 | 107 | 108 |
| 4-nitroquinoline-N-oxyde | | 0.5 | 122 ** | 120 ** | NA | NA | NA | NA | NA | NA | NA | NA | 1010 ** | 613 ** |
| sodium azide | | 8 | NA | NA | 1280 ** | 1252 ** | 2113 ** | 2325 ** | NA | NA | NA | NA | NA | NA |
| 9-aminoacridine | | 100 | NA | NA | NA | NA | NA | NA | 1418 ** | 2609 ** | NA | NA | NA | NA |
| mitomycin C | | 0.1 | NA | NA | NA | NA | NA | NA | NA | NA | 278 ** | 251 ** | NA | NA |
| Sterile Water for Injection | With S9 | 0 | 37 | 41 | 170 | 190 | 11 | 15 | 12 | 14 | 84 | 75 | 161 | 135 |
| S 9490-6 spiked with (b) (4) | | 50 | 36 | 38 | 175 | | 9 | 13 | 11 | 13 | 75 | 67 | 159 | 131 |
| | | 150 | 38 | 35 | 162 | | 14 | 9 | 9 | 13 | 82 | 69 | 154 | 147 |
| | | 500 | 35 | 40 | 171 | | 8 | 12 | 14 | 13 | 77 | 70 | 162 | 152 |
| | | 1000 | | | | 202 | | | | | | | | |
| | | 1500 | 37 | 46 | 181 | 205 | 9 | 7 | 9 | 11 | 76 | 80 | 164 | 145 |
| | | 2500 | | | | 210 | | | | | | | | |
| | | 3500 | | | | 227 | | | | | | | | |
| | | 5000 | 43 | 44 | 228 ** | 220 | 4 | 9 | 7 | 7 | 80 | 67 | 157 | 149 |
| 2-anthramine | | 2.5 | 3771 ** | 4249 ** | 4987 ** | 5272 ** | 280 ** | 404 ** | 449 ** | 583 ** | NA | NA | 871 ** | 957 ** |
| | | 15 | | NA | NA | NA | NA | NA | NA | NA | 302 ** | 426 ** | NA | NA |

* p<0.05 vs negative control; ** p<0.01 vs negative control

6. S 9490-6 (PERINDOPRIL ARGININE) SPIKED WITH (b) (4): DETECTION OF MUTATIONS AT THE THYMIDINE KINASE (TK) LOCUS OF MOUSE LYMPHOMA L5178Y CELLS (Study # 6795)

This GLP study (# 6795) was conducted at (b) (4) during May – Jun, 2011.

Perindopril arginine was spiked with (b) (4) % of impurity (b) (4) (S 9490-6, Batch No. L0037679). S 9490-6 of the batch L0037679 ranging from 992.48 to 3685 ug/mL was tested in the absence and presence of S-9 for mutation potentials at the *tk* locus (5-trifluorothymidine resistance) in mouse lymphoma cells using a fluctuation protocol. A 3-hour treatment incubation period was used for two independent experiments in the presence of S-9. In the absence of S-9, Experiment 1 was performed using a 3-hour treatment incubation and Experiment 2 was performed using a 24-hour treatment incubation. The maximum concentration of the test article (3685 ug/mL, equivalent to 10 mM) was based on data from a previous study. Negative (solvent) and positive control



Reference ID: 3008317

treatments were included in each mutation experiment in the absence and presence of S-9.

As shown in Table 8, all doses were selected to determine viability and 5-trifluorothymidine (TFT) resistance 2 days after treatment. In the first experiment (3 hour treatment), the highest concentration tested (3685 ug/mL) yielded 97% and 37% adjusted relative total growth in the absence and presence of S-9, respectively. In the second experiment, the highest dose level tested (2180.47 or 3685 ug/mL) yielded 24% and 66% relative total growth in the absence and presence of S-9, respectively.

Mutant frequencies (MF) in negative controls fell within acceptable ranges, and clear increases in mutation were induced by the positive control chemicals mitomycin C (0.025 and 0.2 ug/ml, without S-9) and cyclophosphamide monohydrate (3.5 ug/ml, with S-9). No statistically significant increases in mutant frequency were observed following treatment with the test article at any dose level tested, in the absence or presence of S-9, in Experiment 1 or 2 (Table 8). Thus, under the conditions employed in this study, perindopril arginine containing (b) (4) was not mutagenic in this test system in the absence or presence of S-9.

Table 8. Summary of growth and mutant frequencies

| | Treatment Duration (Recovery) | Test Article | Concentration (µg free acid/ml) | Mean RTG (%) | | Mean Mutation Frequency /$10^{-5}$ cells | |
|---|---|---|---|---|---|---|---|
| | | | | -S-9 | +S-9 | -S-9 | +S-9 |
| Experiment 1 | 3h (48h) | Sterile Water for Injection | Vehicle control | 100 | 100 | 52 | 46 |
| | | S 9490-6 spiked with (b) (4) | 992.48 | 110 | 96 | 43 | 58 |
| | | | 1290.22 | 105 | 74 | 56 | 71 |
| | | | 1677.29 | 100 | 77 | 50 | 79 |
| | | | 2180.47 | 116 | 71 | 56 | 79 |
| | | | 2834.62 | 103 | 62 | 55 | 81 |
| | | | 3685.00 | 97 | 37 | 65 | 109 |
| | | MITOMYCIN C | 0.2 µg/ml | 21 | | 1129 | |
| | | CYCLOPHOSPHAMIDE MONOHYDRATE | 3.5 µg/ml | | 26 | | 582 |
| Experiment 2 | 24h (48h) | Sterile Water for Injection | Vehicle control | 100 | | 47 | |
| | | S 9490-6 spiked with (b) (4) | 992.48 | 67 | | 51 | |
| | | | 1290.22 | 51 | | 60 | |
| | | | 1677.29 | 44 | | 75 | |
| | | | 2180.47 | 24 | | 68 | |
| | | MITOMYCIN C | 0.025 µg/ml | 18 | | 900 | |
| | 3h (48h) | Sterile Water for Injection | Vehicle control | | 100 | | 49 |
| | | S 9490-6 spiked with (b) (4) | 1480.92 | | 96 | | 44 |
| | | | 1777.10 | | 97 | | 55 |
| | | | 2132.52 | | 95 | | 64 |
| | | | 2559.03 | | 69 | | 65 |
| | | | 3070.83 | | 53 | | 78 |
| | | | 3685.00 | | 66 | | 91 |
| | | CYCLOPHOSPHAMIDE MONOHYDRATE | 3.5 µg/ml | | 36 | | 666 |



Reference ID: 3008317

7. S 9490-6 (PERINDOPRIL ARGININE) SPIKED WITH Y 31: TOXICITY STUDY BY REPEATED ORAL ADMINISTRATION FOR 4 WEEKS IN WISTAR RATS (Study #6782)

This GLP study (# 6782, draft) was conduct at [redacted] (b) (4), initiated in March 2011.

Seven groups of 9 male and 9 female Wistar rats received vehicle (purified water), S9490-6 alone (PER, batch L0009275), or S9490-6 spiked with [redacted] (b) (4) (PER SPIKED, batch L0037679), daily by gavage at the dose-level of 0.8, 8 or 33 mg free acid /kg/day for 4 weeks. The animals were checked daily for mortality and clinical signs. Body weight and food consumption were recorded once a week. Ophthalmologic examinations were performed once during the acclimation phase and once in week 4. Hematology, blood biochemistry and urinalysis were performed at the end of the treatment period. On completion of the treatment period, the animals were killed and submitted to a full macroscopic post-mortem examination. Weights of adrenal glands, brain, epididymides, heart, kidneys, liver, lungs, ovaries, pituitary gland, prostate, salivary glands (mandibular/sublingual), seminal vesicles, spleen, testes, thymus, thyroid glands, and uterus were recorded. Tissues/organs listed in table 9 were preserved and histologically processed. All listed organs/tissues (Table 9) and macroscopic anomalies of all animals from the control and high dose groups, and all macroscopic anomalies, kidneys and glandular stomach of all animals from the low and intermediate dose groups were examined under microscope by both external and internal professionals.

Table 9. List of tissue/organ for histological processes and examination (from the submission)

| | | | |
|---|---|---|---|
| Adrenal glands | Jejunum | Peyer's patch | Testes |
| Aorta | Kidneys | Pituitary gland | Thymus |
| Brain | Larynx | Prostate | Thyroid glands |
| Caecum | Liver | Salivary glands (parotid | Tongue |
| Colon | Lungs | and mandibular | Trachea |
| Duodenum | Lymph nodes | /sublingual) | Ureters |
| Epididymides | (mandibular, mesenteric) | Sciatic nerve | Urinary bladder |
| Eyes | Mammary gland | Seminal vesicles | Uterus |
| Femur and stifle joint | Oesophagus | Skeletal muscle | Vagina |
| Forestomach | Optic nerves | Skin | |
| Glandular stomach | Ovaries | Spinal cord | |
| Harderian gland | Oviducts | Spleen | |
| Heart | Pancreas | Sternum | |
| Ileum | Parathyroid glands | | |

No death occurred and no relevant clinical signs were observed. No differences were noted in bodyweight, food intake, ophthalmoscopy, hematology, clinical chemistry and urinalysis between the PER and PER SPIKED-dosed groups.

Minimally lower absolute and relative heart weights were seen in all PER or PER SPIKED dosed groups (Table 10). These changes, similar between PER and PER SPIKED at each of the dose levels, were considered secondary to the pharmacological

effect of perindopril arginine and of no toxicological relevance. Weights of epididymides were slightly higher in the PER or PER SPIKED dosed groups (Table 10), which were not dose-related, similar between the PER or PER SPIKED dosed groups, and of no toxicological relevance.

Table 10. Differences in organ weights

| | Dose levels | | 0 mg/kg | 0.8 mg/kg PER | 8 mg/kg PER | 33 mg/kg PER | 0.8 mg/kg SPIKED | 8 mg/kg SPIKED | 33 mg/kg SPIKED |
|---|---|---|---|---|---|---|---|---|---|
| Males | HEART ..... | Mean (g) | 0.8850 | 0.8103 | 0.7898** | 0.8233** | 0.7908* | 0.8097** | 0.7486** |
| | | S.D. | 0.0836 | 0.0650 | 0.0918 | 0.0783 | 0.0795 | 0.1150 | 0.0809 |
| | | Mean (%) | 0.31 | 0.30 | 0.28** | 0.28** | 0.29* | 0.29** | 0.27** |
| | | S.D. | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.03 | 0.02 |
| | EPIDIDYMIDES | Mean (g) | 0.8030 | 0.8752 | 0.8867 | 0.9122 | 0.9220* | 0.8663* | 0.8822* |
| | | S.D. | 0.1073 | 0.1409 | 0.1228 | 0.1302 | 0.1275 | 0.1228 | 0.0580 |
| | | Mean (%) | 0.28 | 0.32 | 0.32 | 0.31 | 0.34* | 0.31* | 0.32* |
| | | S.D. | 0.02 | 0.04 | 0.04 | 0.03 | 0.04 | 0.04 | 0.02 |
| Females | HEART ..... | Mean (g) | 0.6242 | 0.6114 | 0.5522** | 0.5450** | 0.5899* | 0.5600** | 0.5591** |
| | | S.D. | 0.0219 | 0.0632 | 0.0558 | 0.0282 | 0.0588 | 0.0438 | 0.0412 |
| | | Mean (%) | 0.347 | 0.334 | 0.308** | 0.299** | 0.329* | 0.315** | 0.307** |
| | | S.D. | 0.015 | 0.037 | 0.028 | 0.012 | 0.019 | 0.023 | 0.010 |

At 33 mg free acid/kg PER or PER SPIKED, minimal hypertrophy of the juxtaglomerular apparatus was seen at microscopic examination in the kidneys of some rats and a minimally higher incidence of minimal to slight focal erosions of the glandular stomach were observed, occasionally correlating with macroscopic changes (depressed dark red foci). These kidney and glandular stomach histopathological changes were considered as being similar between PER and PER SPIKED-dosed groups (Table 11).

Under the conditions of this study, the NOAEL was 8 mg free acid/kg of PER and PER SPIKED. No toxicity was attributable to the presence of (b) (4)



Reference ID: 3008313

Table 11. Gross and histological findings

| | | | PER | | | PER SPIKED | | |
|---|---|---|---|---|---|---|---|---|
| Dose levels, mg/kg/day | | 0 | 0.8 | 8 | 33 | 0.8 | 8 | 33 |
| Number of Animals on Study : | | 9 | 9 | 9 | 9 | 9 | 9 | 9 |
| Number of Animals Completed: | | (9) | (9) | (9) | (9) | (9) | (9) | (9) |
| **Gross observations** | | | | | | | | |
| GLANDULAR STOMACH; | | | | | | | | |
| FOCUS, DARK RED | Male | 0 | 1 | 1 | 1 | 0 | 0 | 1 |
| | Female | 0 | 0 | 1 | 1 | 0 | 1 | 2 |
| KIDNEYS; | | | | | | | | |
| CYST, SEROUS | Male | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| FOCUS, BROWNISH | Male | 0 | 0 | 0 | 0 | 1 | 1 | 0 |
| DISCOLOURED, DARK BROWN / BLACK | Female | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| FOCUS, BROWNISH | Female | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| **Microscopic findings** | | | | | | | | |
| GLANDULAR STOMACH | | | | | | | | |
| Focal erosion - fundic mucosa | Male | 0 | 0 | 0 | 2 | 0 | 0 | 2 |
| Focal erosion - antral mucosa | Male | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| Submucosal inflammatory cell infiltration | Male | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| Submucosal oedema | Male | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Focal mineralisation | Male | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Focal erosion - fundic mucosa | Female | 0 | 0 | 1 | 2 | 0 | 0 | 1 |
| Focal erosion - antral mucosa | Female | 0 | 1 | 0 | 2 | 0 | 1 | 0 |
| Submucosal inflammatory cell infiltration | Female | 0 | 0 | 0 | 2 | 0 | 0 | 1 |
| Submucosal oedema | Female | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Focal mucosal hyperplasia | Female | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Dilated fundic glands | Female | 1 | 2 | 1 | 0 | 2 | 2 | 2 |
| KIDNEYS | | | | | | | | |
| Hypertrophy of the juxtaglomerular apparatus | Male | 0 | 0 | 0 | 4 | 0 | 0 | 5 |
| Intraluminal mineral deposits | Male | 2 | 2 | 5 | 6 | 2 | 5 | 3 |
| Focal cortical inflammatory cell infiltration | Male | 0 | 0 | 2 | 4 | 1 | 3 | 2 |
| Hypertrophy of the juxtaglomerular apparatus | Female | 0 | 0 | 0 | 1 | 0 | 0 | 4 |
| Intraluminal mineral deposits | Female | 7 | 7 | 6 | 7 | 6 | 5 | 9 |

## SUMMARY AND EVALUATION

Perindopril arginine (S 9490-6) spiked with impurities [redacted] (b) (4) (batch FI 150) or spiked with impurity [redacted] (b) (4) (batch L0037679) did not show mutation potentials in the AMES test and the mouse lymphoma thymidine kinase gene mutation assay. In the 4-week oral toxicity study in rats, treatment with perindopril arginine containing impurities [redacted] (b) (4) was associated with minimal but dose-related lower glucose levels in both sexes (about -19% at 33 mg/kg/day, $p<0.05$ vs control). Treatment of rats with oral perindopril arginine or perindopril arginine containing [redacted] (b) (4) for 4 weeks resulted in higher incidences of

minimal hypertrophy of kidney juxtaglomerular apparatus and minimal to slight focal erosion of glandular stomach at the dose 33 mg/kg/day. The changes were similar between perindopril arginine alone and perindopril arginine containing (b) (4). NOAELs of perindopril arginine alone, perindopril arginine containing (b) (4) and perindopril arginine containing (b) (4) in these repeat oral dose studies in rats were 8 mg/kg/day.

The studies were well designed and conducted. Perindopril arginine containing impurities (b) (4) or containing impurity (b) (4) did not demonstrate mutation potential, and showed toxicity profiles similar to perindopril arginine alone in rats. No new issue of safety concern was aroused from the results of these preclinical studies.

**RECOMMENDATIONS**

None at this time.



Reference ID: 3008317

---

**This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.**

---

/s/

---

BAICHUN YANG
01/06/2012

THOMAS PAPOIAN
01/06/2012
I concur.

---

**This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.**

---

/s/

---

BAICHUN YANG
08/04/2014

THOMAS PAPOIAN
08/04/2014
Concur.

# PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA or Supplement

**NDANumber: 205003** **Applicant: Symplmed Pharmaceuticals, LLC** **Stamp Date: 3/21/2014**

**Drug Name: Prestalia (Perindopril arginine/ amlodipine besylate) tablets** **NDA Type: 505 (b)(2)**

On **initial** overview of the NDA application for filing:

| | Content Parameter | Yes | No | Comment |
|---|---|---|---|---|
| 1 | Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin? | Yes | | |
| 2 | Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin? | Yes | | |
| 3 | Is the pharmacology/toxicology section legible so that substantive review can begin? | Yes | | |
| 4 | Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)? | Yes | | |
| 5 | If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA). | | | N/A |
| 6 | Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route? | Yes | | |
| 7 | Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations? | Yes | | |



Reference ID: 3494085

# PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA or Supplement

| | Content Parameter | Yes | No | Comment |
|---|---|---|---|---|
| 8 | Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions? | Yes | | |
| 9 | Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m$^2$ or comparative serum/plasma levels) and in accordance with 201.57? | Yes | | No dose details under section 8 – to be discussed. |
| 10 | Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.) | Yes | | |
| 11 | Has the applicant addressed any abuse potential issues in the submission? | | | N/A |
| 12 | If this NDA is to support a Rx to OTC switch, have all relevant studies been submitted? | | | N/A |

**IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? Yes**

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Baichun Yang 4/23/2014
Reviewing Pharmacologist (DCRP) Date

Thomas Papoian 4/23/2014
Team Leader/Supervisor (DCRP) Date



Reference ID: 3494085

---

**This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.**

---

/s/

---

BAICHUN YANG
04/23/2014

THOMAS PAPOIAN
04/23/2014
Concur.