

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

21-985

PHARMACOLOGY REVIEW(S)

MEMORANDUM

Feb. 28, 2007

TO: File

FROM: Kenneth L. Hastings, Dr.P.H., D.A.B.T.

SUBJECT: NDA 21-985

I concur with the primary pharmacology/toxicology reviewer, Dr. G. Jagadeesh and the pharmacology/toxicology supervisor, Dr. Charles Resnick, that the marketing application for aliskiren hemifumarate (Rasilez[®] tablets) may be approved based on review of submitted nonclinical data. The product label should be amended as recommended by Dr. Jagadeesh in order to more accurately describe effects observed in nonclinical studies and the appropriate human equivalent doses at which these effects were observed.

Kenneth L. Hastings, Dr.P.H., D.A.B.T.

Associate Director
Office of New Drugs

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this page is the manifestation of the electronic signature.**

/s/

Kenneth Hastings
2/28/2007 04:04:22 PM
PHARMACOLOGIST

SUPPLEMENTAL PHARMACOLOGY/TOXICOLOGY REVIEW

NDA Number: 21,985

Dates of Submission: 2-10-06 to 11-7-06

Sponsor: Novartis Pharmaceuticals Corporation

Manufacturer of Drug Substance: Novartis Pharmaceuticals Corporation

Reviewer: G. Jagadeesh, Ph.D.

Division: Division of Cardiovascular and Renal products

Review Completion Date: February 12, 2007

Drug Product: Tekturna[®] Tablets

Drug Substance:

Generic name: Aliskiren hemifumarate

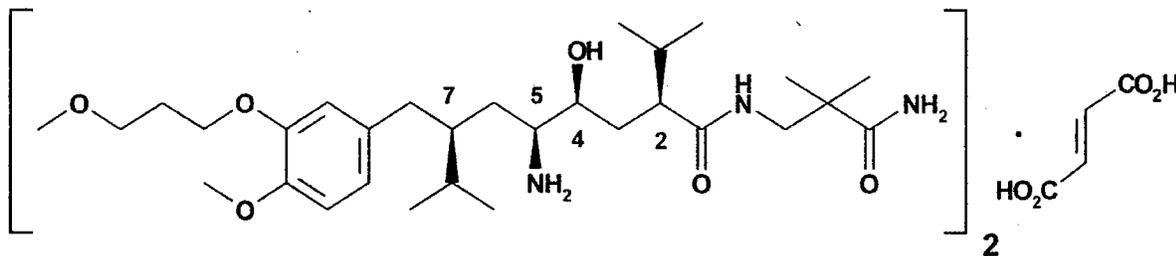
Code name: SPP100 (base), SPP100B (hemifumarate), CGP60536B

Chemical name: (2S,4S,5S,7S)-N-(3-amino-2,2-dimethyl-3-oxopropyl)-2,7-diisopropyl-4-hydroxy-5-amino-8-[4-methoxy-3-(3-methoxypropoxy)phenyl]octanamide hemifumarate.

Chemistry: Aliskiren is a single diastereoisomer having 4 chiral centers, all S-configured. The active substance is the hemi-fumarate salt (2:1 ratio) of the corresponding amine and is white to slightly yellowish powder. It is hygroscopic and very soluble in aqueous media from pH 1 to 7.6.

CAS registry number: 173334-58-2

Molecular formula/molecular weight: C₃₀H₅₃N₃O₆ · 0.5 C₄H₄O₄
551.8 (free base), 609.8 (hemifumarate)



Related INDs/NDAs/DMFs: Novartis' IND 62,976 for aliskiren hemifumarate for the treatment of hypertension.

Drug Class: Renin inhibitor

Intended Clinical Population: Hypertensive subjects

Clinical Formulation: Aliskiren hemifumarate immediate release film-coated tablets will be supplied in two dosage strengths (150 mg and 300 mg of aliskiren) with the following inactive ingredients: colloidal silicon dioxide, crospovidone, hypromellose, iron oxide colorants, magnesium stearate, microcrystalline cellulose, PEG, povidone, talc and titanium dioxide.

Route of Administration: Oral

Proposed Dosage Regimen: One tablet daily.

Disclaimer: Unless indicated otherwise, tables and graphs (with or without editorial corrections by the reviewer) are taken from the sponsor's submission.

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INTRODUCTION

The major findings from the studies reviewed in the original NDA were the presence of microscopic gastrointestinal changes in both the 52 week and 104 week segments of the dietary carcinogenicity study in rats. Treatment with aliskiren hemifumarate resulted in dose-related increases in the incidence and severity of mucosal epithelial hyperplasia in the cecum and colon relative to control in both sexes at doses of 750 or more mg aliskiren/kg/day. Furthermore, after 104 weeks of treatment with aliskiren hemifumarate, a colonic adenoma and a cecal adenocarcinoma were observed ($p > 0.05$) in males receiving 1500 mg/kg/day. In contrast, marmosets and humans developed diarrhea (with no associated histopathological changes in the marmosets) with aliskiren administration. Diarrhea rates in humans increased with increases in dose. The sponsor considers these findings to be associated with the known local irritation potential of aliskiren and may be species (rat) specific. The changes observed in the gastrointestinal tract were further assessed by conducting mechanistic toxicity studies of varying duration in rats. Reports of these studies were later submitted as amendments to the original NDA. These studies were to explore the susceptibility of rat GI tract tissues to effects of aliskiren on epithelial integrity (transepithelial tissue conductance); onset, severity and reversibility (adaptation) of mucosal hyperplasia. The mechanistic aspects of hyperplasia were further assessed by immunohistochemistry and image analyses and gene expression profiling of GI tract tissues. The results of these studies were further intended to bridge preclinical exposure margins to humans.

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1. Effect of Aliskiren on Transepithelial Tissue Conductance and Chloride Flux in Isolated Epithelial Preparations of Rat and Human Colon

Key Study Findings: Exposure of rat colon mucosa to high aliskiren concentrations (10 mM mucosal/ 1 mM serosal) impaired epithelial integrity and compromised transepithelial chloride/water secretion. In contrast, a similar effect was not noted in human colonic mucosa.

Introduction: The results of a 104 week carcinogenicity study in rats revealed inflammatory and proliferative changes in the gastrointestinal tract following aliskiren administration. The present investigations were conducted (*in vitro*) to examine any local or direct effects of aliskiren on intestinal mucosa. Intestinal epithelial integrity (conductance) and chloride/water secretion (chloride flux) were determined in isolated mucosa/submucosa preparations from both rat and human colon.

Study No.: Report #RD-2006-00536

Conducting Laboratory and Location: Not given

Dates of Study: January to April 2006

GLP Compliance: No

Drug, Batch #: Aliskiren hemifumarate; batch #0444029

Methods

Rats: Male Wistar rats (body weight 260-290 gm) were decapitated and colon (region not specified) was removed.

Human: Parts of descending colon were obtained from 3 male and 3 female patients (aged 37 to 75 years) undergoing bowel resection for carcinoma or diverticulitis.

Immediately after excision, histologically normal specimens were transported to the laboratory in oxygenated Krebs-Henseleit solution, pH 7.4.

General Procedure: Segments of colon were opened along the longitudinal axis, rinsed free of luminal contents and then pinned flat on a Sylgard covered petri dish. Mucosa/submucosal sheets with intact submucosal plexus were removed (after separating from longitudinal and circular muscle layers) by dissection and placed as a flat sheet between two halves of an Ussing diffusion chamber. The mucosal and serosal sides of the tissue were bathed separately with Krebs-Henseleit solution (at 37°C, aerated with 95% oxygen and 5% carbon dioxide) delivered to the chamber from reservoirs mounted above the chamber. Each half of the chamber was equipped with one Ag/AgCl electrode for sensing transepithelial potential difference (PD) and a current passing Ag electrode.

The integrity of epithelial cells was studied by two permeability parameters: (1) tissue conductance (G) and (2) chloride flux. Tissue conductance (as mS/cm^2 tissue surface area) was determined by applying Ohm's law every 10 min (apply a voltage and measure the resulting change in current). This is because epithelial tissues transport ions and generate a transepithelial voltage (or active transport potential). The second parameter, chloride flux, was measured continuously as short circuit current (I_{sc}). [The short circuit current is the charge flow per time when the tissue is short circuited, clamping PD to zero. Note that the flow of ions

per time is a more accurate reflection of the absorptive or secretory capacity of the tissue.]

The preparations were equilibrated for 30 min with intermediate wash every 15 min. Baseline values of transepithelial tissue conductance (G) and chloride flux (I_{sc}) were determined. Cumulative concentration response curves were elicited with aliskiren, 0.1 μ M to 10 mM, at 10 min intervals. Increasing concentrations of aliskiren were added to the mucosal side the chamber (maximum 10 mM) relative to the serosal side of the chamber (maximum 1 mM). The mucosal and serosal bath concentrations of aliskiren were in the range of concentrations measured in the intestinal content and in the mucosa of rats from toxicology studies (study reference not given, however, it refers to section 2, Table 2.5, this review).

At the end of each experiment, tissue preparation was challenged with carbachol (10 μ M) added to the serosal side of the chamber to stimulate maximum responses to chloride flux. Cytochalasin B (a cytoskeletal modulator) was used as a reference compound which is known to increase the permeability of intestinal epithelial tight junctions by disrupting perijunctional actin filaments. Control studies were performed with vehicle (saline). Both aliskiren and carbachol were dissolved in saline.

Results

Rat Colon: Following cumulative applications of aliskiren, only the highest concentration (10 mM mucosal/ 1 mM serosal) significantly increased transepithelial tissue conductance relative to vehicle by 11.1 ± 2.5 mS/cm² (Fig. 1.1). Lower concentrations of aliskiren (≤ 1 mM mucosal/ 0.1 mM serosal) had no effect on conductance. In control experiments run in parallel, cytochalasin B (10 μ M) significantly increased tissue conductance (11.8 ± 4.6 mS/cm² relative to vehicle). This effect was similar to aliskiren, thus demonstrating impairment in epithelial integrity.

Aliskiren had no significant effects on transepithelial chloride flux up to a maximum concentration 10 mM (mucosal)/ 1 mM (serosal) though a consistent trend for transient increase was noted relative to control preparation at high concentrations (Fig. 1.2). On the other hand, aliskiren at the maximum concentration, almost abolished carbachol-stimulated transepithelial chloride flux (18.7 ± 11.6 μ A/cm² and 312.2 ± 28.8 μ A/cm² in the presence and absence of aliskiren, respectively, $p < 0.05$). Cytochalasin B had no significant effects on either native or carbachol-stimulated chloride flux (Fig. 1.2, right panel, histogram).

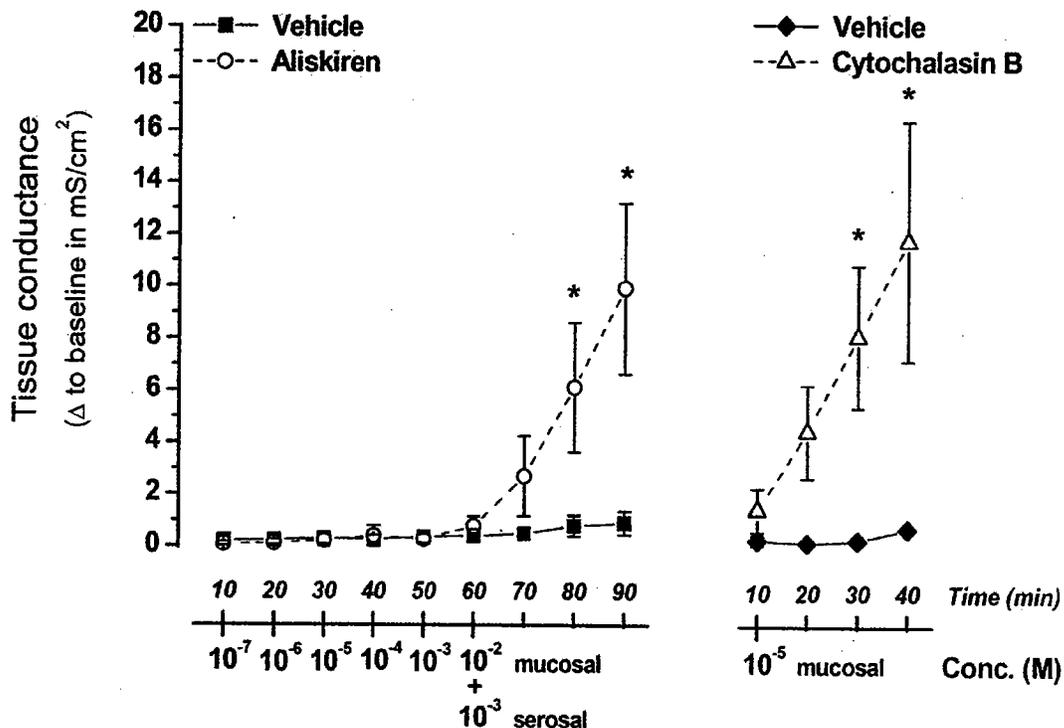


Fig.1.1.: Transepithelial tissue conductance following cumulative application of aliskiren (left side) and a single application of cytochalasin B (right side) in the rat colon. Data are shown as mean ± SEM; preparations from n = 4 to 5 animals; * significant increase vs. vehicle (P < 0.05, ANOVA, post-hoc Dunnett's test).

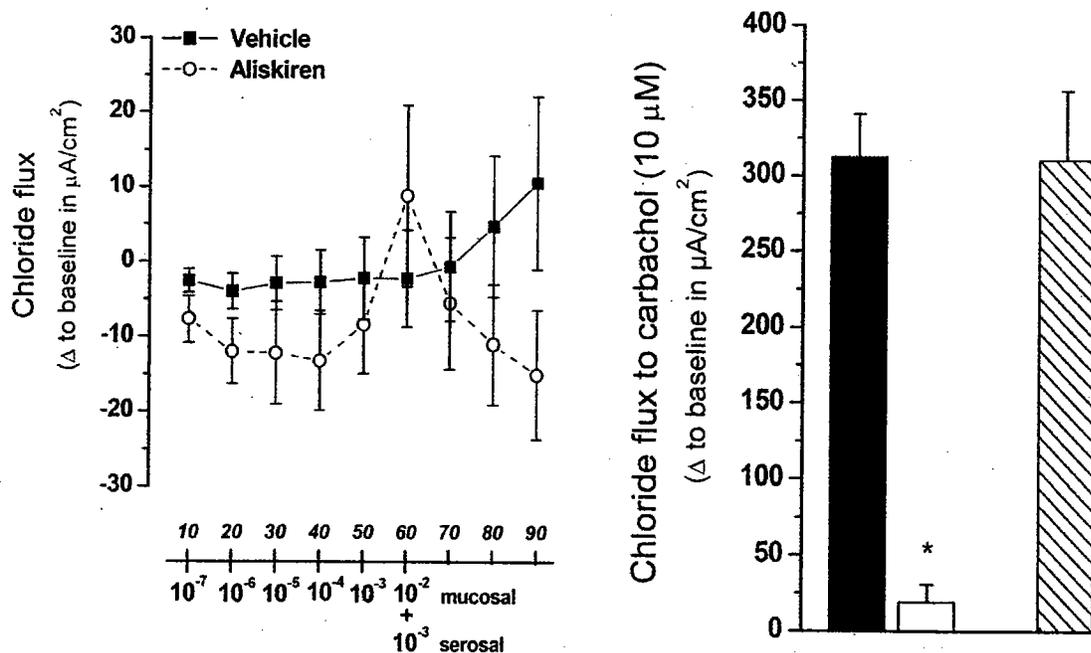


Fig.1.2.: Transepithelial chloride flux following cumulative application of aliskiren (left side). The right side figure shows maximum chloride flux to carbachol in the presence (blank column) and absence (dark column) of aliskiren or presence of cytochalasin B (hatched column) in the rat colon. Data are shown as mean ± SEM; preparations from n = 4 to 5 animals; * significant inhibition vs. vehicle (P < 0.05, ANOVA, post-hoc Dunnett's test).

Human Colon: Following cumulative application, aliskiren had no significant effect on transepithelial tissue conductance in human colon. The increase in transepithelial tissue conductance was similar to that of vehicle (Fig. 1.3 left panel). In contrast, cytochalasin B (10 μ M) significantly increased tissue conductance relative to vehicle (Fig. 1.3 right panel), an effect similar to that noted in rat colon preparations (Fig. 1.1, right panel). Additionally, aliskiren (up to a maximum concentration of 10 mM mucosal/ 1 mM serosal) had no significant effects on transepithelial chloride flux either *per se* (Fig. 1.4 left panel) or in the presence of carbachol (Fig. 1.4, right panel). Cytochalasin B had no significant effects on either native or carbachol-stimulated chloride flux (Fig. 1.4, right panel, histogram).

In summary, in the rat colon, aliskiren at very high concentrations increased transepithelial tissue conductance and impaired transepithelial chloride flux to cholinergic stimulation. Such effects were not noted in human colon preparations.

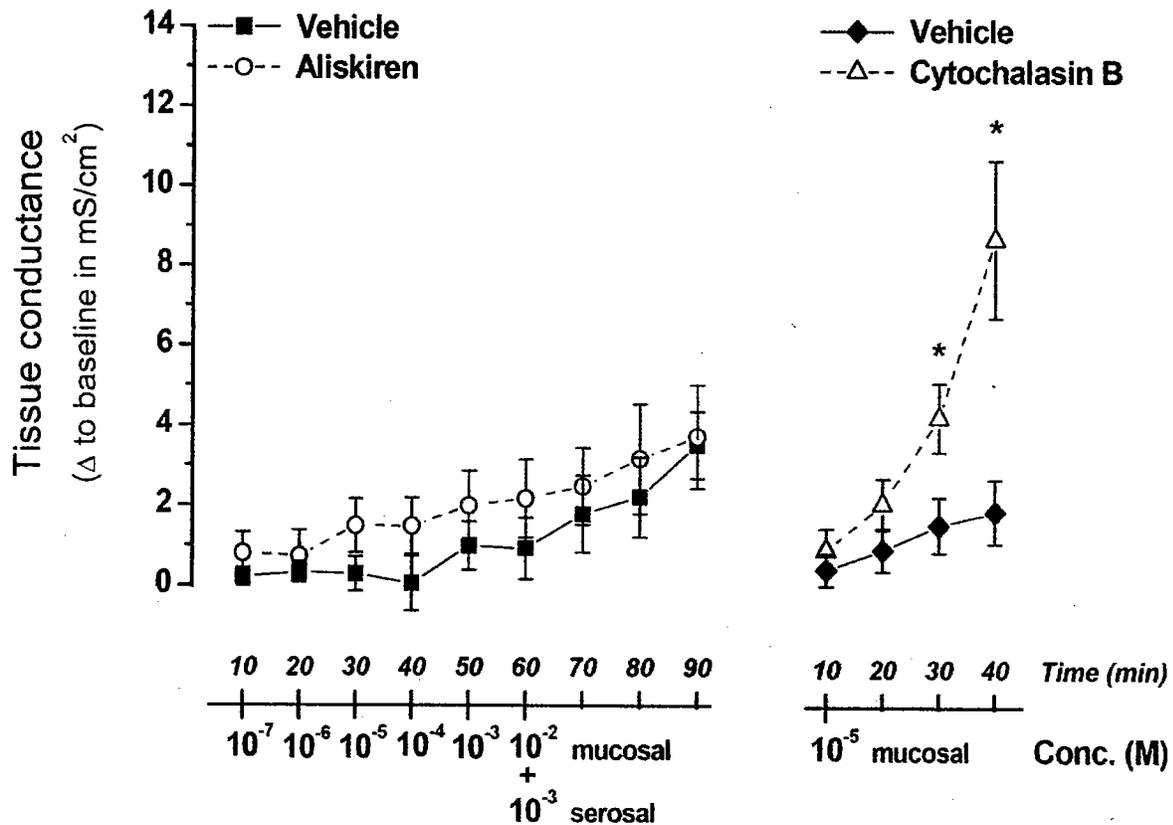


Fig.1.3.: Transepithelial tissue conductance following cumulative application of aliskiren (left side) and a single application of cytochalasin B (right side) in human colon. Data are shown as mean \pm SEM; preparations from 6 patients; * significant increase vs. vehicle ($P < 0.05$, ANOVA, post-hoc Dunnett's test).

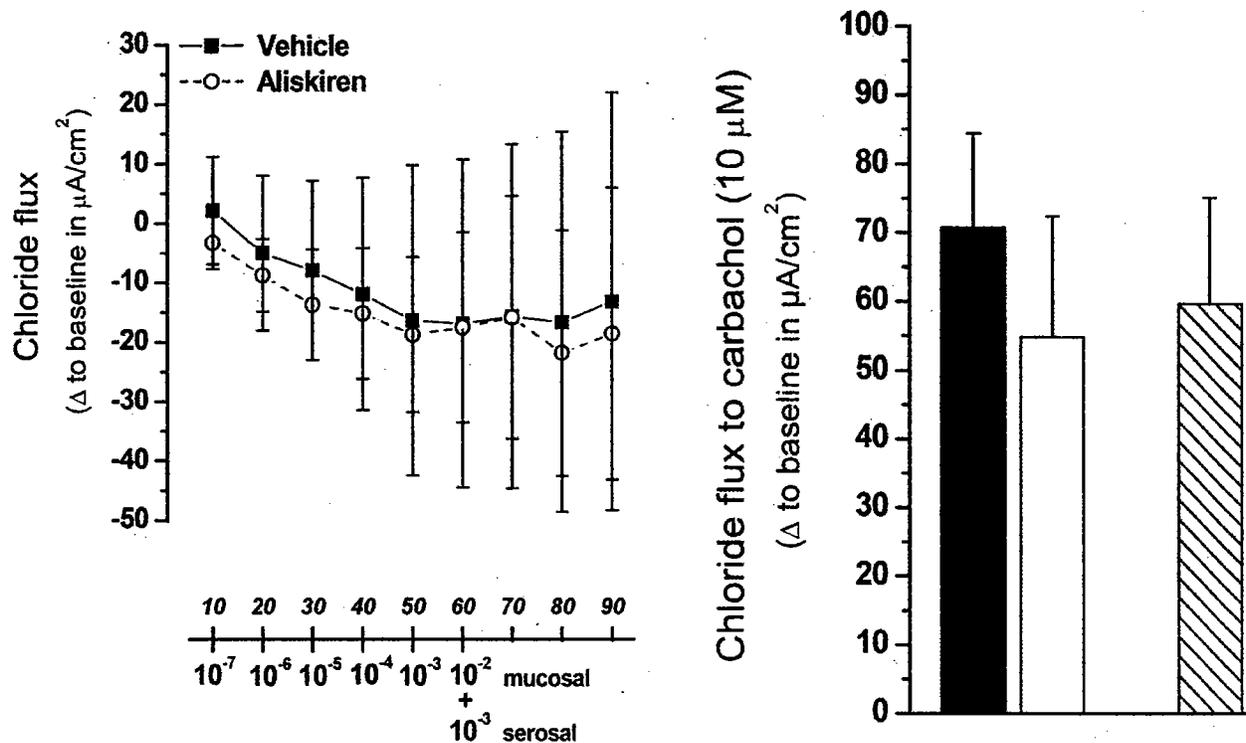


Fig.1.4.: Transepithelial chloride flux following cumulative application of aliskiren (left side) to human colon. The right side figure shows maximum chloride flux to carbachol in the absence (dark column) or in the presence of aliskiren (blank column) or cytochalasin B (10 μM mucosal, hatched column) in human colon. Data are shown as mean ± SEM; preparations from 6 patients.

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2. 1-Week Oral (feed admixture) Mechanistic Study in Male Rats

Key Study Findings: Aliskiren administered as feed admixtures to rats for up to one week resulted in histopathological changes in the gastrointestinal mucosa in general and cecum and colon in particular at doses 750 or more mg/kg/day. Standard microscopic examination revealed mucosal hyperplasia in the cecum after 3 days at doses of 750 or more mg/kg/day and colon at a dose of 1500 mg/kg/day. Immunostaining with BrdU confirmed mucosal effects at 1500 mg/kg/day in both the cecum and colon and also demonstrated an increase in cell proliferation in the cecum following one day of treatment. Analysis of intestinal content and mucosa showed no significant differences in tissue exposure between locations.

Introduction: The aim of the study was to investigate the potential effects of aliskiren on the gastrointestinal tract following administration to male rats for a week or less. The study included image analysis of GI tract tissues, toxicokinetics and GI tract tissue concentration analyses.

Study No.: 0570340

Conducting Laboratory and Location: In-life study at Safety Profiling and Assessment, Novartis Pharmaceuticals Corporation, East Hanover, New Jersey; Immunohistochemistry and image analysis at Safety Profiling and Assessment, Toxicology, Novartis Pharma AG, Basel, Switzerland; gene expression profiling of GI tract tissues at Department of Biomarker Development, Novartis Pharma AG, Basel, Switzerland.

Dates of Study: Dosing was initiated on November 29, 2005 and terminal necropsies were conducted from November 30 to December 7, 2005

GLP Compliance: Yes (only in-life study)

QA Report: yes (X) only in-life part of the study including standard histopathology

Drug, Batch #, and % Purity: Aliskiren hemifumarate; batch #0544033; drug content: —

Formulation: Aliskiren hemifumarate was admixed with the diet. The formulation was analyzed for test article concentration during the week of study to determine stability.

Animals

Species/Strain: IGS Wistar Hannover rats. — WI(Glx/BRL/Han)IGS BR from —

Number/Sex/Group: 55 males

Age: 8 weeks at initiation of dosing

Weight: males: 151.7 to 263.1 g

Husbandry: Animals were housed single in stainless steel cages. Food and water *ad libitum* throughout the study period.

Dosing

Groups of 55 male rats were given aliskiren hemifumarate admixed with the diet at target doses of 276.3, 828.8 or 1657.5 mg/kg/day (250, 750 or 1500 mg/kg/day of base, respectively) for 1, 3 or 7 days. The control group received untreated feed. The doses for this study were the same doses utilized in the 104 week dietary carcinogenicity study in rats. In that study, changes in the GI tract were noted following 52 weeks of dietary administration at doses of 750 or more mg aliskiren/kg/day.

Observations / Measurements

Mortality: Twice daily on week days and once daily on all other days

Clinical Signs: All animals were observed at least once a day.

Body Weights: Recorded once before treatment, on the first day of dosing, during dosing and on the day of scheduled necropsy.

Food Consumption: Recorded for each animal on study day 1 and on the day of necropsy (day 2, 4 or 8).

Necropsy: Animals were not fasted before necropsy. Necropsies were performed on study days 2, 4 and 8 (see Table 2.1 for details). For each day of sacrifice, the first 10 animals in each group were bromodeoxyuridine (BrdU) designated animals for immunohistochemistry (IHC) and image analyses in addition to standard hematoxylin and eosin stained (H & E) processing of tissues. All of these animals received 100 mg BrdU/kg intraperitoneally 60 min prior to sacrifice. The duodenum, jejunum, colon and cecum were separated and flushed with saline to remove contents and then immersed in formalin. The next 5 males in each group were used to collect samples of blood, liver, duodenum, ileum, jejunum, cecum, colon and stomach (fundic) for possible investigational gene expression analysis in addition to collection of designated tissues for H & E processing. For toxicokinetics designated animals, 5 males in each group were used on study days 2 and 8 to collect samples of ileum, jejunum, cecum, colon (approximately 200 mg each if possible) and kidney for aliskiren concentration analysis. Blood was collected from the abdominal aorta prior to exsanguination. There were no tissues saved in formalin nor slides made for the toxicokinetics designated animals. Complete necropsies were performed with macroscopic findings noted for all animals. Protocol -specified tissues (Table 2.2) were collected from all animals with the exception of TK designated animals.

TABLE 2.1
STUDY ANIMAL DESIGNATIONS FOR NECROPSY PROCESSING

Day of Necropsy	Animal #/gp	Designation/Processing
Day 2	01-10	BrdU/IHC and standard H & E processing per tissue list
	11-15	Genomics* and standard H & E processing per tissue list
	16-20	TK blood sampling, tissues collected for aliskiren concentration analysis
Day 4	21-30	BrdU/IHC and standard H & E processing per tissue list
	31-35	Genomics* and standard H & E processing per tissue list
Day 8	36-45	BrdU/IHC and standard H & E processing per tissue list
	46-50	Genomics* and standard H & E processing per tissue list
	51-55	TK blood sampling, tissues collected for aliskiren concentration analysis

*: Results of gene expression changes are not included in the current submission.

Histopathology: Microscopic examination was performed on all H & E processed tissue sections (Table 2.2) from all animals from all dose groups (Table 2.1).

Immunohistochemistry (IHC) and image analysis of the gastrointestinal tract tissues were evaluated and quantified for control and high dose group animals.

TABLE 2.2
TISSUE LIST: FOR COLLECTION, PROCESSING (P) AND/OR GENOMICS (G)

G		blood
G	P	Cecum
G	P	colon
G	P	Duodenum
G	P	Ileum
G	P	Jejunum
G	P	Stomach
G		Liver
		animal identification

Results

Analysis of Formulations: Feed admixture was stable at room temperature for 21 days and formulation homogeneity was confirmed. The achieved concentration of test substance in the dietary formulation was — of the targeted concentration.

Mortality: None

Clinical signs: There were no clinical observations following treatment with the test substance.

Body Weights: Dose-dependent reductions in body weight gain relative to control were noted at doses of 750 or more mg/kg/day on all days of measurement. However, on day 8 statistical significance was noted only for the high dose group (9% gain compared to 21% gain for control). Reduction in body weight gain, according to the sponsor, was in part related to the palatability of the feed admixture and resulted in low food consumption for 4 or more days.

Food Consumption: Statistically significant decreases in food consumption relative to concurrent control were seen at doses of 750 or more mg/kg/day on study 8 and at 1500 mg/kg/day on days 4 and 8.

Pathology: There were no treatment-related macroscopic observations. Treatment for one day was not associated with any treatment-related microscopic changes to the GI tract at any dose level relative to control. Rats necropsied after 3 days demonstrated dose-related increases in the incidence and severity of mucosal hyperplasia in the cecum and colon at doses of 750 or more mg/kg/day with increased severity of the hyperplasia in the colon at 1500 mg/kg/day. Treatment for 7 days further escalated the incidence and severity of hyperplasia (Table 2.3). Mucosal hyperplasia of the ileum was noted only at a dose of 1500 mg/kg/day after 7 days of treatment. Hyperplasia was characterized by increased crypt basophilia, numbers of mitotic figures and mucosal thickness.

TABLE 2.3
NOTEWORTHY MICROSCOPIC INTESTINAL CHANGES

Dose (mg/kg/day)	0	250	750	1500
Treated 1 day and necropsied on day 2				
Cecum: mucosal hyperplasia	2/15	0/15	1/15	3/15
Mean severity	1.0		1.0	1.0
Cecum: inflammation	1/15	0/15	0/15	0/15
Treated 3 days and necropsied on day 4				
Cecum: mucosal hyperplasia	3/15	0/15	10/15	15/15
Mean severity	1.0		1.0	1.4
Cecum: inflammation	0/15	0/15	1/15	6/15
Colon: mucosal hyperplasia	0/15	0/15	1/14	2/15
Mean severity			1.0	1.0
Treated 7 days and necropsied on day 8				
Cecum: mucosal hyperplasia	3/15	3/15	15/15	15/15
Mean severity	1.0	1.0	1.0	1.5
Cecum: inflammation	2/15	1/15	0/15	5/15
Colon: mucosal hyperplasia	0/15	0/15	1/15	10/15
Mean severity			1.0	1.3
Ileum: mucosal hyperplasia	0/15	0/15	0/15	3/15
Mean severity				1.0

Immunohistochemistry and Image Analysis: The histopathological analysis of the cecum and colon sections immunostained with anti-BrdU antibody conducted on control and high dose animals showed that cell proliferation was confined to the mucosa. A statistically significant increase in mucosal cell proliferation relative to control was noted at 1500 mg aliskiren/kg/day in cecum after 1, 3 and 7 days of treatment and in colon after 3 and 7 days of treatment.

Toxicokinetics: Plasma: All animals in the treated group were exposed to the drug. The plasma concentrations of aliskiren increased in a dose-dependent manner on day 2 and day 8 of treatment. Dose normalized concentrations were similar between dose groups on day 2 (0.0535 to 0.0672 ng/ml). However, a trend to over proportionality was observed on day 8 (0.04 to 0.265 ng/ml). Based on mean concentrations, exposure of the low dose group was similar on days 2 and 8 but exposure was higher on day 8 compared to day 2 for the mid and high dose groups (Table 2.4)

TABLE 2.4
MEAN (± SD) ALISKIREN CONCENTRATIONS IN RAT PLASMA (N=5)

Necropsy on	Dose (mg/kg/day)		
	250	750	1500
day 2	13.4 ± 6.3	50.4 ± 21.6	80.2 ± 30.7
day 8	10.1 ± 1.6	128 ± 29.4	398 ± 103

Concentrations expressed as ng/ml

Matrices: Quantifiable concentrations of aliskiren were noted in all matrices from all animals from the treated groups. No significant difference between the concentrations measured in the content from the different part of the intestine was evident. Part of the reason was due to large variations between individual sample concentrations. However,

mean concentrations in intestinal mucosa per dose group measured on days 2 and 8 showed a trend to slightly higher (not statistically significant) concentrations in the mucosa from the colon for the mid and high dose groups (Table 2.5). Drug concentrations in intestinal content and intestinal mucosa depended on dose and the drug contact time (day 2 or day 8). Mean concentrations in kidney and ratios between kidney and plasma concentrations were much lower than that noted for GI tract.

TABLE 2.5
MEAN (\pm SD) GASTROINTESTINAL AND KIDNEY ALISKIREN CONCENTRATIONS IN MALE RAT (N=5) NECROPSIED ON DAY 2 OR DAY 8

Matrix	Dose (mg/kg/day)					
	250		750		1500	
	day 2	day 8	day 2	day 8	day 2	day 8
<u>Content</u>						
Ileum	315 \pm 83.5	783 \pm 397	879 \pm 261	3270 \pm 228	1140 \pm 495	4840 \pm 365*
Jejunum	640 \pm 155	672 \pm 167	3180 \pm 534	2800 \pm 1170	3220 \pm 1530	4550 \pm 1230
Cecum	1040 \pm 508	457 \pm 322	3700 \pm 1230	2920 \pm 891	2820 \pm 1610	1960 \pm 922
Colon	442 \pm 109	502 \pm 190	2560 \pm 521	1840 \pm 827	4790 \pm 1240	3440 \pm 1190
<u>Mucosa</u>						
Ileum	56.2 \pm 15.3	99.3 \pm 27.4	122 \pm 85.2	343 \pm 285	241 \pm 168	288 \pm 72.1
Jejunum	61.0 \pm 30.7	70.5 \pm 33.3	222 \pm 149	266 \pm 155	299 \pm 120	447 \pm 445
Cecum	1010 \pm 525	135 \pm 57.5	515 \pm 163	336 \pm 156	365 \pm 179	337 \pm 147
Colon	159 \pm 89.7	132 \pm 64.7	654 \pm 343	513 \pm 226	844 \pm 316	697 \pm 331
Kidney	2.64 \pm 0.855	3.87 \pm 6.51	3.86 \pm 1.7	14.0 \pm 15.3	2.12 \pm 0.702	13.2 \pm 3.46

Values expressed as μ g/g tissue weight, *n = 4

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3. 4-Week Oral (feed admixture) Mechanistic Study in Male Rats

Key Study Findings: Aliskiren hemifumarate administered as feed admixtures to rats for up to four weeks resulted in histopathological changes in cecum and colon at doses of 250 or more mg aliskiren/kg/day. Standard microscopic examination revealed dose-related increases in the incidence and severity of mucosal hyperplasia in the cecum relative to control at 250 or more mg/kg/day plus a similar lesion in the colon at 1500 mg/kg/day. Immunostaining with BrdU and image analysis confirmed mucosal hyperplasia in the cecum and colon at 1500 mg/kg/day. Gene expression changes (up-regulation) reflected an inflammatory process as they were major in the cecum at all dose levels and major in the colon at the high dose. Furthermore, the gene expression profiling confirmed the presence of a stress or toxic injury to the mucosal epithelium, potentially leading to an injury/repair/proliferation cycle. The study failed to establish a NOAEL.

Introduction: The aim of the study was to investigate the potential effects of aliskiren on the gastrointestinal tract following administration to male rats for 4 weeks. The study included immunohistochemistry and image analyses, gene expression profiling of GI tract tissues, and toxicokinetics analyses of plasma and fecal samples.

Study No.: 0570277 (in-life part of the study including pathology); Report #BMD R0570277 (gene expression analysis).

Conducting Laboratories and Locations: In-life study by Safety Profiling and Assessment, Novartis Pharmaceuticals Corporation, East Hanover, New Jersey; Immunohistochemistry and image analysis of cecum and colon by _____ gene expression profiling of GI tract tissues by Department of Biomarker Development, Novartis Pharma AG, Basel, Switzerland.

Dates of Study: Dosing was initiated on September 16, 2005 and terminal necropsy was conducted on October 17 and 18, 2005

GLP Compliance: The in-life study and plasma concentration analyses were GLP compliant. Immunohistochemistry, image analysis and gene expression profiling of GI tract tissues and fecal concentration analyses were non-GLP.

QA Report: yes for the in-life study (including standard histopathology)

Drug, Batch #, and % Purity: Aliskiren hemifumarate; batch #0544033; drug content: —

Formulation: Aliskiren hemifumarate was admixed with the diet. The formulation was analyzed for test article concentration and stability during the weeks 1 to 4 of study.

Animals

Species/Strain: IGS Wistar Hannover rats, — WI(Glx/BRL/Han)IGS BR from —

Number/Sex/Group: 20 males

Age: 9 weeks at initiation of dosing

Weight: males: 214.2 to 270 g

Husbandry: Animals were housed in pairs (except during fecal sample collection procedure) in stainless steel cages. Food and water *ad libitum* except for study-defined fasting procedures.

Dosing

Groups of 20 male rats were given aliskiren hemifumarate admixed with the diet at target doses of 276.3, 828.8 or 1657.5 mg/kg/day (250, 750 or 1500 mg/kg/day of base, respectively) for 4 weeks. The control group received untreated feed. The doses for this study were the same doses utilized in the 104 week dietary carcinogenicity study in rats (see main review, section 3.3.1). In that study, changes in the GI tract were noted following 52 weeks of dietary administration at doses of 750 or more mg aliskiren/kg/day.

Observations / Measurements

Mortality: Twice daily on week days and once daily on all other days.

Clinical Signs: All animals were observed at least once a day.

Body Weights: Recorded once before treatment, once weekly during dosing and on the day of scheduled necropsy.

Food Consumption: Calculated from feeder weights collected once weekly.

Hematology^a and Clinical Chemistry^b: Blood samples were collected from the sublingual vein from anesthetized animals in week 4.

Necropsy: Animals were fasted overnight before necropsy. The first 10 animals in each group were bromodeoxyuridine (BrdU) designated animals for immunohistochemistry (IHC) and image analyses of the cecum and colon in addition to standard hematoxylin and eosin stained (H & E) processing of protocol tissues (Table 3.1). All of these animals received 100 mg BrdU/kg intraperitoneally 60 min prior to sacrifice. The duodenum, jejunum, colon and cecum were separated and flushed with saline to remove contents and then immersed in formalin. The remaining 10 males in each group were used to collect samples of duodenum, jejunum, ileum, cecum and colon for possible investigational gene expression analysis in addition to collection of designated tissues (Table 3.1) for standard H & E processing.

Histopathology: Microscopic examination was performed on H & E processed tissue sections (Table 3.1) from all animals in all dose groups. Immunohistochemistry (IHC) and image analysis of the cecum and colon were evaluated and quantified for control and high dose group animals.

Investigational Gene Expression Analyses: Total RNA extracted from frozen tissues (duodenum, jejunum, ileum, cecum and colon) was purified. A part of each individual RNA sample was kept for the analysis of genes

_____ this was
_____ was scanned, processed
_____ and the data was converted to expression levels.

^a erythrocytes, hematocrit, hemoglobin, reticulocytes, Wintrobe indices, red cell distribution width (RDW), white blood cell, white blood cell differential

^b ALT, AST, AP, total bilirubin, bile acids, total protein, albumin, globulin, A/G ratio, glucose, urea, creatinine, sodium, potassium, chloride, calcium, inorganic phosphorus, triglycerides, cholesterol

TABLE 3.1
TISSUE LIST: FOR COLLECTION, PROCESSING (P) AND/OR GENOMICS (G)

		adrenal			pancreas	
		aorta			parathyroid	
		bone marrow (in bone)			pituitary	
		brain			prostate	
G	P	cecum		P	rectum	
G	P	colon			salivary gland	
G	P	duodenum			sciatic nerve	
		epididymis			seminal vesicle	
		esophagus			skeletal muscle	
		eye			skin	
		femur/tibia			spinal cord	
		harderian gland			spleen	
		heart			sternum	
G	P	ileum		G	P	stomach
G	P	jejunum				testis
		kidney				thymus
		lacrimal gland				thyroid
G		liver				tongue
		lung				trachea
		lymph node - bronchial				urinary bladder
		lymph node - mandibular				macroscopic lesions
		lymph node - mesenteric				animal identification
		mammary gland		G		blood
		nasal passage				

Toxicokinetics: Blood samples for test substance determination were collected at 4, 8, 16 and 24 hours after the lights were turned on (5:30 AM) during study week 3 (5 animal/group/time point). Blood was collected from the sublingual vein of anesthetized animals. Fecal samples were collected from the first 3 surviving control males and the first 5 surviving males in the treated dose groups in study week 4. The designated males were placed in individual metabolism cages overnight with feed and remained in these cages until an adequate sample was obtained. The concentration of aliskiren in the fecal samples was determined by HPLC/MS/MS.

Results

Analysis of Formulations: Feed admixture was stable at room temperature for 21 days and formulation homogeneity was confirmed. The achieved concentration of test substance in the dietary formulation was _____ of the targeted concentration.

Mortality: None

Clinical signs: Two males in the high dose group were noted to be thin in appearance. The signs correlated with significantly decreased body weight and were considered related to the test substance.

Body Weights: Dose-dependent, statistically significant reductions in body weight relative to control were noted at doses of 750 or more mg/kg/day on study days 22 and 29 and at 1500 mg/kg/day on study day 8 through study day 29. The reductions in mean body weight relative to control for the 750 and 1500 mg/kg/day groups on study day 29 were approximately 6 and 17%, respectively (Table 3.2). In addition, statistically significant dose-dependent reductions in body weight gain relative to control were noted at doses of 750 or more mg/kg/day beginning on day 8. Mean reductions in body weight gain on study day 29 were approximately 19 and 64% of control values at 750 and 1500 mg/kg/day, respectively.

TABLE 3.2
MEAN ANIMAL BODY WEIGHT (gm)

Group(s)		Day of Phase					
		21	1*	8	15	22	29
1	(N)	20	20	20	20	20	20
	Means	229.0	242.1	269.5	292.6	314.1	328.4
	Sdevs	11.78	13.16	16.51	18.80	21.33	23.97
2	(N)	20	20	20	20	20	20
	Means	228.6	240.5	268.0	291.1	309.3	322.1
	Sdevs	11.68	12.95	14.25	17.55	21.19	23.45
3	(N)	20	20	20	20	19	19
	Means	228.4	239.4	259.4	278.5	295.8*	309.6*
	Sdevs	11.51	13.15	16.73	20.38	25.16	27.32
4	(N)	20	20	20	20	20	20
	Means	228.6	242.0	242.2+	253.3+	265.9+	273.5+
	Sdevs	12.17	13.84	13.09	20.78	18.01	19.54

Note: ! = pretest phase; * = treatment phase
 *(+) = mean value of group was significantly different from control at P = 0.05(0.01) with Dunnett's test of significance
 ‡(\$) = mean value of group was significantly different from control at P = 0.05(0.01) with Modified T test of significance

Food Consumption: A slight decrease in food consumption relative to concurrent control was noted at 1500 or more mg/kg/day during the first week of the study but did not attain statistical significance.

Hematology and Clinical Chemistry: Test substance-related changes relative to control were not noted for either hematology or clinical chemistry parameters.

Gross Pathology: There were no treatment-related macroscopic findings.

Histopathology: A dose-related increase in the incidence and severity of mucosal hyperplasia of the cecum was noted in animals dosed at 250 or more mg/kg/day (4/20, 11/20, 18/20 and 20/20 from control, 250, 750 and 1500 mg/kg/day, respectively). Hyperplasia was characterized by increased crypt basophilia, numbers of mitotic figures and mucosal thickness. Animals in the high dose group also demonstrated minimal to slight diffuse mucosal hyperplasia of the colon (14/20), mucosal inflammation in the cecum (4/20) and hyperplasia in the ileum (4/20) (Table 3.3).

TABLE 3.3
INCIDENCE AND SEVERITY OF INTESTINAL MICROSCOPIC FINDINGS AT TERMINAL NECROPSY

SEX :		1	2	3	4
DOSE GROUP:		1	2	3	4
NO. ANIMALS:		20	20	20	20
CECUM :		20	20	20	20
N.A.D. :		14	7	2	-
.....					
-	Hyperplasia, Lymphoid:	2	1	5	4
	Grade 1:	-	1	2	2
	Grade 2:	2	-	3	2
-	Dilatation :	1	1	1	1
	Grade 1:	-	-	-	1
	Grade 2:	-	1	1	-
	Grade 3:	1	-	-	-
-	Hyperplasia, Mucosal :	4	11	18	20
	Grade 1:	4	9	8	4
	Grade 2:	-	2	10	16
-	Inflammation, Mixed :	-	-	-	4
	Grade 1:	-	-	-	3
	Grade 2:	-	-	-	1
.....					
COLON :		20	20	20	20
N.A.D. :		19	17	18	5
.....					
-	Hyperplasia, Mucosal :	-	-	2	14
	Grade 1:	-	-	2	9
	Grade 2:	-	-	-	5
-	Hyperplasia, Lymphoid:	1	3	-	-
	Grade 1:	-	1	-	-
	Grade 2:	1	1	-	-
	Grade 3:	-	1	-	-
-	Dilatation :	-	-	-	1
	Grade 1:	-	-	-	1
.....					
ILEUM :		20	20	20	20
N.A.D. :		20	19	19	16
.....					
-	Hyperplasia, Mucosal :	-	-	-	4
	Grade 1:	-	-	-	2
	Grade 2:	-	-	-	2
-	Dilatation :	-	-	1	-
	Grade 1:	-	-	1	-
-	Hyperplasia, Lymphoid:	-	1	-	-
	Grade 1:	-	1	-	-

Group 1: Control; Group 2: 250 mg/kg/day; Group 3: 750 mg/kg/day; Group 4: 1500 mg/kg/day

Immunohistochemistry and Image Analysis: The histopathological analysis of the cecum and colon sections immunostained with anti-BrdU antibody conducted on control and high dose animals showed that cell proliferation was mainly confined to the mucosa. A statistically significant increase in mucosal cell proliferation relative to control was noted at 1500 mg aliskiren/kg/day in the mucosa of both cecum and colon, confirming the histopathological examination in which hyperplasia was documented in the mucosa of both of these tissues.

Toxicokinetics: Plasma: The plasma concentrations of aliskiren increased with increasing dose but were greater than dose proportional (Table 3.4). Inter-animal variability was high (CV up to 111%). Measurable concentrations of aliskiren were noted in 9 of 20 animals in the control group. The maximum concentration measured in these animals was less than half of the C_{max} noted for the low dose group. The nature of contamination is not known.

TABLE 3.4
TOXICOKINETIC PARAMETERS OF ALISKIREN IN RAT PLASMA

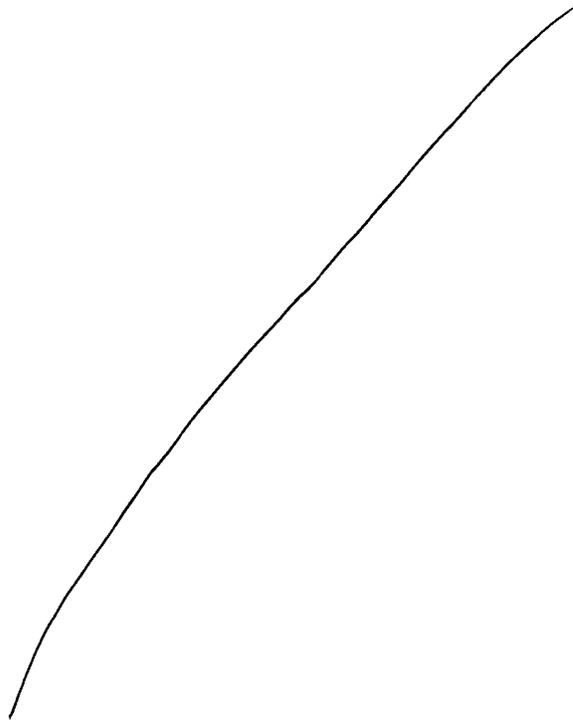
Parameter	Unit	Low	Mid	High
		(250 mg/kg/day)	(750 mg/kg/day)	(1500 mg/kg/day)
t _{max}	hour	16	16	8
C _{max}	ng/mL	166	827	1960
C _{max} /Dose	(ng/mL)/(mg/kg/day)	0.664	1.10	1.31
AUC _(0-24 hr)	ng.hr/mL	2590	12400	36300
AUC _(0-24 hr) /Dose	(ng.hr/mL)/(mg/kg/day)	10.3	16.5	24.2

Fecal aliskiren concentrations increased with increasing dose but were less than dose proportional (16.9, 25.8 and 39.5 mg aliskiren/g of feces at doses 250, 750 and 1500 mg/kg/day, respectively).

Gene Expression Analysis: Increase and decrease in expression refers to RNA expression levels. Only those genes showing statistically significant changes in the expression between control and treated groups are reported here.

No major gene expression changes were observed after 4 weeks of treatment with aliskiren in the duodenum or jejunum at any dose level or in the ileum at low and mid dose levels. Significant changes were noted in the ileum for the high dose group, which were similar to those in the cecum. Dose-dependent changes with a major impact in the high dose treatment group were noted in the cecum and colon. Gene expression changes noted in all three tissues (ileum, cecum and colon) were consistent, dose-dependent (minor at the lowest dose and major at the highest dose) and pointed to different mechanisms. The **inflammatory process** consisted of an up-regulation (over-expression) of genes related to inflammatory cytokines, chemokines and phagocytes-secreted proteins (Tables 3.5, 3.6). These mediators of inflammatory cells reflected the presence of stress or toxic injury to the mucosal epithelium triggering an **epithelial reaction**, resulting in up-regulation of cell-proliferation-related genes and the activation of local **growth factors** (Tables 3.7, 3.9). The over expression of **transcription factors** (Tables 3.8, 3.9)

led to modification in **signal transduction pathways**. Gene expression related to cell survival and apoptosis showed significant dose-dependent changes (Tables 3.10, 3.11). The mediators of inflammatory cells disrupt normal tissue homeostasis, acting directly on epithelial cells and indirectly on stromal cells and extracellular matrix (ECM) components (**epithelial damage**). Gene expression related to ECM remodeling (such as degrading proteases, matricellular proteins) was up-regulated in a dose-dependent manner. In addition, gene expression related to cell adhesion was up-regulated (Tables 3.12, 3.13). Changes in epithelial remodeling were major in the cecum and minor in the colon. Gene expression linked to innate immunity was up-regulated (dose-dependent up-regulation of toll-like receptor and down regulation of a negative regulator of toll-like receptor signaling) in the cecum. Additionally, changes in the expression of some genes related to cytoskeleton, transporters and metabolism in both cecum and colon, and intracellular trafficking and GI specific functions in the cecum were noted for both mid and high dose treated animals.



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4. 13-Week Oral (feed admixture) Mechanistic Study in Male Rats

Key Study Findings: Aliskiren hemifumarate administered as feed admixtures to rats for up to thirteen weeks produced a dose-related mucosal hyperplasia of the cecum at doses of 250 or more mg aliskiren/kg/day and the colon at 750 or more mg aliskiren/kg/day. Immunostaining with BrdU and image analysis confirmed mucosal hyperplasia in the cecum and colon at 1500 mg/kg/day. Hyperplasia was reversible after a 4 week recovery period. Gene expression profiling of the GI tract demonstrated a proliferative epithelium (slight up-regulation in some growth factors) without inflammation in the cecum and colon at the mid and high doses (750 and 1500 mg aliskiren/kg/day). Loss of body weight and reduced body weight gain was noted for animals receiving 750 or more mg/kg/day. The study failed to establish a NOAEL for mucosal hyperplasia in the cecum.

Introduction: The aim of the study was to investigate the potential effects of aliskiren on the gastrointestinal tract following administration to male rats for 13 weeks and the reversal of any toxic effects after 4 weeks of recovery. The study included immunohistochemistry and image analyses, gene expression profiling of GI tract tissues, and toxicokinetics analyses of plasma and fecal samples.

Study No.: 0570299

Conducting Laboratories and Locations: In-life study by Safety Profiling and Assessment, Novartis Pharmaceuticals Corporation, East Hanover, New Jersey; fecal concentration analyses by DMPK/BioAnalytics, Novartis Pharma, France; immunohistochemistry and image analysis of cecum and colon by _____ gene expression profiling of GI tract tissues by Department of Biomarker Development, Novartis Pharma AG, Basel, Switzerland.

Dates of Study: Dosing was initiated on September 20, 2005 and terminal necropsies were conducted on January 20, 2006.

GLP Compliance: Yes for the in-life study and plasma concentration analyses.

Immunohistochemistry, image analysis and gene expression profiling of GI tract tissues and fecal concentration analyses were non-GLP.

QA Report: yes for in-life study (including standard histopathology)

Drug, Batch #, and % Purity: Aliskiren hemifumarate; batch #0544033; drug content: _____

Formulation: Aliskiren hemifumarate was admixed with the diet. The formulation was analyzed for test article concentration and stability at the start of weeks 1 or 2 and 13 of study.

Animals

Species/Strain: IGS Wistar Hannover rats, _____ WI(Glx/BRL/Han)IGS BR from _____

Number/Sex/Group: 20 males

Age: 9 weeks at initiation of dosing

Weight: 221.4 to 289.2 g

Husbandry: Animals were housed in pairs (except during fecal sample collection procedure) in stainless steel cages. Food and water *ad libitum* except for study-defined fasting procedures.

Dosing

Groups of 20 male rats were given aliskiren hemifumarate admixed with the diet at target doses of 276.3, 828.8 or 1657.5 mg/kg/day (250, 750 or 1500 mg/kg/day of base, respectively) for 13 weeks. The control group received untreated feed. Recovery phase animals (first 7 animals in each group) were treated for 13 weeks and were then allowed at least 4 weeks of recovery following the cessation of treatment; these animals were killed on recovery day 29 (Table 4.1). The doses for this study were the same doses utilized in the 104 week dietary carcinogenicity study in rats (see main review, section 3.3.1). In that study, changes in the GI tract were noted following 52 weeks of dietary administration at doses of 750 or more mg aliskiren/kg/day.

TABLE 4.1
STUDY DESIGN
STUDY ANIMALS (N=20) ALLOCATED FOR MEASUREMENTS AND NECROPSY PROCESSING

Group	Treatment	Dosage* mg/kg/day	During first 13 weeks of dosing				Recovery Phase	
			Hematology & Clin Chem	Toxicoki netics	BrdU/IHC and Std Histopathol	Genomics	BrdU/IHC and Std Histopathol	Genomics [§]
1	Control	0	#1-10	#11-20 [†]	#8-15	#16-20	#1-4	#5-7
2	Aliskiren	250	#1-10	#11-20 [†]	#8-15	#16-20	#1-4	#5-7
3	Hemi-	750	#1-10	#11-20 [†]	#8-15	#16-20	#1-4	#5-7
4	fumarate	1500	#1-10	#11-20 [†]	#8-15	#16-20	#1-4	#5-7

*: Target dose levels (as base); [†]: Blood was collected from all 10 animals, while fecal samples were collected from #16-20 (5 animals/group); [§]: results not reported in the current submission

Observations / Measurements

Mortality: Twice daily on week days and once daily on all other days.

Clinical Signs: All animals were observed at least once a day.

Body Weights: Recorded once before treatment, once weekly during dosing and on the day of scheduled necropsy. Recovery animals were weighed weekly.

Food Consumption: Calculated from feeder weights collected once weekly including recovery animals.

Hematology^c and Clinical Chemistry^d: Blood samples were collected from the sublingual vein of anesthetized animals in week 13 (from the first 10 animals in each group). No blood samples were collected from the recovery animals during the recovery phase.

Necropsy: Animals were fasted overnight (18 hr) before necropsy. A full necropsy was performed with a recording of macroscopic abnormalities for all protocol tissues. The first 7 animals in each group were allocated to the recovery phase. The next 8 animals in each group from the main study and the first 4 in the recovery groups received 100 mg

^c erythrocytes, hematocrit, hemoglobin, reticulocytes, Wintrobe indices, red cell distribution width (RDW), white blood cell, white blood cell differential

^d ALT, AST, AP, total bilirubin, bile acids, total protein, albumin, globulin, A/G ratio, glucose, urea, creatinine, sodium, potassium, chloride, calcium, inorganic phosphorus, triglycerides, cholesterol

bromodeoxyuridine (BrdU)/kg intraperitoneally 60 min prior to sacrifice at the end of 13 weeks of dosing or at the end of the 4 week recovery period, respectively (see Table 4.1). These animals underwent necropsy with tissues taken for standard hematoxylin and eosin (H & E) processing of protocol tissues (Table 4.2) as well as special histopathological processing for BrdU/immunohistochemistry (IHC) and image analyses of the cecum and colon. The last 5 males in each main study group (#16-20) and the last 3 males from the recovery groups (#5-7) were used to collect samples of blood, jejunum, ileum, cecum and colon for investigational gene expression analysis in addition to collection of designated tissues (Table 4.2) for standard H & E processing.

TABLE 4.2
TISSUE LIST: FOR COLLECTION, PROCESSING (P) AND/OR GENOMICS (G)

	adrenal		pancreas
	aorta		parathyroid
	bone marrow (in bone)		pituitary
	brain		prostate
G	P cecum	P	rectum
G	P colon		salivary gland
G	P duodenum		sciatic nerve
	epididymis		seminal vesicle
	esophagus		skeletal muscle
	eye		skin
	femur/tibia		spinal cord
	harderian gland		spleen
	heart		sternum
G	P ileum	G	P stomach
G	P jejunum		testis
	kidney		thymus
	lacrimal gland		thyroid
G	liver		tongue
	lung		trachea
	lymph node – bronchial		urinary bladder
	lymph node – mandibular	P	macroscopic lesions
	lymph node – mesenteric		animal identification
	mammary gland	G	blood
	nasal passage		

Histopathology: Microscopic examination was performed on all tissue sections processed with H & E (Table 4.2) from all animals in all dose groups. Immunohistochemistry (IHC) and image analysis of the cecum and colon were evaluated and quantified for control and high dose group animals.

Investigational Gene Expression Analyses: Total RNA extracted from blood and frozen tissues (jejunum, ileum, cecum and colon) was purified. A part of each individual RNA sample was kept for the analysis of genes

_____ was scanned, processed and the data was converted to expression levels.

Toxicokinetics: Blood samples for test substance determination were collected from the last 10 animals in each group in study week 11. Three animals/group were bled at approximately 0 and 16 hr (3 animals/group/time point) and 4 animals/group were bled at approximately 8 hr after the lights turned on (5:30 AM). No blood samples for toxicokinetic evaluation were collected during the recovery period. Blood was collected from the sublingual vein of anesthetized animals. Fecal samples were collected from the last 5 males per group in study week 13. The designated males were placed in individual metabolism cages overnight with feed and remained in these cages until an adequate sample was obtained. The concentration of aliskiren in the fecal samples was determined by HPLC/MS/MS.

Results

Analysis of Formulations: Feed admixture was stable at room temperature for 21 days and formulation homogeneity was confirmed. The achieved concentration of test substance in the dietary formulation for the entire study was _____ of the targeted concentration.

Mortality: None

Clinical signs: Treatment-related clinical signs such as fecal changes (soft feces, diarrhea) and stained fur/perineal staining were noted at doses 750 or more mg/kg/day. Additional clinical signs attributed to treatment included thin appearance, dehydration, chromorrhinorhea in a few animals (isolated occurrences) at 1500 mg/kg/day. All of these clinical signs except stained fur were resolved during the recovery period.

Body Weights: Reductions in body weight relative to control were noted at doses of 750 (3-4%, $p > 0.05$) and 1500 mg/kg/day (7-13%, $p < 0.05$) from day 22 or day 8, respectively, to the end of treatment. The mean body weight gain relative to treatment day 1 was significantly ($p < 0.05$) reduced compared to control for the 750 (12-22%) and 1500 (37-96%) mg/kg/day groups throughout the treatment period (Table 4.3). During recovery, mean body weights for the high dose group were still reduced (9-16%, $p < 0.05$) except for study day 29 (Table 4.4). Mean body weight gains for this period also remained reduced compared to control (9-16%, $p > 0.05$ at 750 mg/kg/day and 23-45%, $p < 0.05$ at 1500 mg/kg/day).

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TABLE 4.3
MEAN ANIMAL BODY WEIGHT (gm) DURING TREATMENT PERIOD

Group (s)	Day of Phase												
	1 [†]	1 [‡]	8	15	22	29	36	43	50	57	64	71	78
	Male Animals												
1	(N) 20	20	20	20	20	20	20	20	20	20	20	20	20
	Means 243.1	259.8	280.3	300.5	314.7	332.1	342.4	353.3	362.2	372.4	378.0	381.8	387.1
	Sdevs 13.17	16.49	19.97	22.37	22.73	23.04	23.75	24.31	23.89	24.18	25.12	24.83	25.46
2	(N) 20	20	20	20	20	20	20	20	20	20	20	20	20
	Means 242.4	260.1	279.8	299.7	311.1	325.7	335.2	347.2	359.1	368.9	374.4	382.6	384.8
	Sdevs 13.49	15.69	18.59	21.34	22.93	22.06	21.93	22.90	22.60	23.21	23.09	23.46	24.18
3	(N) 20	20	20	20	20	20	20	20	20	20	20	20	20
	Means 242.7	262.3	278.4	297.1	305.7	321.9	331.5	341.1	347.2	357.7	362.2	370.1	371.8
	Sdevs 12.60	15.53	16.11	19.59	24.01	25.89	27.83	29.63	31.30	32.53	35.23	35.55	35.56
4	(N) 20	20	20	20	20	20	20	20	20	20	20	20	20
	Means 242.5	260.8	261.6 [†]	278.9 [†]	284.8 [†]	304.6 [†]	308.6 [†]	313.9 [†]	318.7 [†]	324.8 [†]	332.6 [†]	339.2 [†]	338.9 [†]
	Sdevs 13.38	15.30	26.04	24.24	24.52	25.61	25.61	27.69	30.16	33.75	35.12	36.77	35.92

Note: † = pretest phase; ‡ = treatment phase
 * (†) = mean value of group was significantly different from control at P = 0.05(0.01) with Dunnett's test of significance
 ‡ (\$) = mean value of group was significantly different from control at P = 0.05(0.01) with Modified T test of significance

TABLE 4.4
MEAN ANIMAL BODY WEIGHT (gm) DURING RECOVERY PERIOD

Group (s)	Day of Phase						
	85 [†]	91	1 [‡]	8	15	22	29
	Male Animals						
1	(N) 20	20	7	7	7	7	7
	Means 394.3	399.4	399.3	405.4	415.7	414.3	418.9
	Sdevs 26.36	26.06	20.03	19.99	19.99	20.96	20.77
2	(N) 20	20	7	7	7	7	7
	Means 393.8	399.3	390.6	397.4	408.2	407.7	413.2
	Sdevs 24.59	25.15	13.25	12.04	14.22	14.49	16.96
3	(N) 20	20	7	7	7	7	7
	Means 379.1	384.6	375.7	385.6	398.8	396.3	404.4
	Sdevs 35.96	37.03	53.20	55.44	56.75	54.89	54.52
4	(N) 20	20	7	7	7	7	7
	Means 345.9 [†]	345.5 [†]	337.0 [‡]	361.9 [‡]	376.2 [‡]	375.2 [‡]	383.0
	Sdevs 36.59	36.33	30.66	32.11	32.52	34.06	33.29

Note: † = treatment phase; ‡ = recovery phase
 * (†) = mean value of group was significantly different from control at P = 0.05(0.01) with Dunnett's test of significance
 ‡ (\$) = mean value of group was significantly different from control at P = 0.05(0.01) with Modified T test of significance

Food Consumption: No significant change; however, spillage was noted, especially for the high dose animals.

Hematology and Clinical Chemistry: Test substance-related changes relative to control were not noted for either hematology or clinical chemistry parameters.

Gross Pathology: There were no treatment-related macroscopic findings.

Histopathology: A dose-related increase in the incidence and severity of mucosal hyperplasia of the cecum was noted in animals dosed at 250 or more mg/kg/day. Animals in the high dose group also demonstrated minimal diffuse mucosal hyperplasia of the colon (7/13) (Table 4.5). Hyperplasia was characterized by increased crypt basophilia, numbers of mitotic figures and mucosal thickness. Incidences and severities of hyperplasia returned to control levels following the 4 week recovery period (Table 4.5).

Immunohistochemistry and Image Analysis: The histopathological analysis of the cecum and colon sections immunostained with anti-BrdU antibody (conducted on control and high dose animals) showed that cell proliferation was mainly confined to the mucosa. A statistically significant increase in mucosal cell proliferation relative to control was noted

after treatment with 1500 mg aliskiren/kg/day for 13 weeks in the mucosa of both cecum and colon confirming the histopathological examination in which hyperplasia was documented in the mucosa of both of these tissues. None of these findings were evident after the 4 week recovery period.

TABLE 4.5
INCIDENCE OF INTESTINAL FINDINGS IN ANIMALS AT SCHEDULED NECROPSIES

Microscopic Findings	Target Dose (mg/kg/day)			
	0	250	750	1500
Week 13 sacrifice				
Cecum: mucosal hyperplasia	4/13	10/13	12/13	13/13
Mean severity	1.0*	1.0	1.0	1.4
Colon: mucosal hyperplasia	0/13	0/13	1/13	7/13
Mean severity			1.0	1.0
Recovery sacrifice				
Cecum: mucosal hyperplasia	1/7	1/7	1/7	2/7
Mean severity	1.0	1.0	1.0	1.0

*Severity: 1=minimal, 2 =slight

Toxicokinetics: Plasma: The plasma concentrations of aliskiren increased over-proportional to the dose range tested especially for the mid and high dose groups (Table 4.6). Inter-animal variability was high. Measurable concentrations of aliskiren were noted in 7 of 10 animals in the control group. The maximum concentration measured in these animals was less than half of the C_{max} noted for the low dose group. The nature of contamination is not known.

TABLE 4.6
TOXICOKINETIC PARAMETERS OF ALISKIREN IN RAT PLASMA

Target Dose		250 mg/kg/day	750 mg/kg/day	1500 mg/kg/day
	Unit	Mean	Mean	Mean
t _{max}	Hour	0	0	16
C _{max}	ng/mL	88.5	1430	2120
C _{max} /Dose	(ng/mL)/(mg/kg/day)	0.354	1.91	1.41
AUC _(0-24 hr)	ng.hr/mL	1400	17800	24500
AUC _(0-24 hr) /Dose	(ng.hr/mL)/(mg/kg/day)	5.61	23.7	16.4

Fecal mean aliskiren concentrations increased with increasing dose but were less than dose proportional (10.9, 15.4 and 15.9 mg aliskiren/g of feces at doses 250, 750 and 1500 mg/kg/day, respectively). Aliskiren was not detected in any of the samples from the control dose group.

Gene Expression Analysis: Increase and decrease in expression refers to RNA expression levels. Only those genes showing statistically significant changes in the expression between control and treated groups are reported here.

Jejunum No major gene expression changes were observed after 13 weeks of treatment with aliskiren.

Ileum No effect at the low dose level. An inflammatory signal was not evident at mid and high doses. Minimal epithelial reaction (that is triggered due to stress or toxic injury to the mucosal epithelium) was noted at both dose levels as indicated by slight up-regulation of mitosis regulators and growth factors. Changes related to extracellular matrix (epithelial damage) were limited to down-regulation of proteases and matricellular proteins.

Cecum Only minor gene expression changes (not pointing to the presence of an inflammatory reaction) were noted at the lowest dose. The main observed changes could be related to a slight epithelial reaction (slight up-regulation of cell proliferation regulation of growth factors and signaling) and minor changes in the expression of ECM (indicating **epithelial damage**) (Table 4.7). In contrast, the gene expression changes in the cecum after 13 weeks of exposure at the mid and high doses were more pronounced and dose-dependent. The gene expression profiling showed a weak **inflammatory** signal (moderate up-regulation of genes related to cytokines, chemokines and phagocytes-secreted proteins). There was no clear proliferative activity or **epithelial reaction**, though slight up-regulation of growth factor and transcription factor-related gene expression was noted (Table 4.7). Some changes related to ECM remodeling, cell adhesion and specific endothelial markers were noted (**epithelial damage**) (Table 4.8).

Colon Very few gene expression changes were noted for the low dose group. No signs of inflammatory reaction, proliferation and growth factor secretion could be detected at this dose level. For the mid dose group, no inflammatory signal could be described but minor changes were noted regarding cell proliferation. In addition, some minor changes were noted in the expression of genes related to ECM, cell adhesion, the cytoskeleton, GI specific proteins, basal metabolism and immune cells. On the other hand, the number of genes expressing changes for the high dose group were approximately 3 times higher than those for the mid and low dose groups. As with the other two dose groups, no signs of inflammation could be detected. However, numerous gene expression changes pointed to a proliferation process (**epithelial reaction**) with an up regulation of mitotic gene expression (Table 4.9). The gene expression related to apoptosis showed an up-regulation of inhibitors of apoptosis. The gene expression related to epithelial matrix remodeling (such as proteases) with cell adhesion changes was up-regulated (**epithelial damage**) (Table 4.10).

Blood Inflammatory signal was absent at any of the tested doses.

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OVERALL SUMMARY AND EVALUATION

Aliskiren is a new class of non-peptide, low molecular weight renin inhibitor for the treatment of hypertension. The results of the 104 week dietary carcinogenicity study in Wistar rats and the 26 week dietary carcinogenicity study in transgenic (rasH2) mice revealed inflammatory and proliferative changes in the gastrointestinal tract in general and cecum and colon in particular (see the original NDA review). In mice, an increased incidence of mucosal hyperplasia/hypertrophy was noted in both sexes in the cecum at 750 and 1500 mg/kg/day and in the duodenum, jejunum, ileum and colon at 1500 mg/kg/day. In rats, an increased incidence of mucosal epithelial hyperplasia was noted in the small and large intestines of both sexes at 250 or more mg/kg/day. An increased incidence of cecal erosion and ulceration was noted in both sexes at 750 or more mg/kg/day. One colonic adenoma and one cecal adenocarcinoma were observed ($p > 0.05$) in male rats (1 of 50 each) receiving 1500 mg/kg/day. The sponsor carried out mechanistic toxicity studies (*in vitro* and *in vivo*) to assess the changes observed in the (male) rat GI tract.

In vitro studies demonstrated that exposure of rat colon to high concentrations of aliskiren (10 mM mucosal/ 1 mM serosal) resulted in significant increases in transepithelial tissue conductance and impaired transepithelial chloride flux to cholinergic stimulation. This indicates that aliskiren can impair epithelial integrity and can compromise transepithelial chloride/water secretion. In contrast, aliskiren did not affect either epithelial integrity or secretory function in human colon preparations. The lack of effect on human colon mucosa suggests a non-specific local toxicity of aliskiren in rat mucosal cells or rat colon, which may be more susceptible to detrimental effects of aliskiren than human colonic mucosa.

Dietary mechanistic toxicity studies in rats after 1 (with sacrifices after 1, 3 and 7 doses), 4 and 13 weeks of treatment demonstrated a dose-related increase in the incidence and severity of minimal to slight diffuse mucosal hyperplasia in the cecum at doses of 250 or more mg/kg/day and in the colon at 750 or more mg/kg/day. Hyperplasia was characterized by increased crypt basophilia, numbers of mitotic figures and mucosal thickness. Animals in the high dose group also displayed mucosal inflammation in the cecum after 1, 3, 7 and 28 days of treatment but not after 13 weeks of treatment. Additionally, mucosal hyperplasia of the ileum was noted in rats after 7 and 28 days of dosing at 1500 mg/kg/day. Bromodeoxyuridine immunohistochemistry and image analysis confirmed the pathological findings at 1500 mg/kg/day (tissues from animals treated with lower doses were not subjected to this procedure) and in addition showed evidence of effects in the cecum as early as day 2 (after one day of treatment); a statistically significant increase in cell proliferation was observed in the cecum on days 1, 3 and 7 of treatment and in the colon after 3 and 7 days of treatment. Gene expression profiling revealed the presence of marked inflammation in the colon and cecum after short term treatment (1 and 4 week studies) but not after 13 weeks of exposure to aliskiren hemifumarate, possibly due to adaptation. Furthermore, complete reversibility of the changes was demonstrated in the 13 week study after a 4 week drug-free period.

Gene expression profiling in rat ileum, jejunum, cecum and colon, obtained during the 4 and 13 week mechanistic toxicity studies, lends support to the hypothesis of an early local irritation triggered by high local concentrations of aliskiren. The observed gene expression changes in the 4 week study revealed an inflammatory process associated with an epithelial reaction, growth factor secretion, modifications in signal transduction and transcription factors. These gene expression changes indicated the presence of a stress or toxic injury to the mucosal epithelium, potentially leading to an injury/repair/proliferation cycle. This epithelial injury had consequences for GI specific functions which were revealed at the gene expression level with changes in the expression of specific proteins, transporters and muscle activities. On the other hand, in the 13 week study, gene expression changes not pointing to the presence of an inflammatory reaction were noted for all of the GI tract tissues at all doses. A slight epithelial reaction was noted in the ileum at mid and high doses, as there was up-regulation of stress-induced specific transcription factors. In the cecum, a weak epithelial reaction with few responses at the transcription factor level was noted at all dose levels in a dose-dependent manner. A maintained proliferative epithelial reaction (up-regulation of some growth factors) was noted for the colon at the mid and high doses and this could have resulted in the chronic inflammatory and proliferative changes observed microscopically at these doses after 104 weeks of treatment.

The rat mechanistic toxicity studies were also designed to measure aliskiren concentrations in feces for estimation of safety margins based on local GI tract exposure. The following table compares rat fecal and GI mucosal concentrations to human fecal and rectal mucosal concentrations at the NOAEL (250 mg/kg/day) for histopathological changes in the GI tract in the rat 104 week carcinogenicity study. None of the 3 mechanistic toxicity studies reviewed here were able to establish a NOAEL due to histopathologic observation (at all doses) of a dose-dependent increase in the incidence of mucosal hyperplasia in the cecum. There was no overlap of human and rat fecal concentrations but large variations were noted between individual samples. Human fecal concentrations ranged from 0.021 mg/g to 5.01 mg/g at 300 mg/day, the maximum recommended human dose. The fecal concentrations measured at the lowest dose in the two rat studies ranged from 8.79 to 18.6 mg/g. For GI tissue concentrations, mucosal concentrations at the NOAEL of 250 mg/kg/day ranged from 58 to 216 µg/g in the cecum and 62 to 197 µg/g in the colon, while human rectal mucosal concentrations ranged from 2 µg/g to 60 µg/g. The wide range of values might further shrink the low multiples.

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TABLE
ALISKIREN GASTROINTESTINAL RAT TO HUMAN EXPOSURE MULTIPLES
MEAN ± S.D FECAL (mg/g) AND GI MUCOSAL (µg/g) ALISKIREN CONCENTRATIONS IN RATS *VERSUS*
HUMANS

Duration/Species (Study #, Rev Sec)	Dose mg/kg/d	Fecal concentration	Multiples	GI mucosal concentration	Multiples
4-week rat study (TOX 0570277; Section 3)	250 ^a	16.94 ± 1.05	11	Not determined	-
13-week rat study (TOX 057029; Section 4)	250 ^a	10.96 ± 1.51	7	Not determined	-
1-Week rat study (TOX 0570340; Section 1)	250			Jejunum 70.5 ± 33	Ileum 99.3 ± 27
	750			Cecum 135 ± 58	colon 132 ± 65
	1500			See footnote b.	
				266 ± 155	343 ± 285
				447 ± 445	336 ± 156
					513 ± 226
					697 ± 331
Human study (CSPP100A2105) ^c	300 mg/day	1.53 ± 1.32	-	Mucosa rectum: 22.2 ± 15.6	

^a: 250 mg/kg/day was the NOAEL dose for the absence of any histopathological changes in the GI tract in the rat 104 week carcinogenicity study. A true NOAEL was not established for the 104 week carcinogenicity study. None of the 3 mechanistic toxicity studies referenced here were able to establish a NOAEL due to dose-dependent increase in the incidence of mucosal hyperplasia in the cecum.

^b: No direct comparison can be made between the results in rats and humans, as mucosal aliskiren concentration was not measured in rat rectum. The rectal mucosal concentration in humans is about six-fold lower (mean data) than in rat colon. The sponsor suggests "rat rectal mucosal aliskiren concentration would be expected to be higher than that in colon due to local differences in luminal drug exposure (rat colonic content: 502 µg/g *versus* rat feces: 10900-16900 µg/g)".

^c: The fecal aliskiren concentrations were taken when plasma aliskiren was at steady state, beginning after dosing on day 8.

Evaluation

Rat cecum and colon had the highest predisposition to develop mucosal hyperplasia though all regions of the gastrointestinal tract were equally exposed to aliskiren in the diet, suggesting tissue differences in aliskiren sensitivity. The onset was very rapid and occurred within 1 to 3 days of treatment. Local irritation triggered by high local concentrations (due to very low absorption) of aliskiren reflects an inflammatory process, and subsequent epithelial reaction with proliferation, growth factor induction and modification in signal transduction pathways due to induction of transcription factors seen 4 weeks into the treatment. However, the 13-week mechanistic study suggested the presence of an adaptation mechanism, leading to a regression of the inflammatory profile identified in the 1 and 4 week studies. In the 13 week study no proliferative or inflammatory changes were noted at 250 mg/kg/day; however, a maintained epithelial reaction

was noted in the cecum and colon at 750 or more mg/kg/day. This could result in the chronic inflammatory and proliferative changes observed at these doses in the carcinogenicity study. A complete reversibility of the GI changes was demonstrated after 4 weeks of recovery.

As in rodents and marmosets, in humans, aliskiren hemifumarate is poorly absorbed (approximately 3%) in the GIT and is predominantly excreted as unabsorbed, unchanged compound. *In vitro* studies showed that human intestinal tissue was less sensitive (in terms of causing cell injury) than rat tissue to the local effects of aliskiren. However, chronic administration of aliskiren may have a local irritant effect on the GI tract tissues. This is evident from clinical studies in which diarrhea was a prominent finding (6-12%) at the 600 mg dose (diarrhea and not hyperplasia was the predominant effect in marmosets). In discussing the GI issues (the gastrointestinal safety review, release date November 24, 2006), the sponsor asserts that a wide exposure margin exists between rat and human fecal concentrations (see the Table above). However, a dose not causing hyperplasia in cecum or colon was not established in the 13-week study. Thus, the basis for calculating the safety margin is questionable (also note wide range of variation between individual samples). On the other hand, as noted in the *in vitro* transepithelial tissue conductance studies, human intestinal tissue is less sensitive than rat tissue to the local cytotoxic effects of aliskiren and this could simply result in diarrhea rather than precipitating inflammatory and proliferative changes as noted in rats.

RECOMMENDATIONS

No change to recommendations contained in original pharmacology/toxicology review.

Reviewer signature: _____

Accepted by _____ on _____

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/s/

Gowra Jagadeesh
2/13/2007 11:24:34 AM
PHARMACOLOGIST

Charles Resnick
2/13/2007 12:51:02 PM
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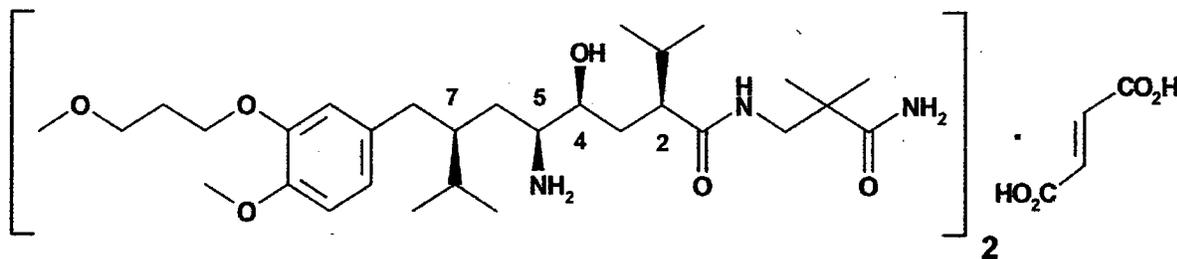


DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER: 21-985
SERIAL NUMBER: EDR
DATE RECEIVED BY CENTER: 2/21/06
DRUG PRODUCT: Rasilez[®] Tablets
DRUG SUBSTANCE: Aliskiren Hemifumarate
INTENDED CLINICAL POPULATION: Hypertensive
SPONSOR: Novartis Pharmaceuticals Corporation
REVIEW DIVISION: Division of Cardiovascular and Renal Drug
Products
PHARM/TOX REVIEWER: G. Jagadeesh, Ph.D.
PHARM/TOX SUPERVISOR: Charles Resnick, Ph.D.
DIVISION DIRECTOR: Norman Stockbridge, M.D., Ph.D.
PROJECT MANAGER: John David

Date of review submission to Division File System (DFS): 9/26/06

NDA Number: 21,985**Date of Submission:** 2-10-06**Center Receipt Date:** 2-21-06**Sponsor:** Novartis Pharmaceuticals Corporation**Manufacturer of Drug Substance:** Novartis Pharmaceuticals Corporation**Reviewer:** G. Jagadeesh, Ph.D.**Division:** Division of Cardiovascular and Renal products**Review Completion Date:****Drug Product:** Rasilez[®] Tablets**Drug Substance:***Generic name:* Aliskiren hemifumarate*Code name:* SPP100 (base), SPP100B (hemifumarate), CGP60536B*Chemical name:* (2S,4S,5S,7S)-N-(3-amino-2,2-dimethyl-3-oxopropyl)-2,7-diisopropyl-4-hydroxy-5-amino-8-[4-methoxy-3-(3-methoxypropoxy)phenyl]octanamide hemifumarate.*Chemistry:* Aliskiren is a single diastereoisomer having 4 chiral centers, all S-configured. The active substance is the hemi-fumarate salt (2:1 ratio) of the corresponding amine and is white to slightly yellowish powder. It is hygroscopic and very soluble in aqueous media from pH 1 to 7.6.*CAS registry number:* 173334-58-2*Molecular formula/molecular weight:* C₃₀H₅₃N₃O₆ · 0.5 C₄H₄O₄
551.8 (free base), 609.8 (hemifumarate)**Related INDs/NDAs/DMFs:** Novartis' IND 62,976 for aliskiren hemifumarate for the treatment of hypertension**Drug Class:** Renin inhibitor**Intended Clinical Population:** Hypertensive subjects**Clinical Formulation:** Aliskiren immediate release film-coated tablets will be supplied in two dosage strengths for oral administration: 150 mg and 300 mg. The composition is provided in the following Table.

COMPOSITION OF ALISKIREN 150 MG AND 300 MG FILM-COATED TABLET

Ingredient	Amount per tablet (mg)		Function
	150mg tablet	300mg tablet	
SPP100 hemifumarate ¹⁾			Drug substance
Cellulose microcrystalline / Microcrystalline Cellulose			
Crospovidone			
Povidone			
Magnesium stearate			
Colloidal Silicon Dioxide			
Core tablet weight			

Route of Administration: Oral

Proposed Dosage Regimen: One tablet daily.

Disclaimer: Unless indicated otherwise, tables and graphs (with or without editorial corrections by the reviewer) are taken from the sponsor's submission.

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EXECUTIVE SUMMARY

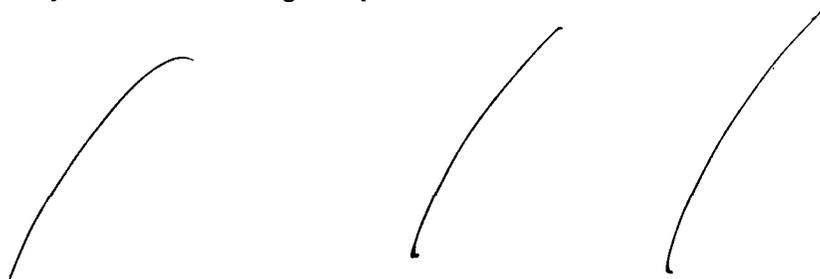
I. Background

Control of blood pressure by the renin angiotensin system (RAS) is exerted through multiple actions of angiotensin II, the central product of the RAS. Angiotensin II is a potent vasoconstrictor and stimulator of aldosterone biosynthesis in the adrenal cortex. It plays a pivotal role in the pathophysiology of hypertension, cardiac hypertrophy and remodeling, heart failure, vascular thickening, atherosclerosis and electrolyte balance. The importance of the RAS is reflected in the observation that angiotensin II levels are elevated in renovascular and malignant hypertension.

Pharmacological agents can interrupt the functioning of the RAS by preventing the actions of angiotensin II by reducing its production or preventing its binding to target receptors. Blocking the actions of angiotensin II at its target sites, AT-1 receptors, is regarded as one of the effective methods in the treatment of hypertension. ACE inhibitors, one level up in the cascade, block the conversion of angiotensin I to angiotensin II and have constituted one of the major advances in the treatment of high blood pressure and cardiac insufficiency. Blocking the action of the enzyme renin, a rate-limiting step in the synthesis of angiotensin I from angiotensinogen, prevents the production of angiotensin peptides by the ACE and non-ACE pathways. Renin is secreted by the kidney in response to a decrease in blood volume and blood pressure. Since renin inhibitors block the RAS at a higher level in the cascade than do ACE inhibitors or angiotensin II receptor blockers, they produce a broader inhibition of the RAS. Aliskiren is a non-peptide, low molecular weight, potent and selective inhibitor of human renin. It displays strong species-dependent substrate specificity. No member of this drug class has yet been approved for marketing. It will be the first in the class, if approved, for the treatment of hypertension.

II. Recommendations

- A. **Recommendation on Approvability:** Approvable
- B. **Recommendation for Nonclinical Studies:** None
- C. **Recommendations on Labeling:** Those sections in the proposed labeling (EDR version dated February 10, 2006) that deal with preclinical studies covered by this review are considered satisfactory with the following exceptions.

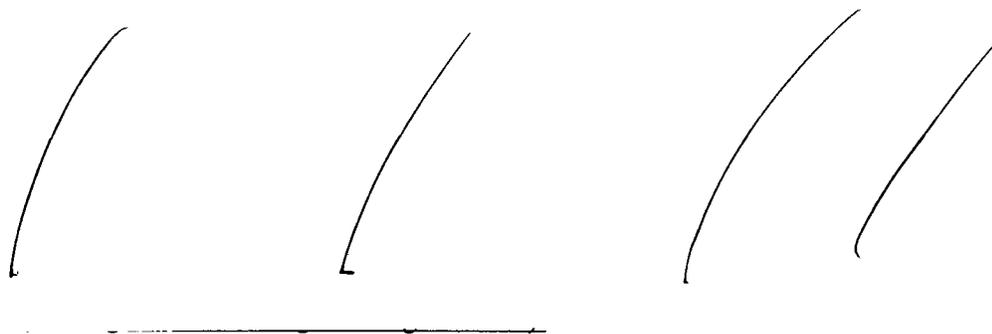


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III. Summary of Nonclinical Findings

- A. **Brief Overview of Pharmacology:** Aliskiren demonstrated high selectivity for human renin ($IC_{50} = 0.6$ nM) compared to renin from marmoset, dog, rabbit, and rat (IC_{50} s of 2.0, 7.0, 11.0, and 80 nM, respectively). The ability of aliskiren to lower mean arterial blood pressure (MAP) and block the RAS was demonstrated in severely and mildly sodium depleted marmosets after single or repeated oral dosing. Evidence of RAS blockade was shown by inhibition of plasma renin activity and increased plasma concentration of renin, a compensatory feedback loop due to less availability of angiotensin II at the AT-1 receptor site. Aliskiren (50 mg/kg/day for 4 weeks *via* subcutaneous osmotic minipump) significantly reduced MAP (37 to 47 mm Hg drop relative to sham vehicle group) in both renin-dependent (Goldblatt model) and -independent (uni-nephrectomy and clipping of both renal arteries) hypertensive, hypercholesterolemic (ApoE^{-/-}) mice¹. Effects of single and repeat oral doses of aliskiren were studied in conscious, unrestrained male double transgenic rats² (dTGR). Aliskiren rapidly and dose-dependently reduced mean arterial blood pressure following single or repeat oral doses. The highest single (100 mg/kg) oral dose of aliskiren resulted in a prolonged antihypertensive effect that lasted for more than 24 hr after dosing. The oral antihypertensive potency of aliskiren in dTGR is similar to that observed in the sodium-depleted marmoset. In safety pharmacology studies, aliskiren (0.3 to 3 mg/kg, i.v.) displayed no significant effects on the ECG, respiratory or renal function in anesthetized normotensive rats. *In vitro* receptor binding assays showed no significant affinity of aliskiren (at 10 μ M) for 16 different

¹ C57/BL/6 background, apolipoprotein E gene knock out

² Human renin does not effectively cleave rat angiotensinogen, and *vice versa*. The single transgenic rats (i.e., transgenic for either human angiotensinogen or renin) are normotensive. However, when cross-bred, the dTGR offspring develop (at 3 weeks of age) severe and sustained hypertension (due to life-long over-expression of human renin and angiotensinogen, i.e., over-stimulated RAS) with severe organ damage and do not live beyond the seventh or eighth week of age (Pilz, B., *et al.* Aliskiren, a human renin inhibitor, ameliorates cardiac and renal damage in double-transgenic rats. *Hypertension* 46: 569-576, 2005).

neurotransmitter receptors and no effect on potassium current in cloned hERG channels.

- B. Brief Overview of Drug Disposition (ADME):** Aliskiren hemifumarate was rapidly absorbed following single or multiple oral administrations in mice, rats, rabbits and marmosets with T_{max} values of 0.25 hr to 2 hr. Oral absorption was highly variable in all species (including human) and ranged from 2 to 25% of the dose. Oral bioavailability of aliskiren, calculated from AUC values, was 3%, 2.4%, 1.5% and 1.9 to 2.6% in marmosets, rats, mice and humans, respectively. The plasma concentration-time profile studies in rats and marmosets indicate a rapid and multi-exponential decline with terminal half-lives of 23 and 36 hr in the rat and the marmoset (human, 24-49 hr), respectively. Whole-body autoradioluminograms in rats showed that the overall exposure to aliskiren was marginal, which is in line with the poor to moderate oral absorption. The highest radioactivity concentration was observed in the lumen of the gastrointestinal tract, especially the colon and cecum, reflecting mostly non-absorbed material. Radioactivity was eliminated within 24 hr from all tissues, except the intestinal wall and brown fat. Embryo-fetal transfer of radioactivity was noted in pregnant rabbits (day 17 of gestation). Significant concentrations of radioactivity were found in placenta, amniotic fluid and fetuses, suggesting a free passage of parent compound and its metabolites. *In vitro* plasma protein binding was highest in marmoset (92%) followed by mouse (71%), rat (62%), rabbit (58%) and human (49-52%). Biotransformation occurred to a low extent since unchanged parent compound accounted for >90% of radioactivity in plasma and excreta. Only a small part of the absorbed aliskiren was metabolized. The primary biotransformation reactions of aliskiren were oxidation at the phenolic moiety and the attached side chain. CYP3A4/5 enzymes catalyzed these oxidations predominantly. Additionally, amide hydrolysis was observed as well as some glucuronidation of oxidated metabolites. All metabolites observed in plasma were also found in the excreta either in free or conjugated form. Aliskiren (200 μ M) showed significant and distinct inhibition of CYP2C9 (32%), CYP2C19 (43%) and CYP2D6 (33%). At more relevant *in vivo* concentrations (25 μ M), none of the investigated cytochromes were substantially inhibited by aliskiren. Aliskiren and its metabolites were excreted mainly *via* the hepato-biliary route (about 70% of the dose in bile) into feces (>85%). Renal excretion was minor in all species (0.3%-15%) including human (0.6%). Aliskiren was excreted mainly unchanged with only minor proportions of metabolites detected in excreta. About 0.08% of the administered dose was estimated to be excreted in the milk of lactating rats between 0 and 72 hours. Assuming a systemic availability of 6.7% after oral administration for total radioactivity, approximately 1.1% of the systemically available radioactivity was eliminated *via* milk in rats.
- C. Brief Overview of Toxicology:** A detailed toxicology evaluation (single dose toxicity, repeat dose toxicity, carcinogenicity, genotoxicity and reproductive toxicity studies) was conducted to support the chronic administration of aliskiren to adult hypertensive patients.

In a single dose toxicity study, aliskiren was well tolerated by rats at the highest tested oral (gavage) dose, 2000 mg/kg.

Repeat dose toxicity studies of aliskiren hemifumarate in mice, rats and marmosets revealed different targets for toxicity (respiratory system, gastrointestinal tract and/or kidney), dependent largely on the route of administration, duration of study and the dosage employed. Irritation of the respiratory tract was manifested by ulceration and inflammatory exudates in the nasal cavities and nasopharynx, tracheo-pulmonary irritation/necrosis and respiratory distress resulting in premature deaths at doses of 200 or more mg/kg/day in oral gavage studies in rats and mice of 13 week or longer duration. Deaths were attributed to aspiration of the dosing solution into the respiratory tract rather than systemic toxic effects. Most of the chronic toxicity studies that evaluated doses in excess of 250 mg/kg/day employed dietary administration of aliskiren hemifumarate and in those studies none of these respiratory tract effects were noted at doses as high as 1500 mg/kg/day, administered for up to 104 weeks to rats and mice. Another target was the gastrointestinal tract of the rat. Histopathological lesions indicative of local gastrointestinal tract (GIT) irritation in rats were observed as minimal inflammatory cell infiltration, atrophy or basophilia in the cecum or colon in rats receiving 2000 mg/kg/day in a comet assay (2nd day sacrifice); mucosal hyperplasia in the cecum or colon at doses of 250 or more mg/kg/day in a 1, 4 and 13 week dietary mechanistic study; erosion and/or ulceration in the cecum and/or colon in animals receiving 750 mg/kg/day in a 13 week oral gavage study; and increased incidence of mucosal hyperplasia of the GIT and erosion/ulceration of the cecum and/or colon at doses of 750 or more mg/kg/day in the rat carcinogenicity study (noted at both 52 and 104 week sacrifices). In transgenic mice, an increased incidence of diffuse mucosal hypertrophy was noted in duodenum, jejunum, cecum and colon in both sexes at 1500 mg/kg/day (findings in cecum at 750 or more mg/kg/day) in a 26 week carcinogenicity study. In addition, focal atypical hyperplasia, a pre-neoplastic finding, was noted in the colons of animals receiving 1500 mg/kg/day (1 male and 3 females). This is not a common spontaneous lesion in this mouse strain. None of the above findings in mice were noted in concurrent control animals. In marmosets, GIT intolerance was reflected as salivation, vomiting and diarrhea at doses of 50 or more mg/kg/day in a 13 week study. The third organ of toxicity was kidney. Degeneration/regeneration of renal cortical tubules and arteriolar hypertrophy (attributed to hypotension and poor renal perfusion resulting from treatment) were noted in moribund and scheduled sacrificed marmosets at doses as low as 20 mg/kg/day administered for 13 weeks or more. The effects were correlated with significantly increased creatinine and blood urea values and significantly increased mean absolute and relative kidney weights. Except for creatinine and BUN values, this pathology was evident in animals following a 4 or 8 week drug free recovery period. The kidney effects were not noted in rodents. Another effect associated with the administration of aliskiren hemifumarate was a significant ($p < 0.05$) reduction in overall body weight gain relative to control at doses as low as 1000 mg/kg/day in 4 or more week studies in mice. In rats, a significant ($p < 0.05$) reduction in group mean body weight relative to control was noted at gavage doses as low as 150 mg/kg/day in 13 or more week studies. Body weights

were less affected with dietary administration than with gavage at equivalent doses in rats. Statistically significant, dose-dependent reductions in mean body weight (6 to 28.5% that of concurrent control) and mean body weight gain (10 to 47% that of concurrent control) were observed at doses of 250 or more mg/kg/day from week 26 onwards. A dose-dependent reduction in body weight gain relative to control (12 to 20% for females, 23 to 67% for males) was noted at doses of 1500 or more mg/kg/day as early as 2 weeks. For marmosets, a significant ($p < 0.05$) reduction in body weight relative to control was observed for females only at doses of 20 or more mg/kg/day as early as 13 weeks.

The 24 month dietary carcinogenicity study in rats found one colonic adenoma and one cecal adenocarcinoma in males at 1500 mg/kg/day. The incidences of rats with adenoma, adenocarcinoma or with either tumor were not significantly increased above concurrent control. Since the incidence of large intestinal adenoma/adenocarcinoma in historical controls is low (<0.1%), a relationship of these tumors to aliskiren-treatment cannot be ruled out. In addition, there were findings (also noted at the 1 year sacrifice) of an increased incidence (mostly dose-dependent) of mucosal epithelial hyperplasia in the duodenum, cecum, colon and rectum of both sexes at doses of 250 or more mg/kg/day. The hyperplasia was associated with inflammation, inflammatory exudates in the lumen and/or erosions/ulcerations in some animals.

The 26 week dietary carcinogenicity study in transgenic (*rasH2*) mice revealed focal atypical mucosal hyperplasia (a pre-neoplastic finding) in the colon at 1500 mg/kg/day (1 male and 3 females). In addition, an increased incidence of minimal to moderate hypertrophy/hyperplasia in the small intestine and cecum of both sexes and atrophic changes in the female reproductive organs were noted at doses of 750 or more mg/kg/day. All of these findings were absent in the concurrent control. Reduced hematopoiesis in the spleen and bone marrow was noted in females receiving 1500 mg/kg/day. There were no increases in the incidence of any tumor type.

Genotoxicity: Aliskiren hemifumarate was negative in all tester strains in the Ames reverse mutation assay both in the absence and presence of metabolic activation. No positive results for clastogenicity were noted in the *in vitro* CHO chromosomal aberration assay, the *in vitro* gene mutation test with Chinese hamster V79 cells, or in the *in vivo* mouse micronucleus assay. In addition, an *in vivo* comet assay, which detects DNA damage, indicated that aliskiren has no potential to induce DNA damage in liver, colon or cecum of rats.

Reproductive Toxicity: Studies evaluated effects of aliskiren on male and female fertility and early embryonic development in rats, teratogenic potential in rats and rabbits and pre- and postnatal development in rats. No adverse effects on fertility of males or females, on early embryonic development or on pre- and postnatal development were observed when aliskiren hemifumarate was administered to rats at doses of up to 250 mg aliskiren/kg/day. This dose did not cause parental toxicity and also had no effect on the reproductive performance of the F₁ generation. No evidence of embryo-fetal toxicity or teratogenicity was noted at doses up to 600 mg aliskiren/kg/day in pregnant rats. A developmental toxicity study in pregnant rabbits

demonstrated maternal toxicity (deaths, abortions, significant dose-dependent reduction in mean body weight gain, food and water intake relative to control) at doses as low as 50 mg aliskiren/kg/day. A group treated with 200 mg aliskiren/kg/day was terminated early (after 7 doses) as a result of a large number of deaths. All of those rabbits showed inappetence and loss of body weight. A statistically significant and dose-dependent decrease (2 to 18%) in mean litter weight was noted at 50 or more mg/kg/day. This study failed to identify a NOAEL for maternal or fetal toxicity. Levels of aliskiren in plasma were not determined in any of the reproductive toxicity studies. The maximum dose evaluated for teratogenic potential in rats (600 mg/kg/day) is, on a mg/m^2 basis, about 22 times the maximum recommended human dose of aliskiren (300 mg/day), but the maximum dose that could be evaluated for teratogenic potential in rabbits (100 mg/kg/day) is, on a mg/m^2 basis, only about 7 times the MRHD of aliskiren. Fetal birth weight was adversely affected in rabbits (not in rats) at doses as low as 50 mg/kg/day, the lowest dose used in the study and on a mg/m^2 basis, only about 3.6 times the MRHD of aliskiren.

D. Nonclinical Safety Issues Relevant to Clinical Use

Test substance-associated pre-neoplastic focal atypical hyperplasia was noted ($p > 0.05$) in the colons of transgenic rasH2 mice (1 of 25 males and 3 of 25 females) at 1500 mg/kg/day. This atypical hyperplasia of the colon was absent in the concurrent control mice and, according to the sponsor, is not a common spontaneous lesion. Additionally, an increased incidence of mucosal hyperplasia/hypertrophy was noted in both sexes in the cecum at 750 and 1500 mg/kg/day and in the duodenum, jejunum, ileum and colon at 1500 mg/kg/day. None of these findings were seen in control animals. Similarly, in rats, an increased incidence of mucosal epithelial hyperplasia was noted in the small and large intestines of both sexes at 250 or more mg/kg/day. Cecal erosion and ulceration were noted with increased incidence in both sexes at 750 or more mg/kg/day. One colonic adenoma and one cecal adenocarcinoma were also observed ($p > 0.05$) in males (1 of 50 each) receiving 1500 mg/kg/day. Rat cecum and colon had the highest predisposition to develop mucosal hyperplasia though all regions of the GIT were equally exposed to aliskiren in the diet, suggesting tissue differences in aliskiren sensitivity. The onset was very rapid and occurred within 1 to 3 days of treatment as demonstrated in 1 and 4 week dietary mechanistic studies. The sponsor attributes rodent specific mucosal hyperplasia/hypertrophy in the gastrointestinal tract to an irritative effect of aliskiren. Local irritation triggered by high local concentrations (due to very low absorption) of aliskiren reflects an inflammatory process, and subsequent epithelial reaction with proliferation, growth factor induction and modification in signal transduction pathways due to induction of transcription factors was seen as early as 1 week into the treatment. However, a 13 week mechanistic study suggested the presence of an adaptation mechanism, leading to a regression of the inflammatory profile identified in the 1 and 4 week studies. Thus, in the 13 week study no proliferative or inflammatory changes were noted at 250 mg/kg/day; however, a maintained epithelial reaction was noted in the cecum and colon at 750 or more mg/kg/day. This, according to the sponsor, could result in the chronic inflammatory and proliferative changes

observed at these doses in the carcinogenicity study. In marmosets, diarrhea was the predominant effect after exposure to high oral dietary doses of 50 or more mg aliskiren/kg/day. As in rodents and marmosets, in humans, aliskiren is poorly absorbed (approximately 3%) in the GIT and is predominantly excreted as unabsorbed, unchanged compound. *In vitro* studies showed that human intestinal tissue was less sensitive than rat tissue to the local effects of aliskiren. However, chronic administration of aliskiren may have a local irritant effect in the GIT in general, and cecum and colon in particular. This is evident from clinical studies in which diarrhea was a prominent finding (6-12%) at the 600 mg dose. We do not have a basis to predict that the local irritant effect of aliskiren in humans will result in the atypical hyperplasia seen in rodents. Kidneys were the target organ for toxicity in marmosets. In 13 and 26 weeks studies, kidneys of marmosets receiving 20 or more mg/kg/day showed evidence of urinary stasis and tubular dilatation with degeneration, arteriolar or juxtaglomerular hypertrophy/hyperplasia and interstitial inflammation. Additionally, treatment with aliskiren was associated with elevated serum creatinine and blood urea nitrogen and increased kidney weights. Though the biochemical changes were reversible, the renal lesions were still evident following a 4 or 8 week recovery period. The sponsor attributes the kidney effect to prolonged periods of markedly reduced blood pressure at doses 28-fold higher (on a body weight basis) than the minimally effective pharmacological dose in the marmoset (0.7 mg/kg). However, that renally-toxic dose in the marmoset (20 mg/kg/day) is only about 4 times the maximum dose administered in the clinical trials (300 mg/day or 5 mg/kg/day in a 60 kg patient).

Local irritating properties of aliskiren (manifested by ulceration and inflammatory exudates in the nasal cavities and nasopharynx, tracheo-pulmonary irritation/necrosis and respiratory distress) following gavage and not dietary administration in rodents may not be relevant to the clinical situation since encapsulated tablets are administered to patients.

Though there was no significant effect of aliskiren during the reproductive toxicity studies in rats and rabbits, the administration of drugs affecting the RAS during the 2nd or 3rd trimester of pregnancy has been associated with fetal malformations and neonatal deaths. A recent publication notes a significant increase in the risk of cardiovascular and central nervous system congenital malformations with the use of ACE inhibitors during the first trimester.³ Additionally, aliskiren and/or its metabolites were found in significant concentrations in the placenta, amniotic fluid and fetuses in pregnant rabbits and in the milk of lactating rats.

³ Cooper, W.O. *et al.*: Major congenital malformations after first-trimester exposure to ACE inhibitors. *N Engl J Med* 354: 2443-51, 2006

Friedman, J.M.: ACE Inhibitors and Congenital Anomalies. *N Engl J Med* 354: 2498-00, 2006.

In conclusion, aliskiren did not demonstrate carcinogenic, genotoxic or teratogenic activity in animal studies. The results of the preclinical studies suggest that aliskiren can be used safely in humans for the treatment of hypertension, at the intended therapeutic dose and in accordance with the proposed product labeling.

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PHARMACOLOGY/TOXICOLOGY REVIEW

1.0. PHARMACODYNAMICS

1.1. Studies Related to Proposed Therapeutic Indication

In vitro studies

1.1.1. Potency and Enzyme Specificity of Aliskiren in vitro

This non-GLP study (report #BS 15/1995, dated September 19, 1995) was conducted at _____

Aliskiren was studied for its inhibitory potency and specificity for human renin.

Human recombinant renin (0.33 ng/ml) was incubated with a synthetic tetradecapeptide substrate (13.33 μ M) for 1 hr at 37°C. Ang I generated during the incubation was measured by radioimmunoassay (RIA). The potency of aliskiren to inhibit various other human aspartic proteinases was also studied. Results were expressed as IC₅₀ values, the concentration of inhibitor reducing 50% of the amount of product generated in the absence of an inhibitor. Aliskiren inhibited human renin with an IC₅₀ value in the subnanomolar range (0.6 nM) and was weakly active against other proteinases (Table 1.1.1.1).

TABLE 1.1.1.1
ENZYME SPECIFICITY OF ALISKIREN

Enzyme	IC ₅₀ (nM)
Renin	0.6
Cathepsin D	5000
Cathepsin E	>10,000
Pepsin	>10,000
HIV-1 peptidase	>10,000

In the second part of the study, the species specificity of aliskiren was tested by determining the concentration that inhibited by 50% (IC₅₀) the activity of endogenous renin in the plasma from human, marmoset, rat, dog, rabbit, cat, pig or guinea pig. Aliskiren was 3.3-fold more potent against human renin than against marmoset renin, while it was moderately active against renin from non-primate species such as dog and rabbit. It was weakly active against renin from rat, cat, pig and guinea pig (Table 1.1.1.2).

TABLE 1.1.1.2
INHIBITORY POTENCY OF ALISKIREN ON PRA IN VARIOUS SPECIES

Animal	IC50 (nM)
Human	0.6
Marmoset	2
Dog	7
Rabbit	11
Guinea pig	63
Rat	80
Pig	150
Cat	8500

In vivo studies

Aliskiren was tested in normotensive marmosets, hypertensive mice and rats, and transgenic rats, primarily for its ability to inhibit the RAS and reduce blood pressure.

1.1.2. Effect of Single Dose Aliskiren in Sodium-Depleted Marmosets

This non-GLP study (study #BS 17/1995, report #RD-1995-03292, dated September 19, 1995) was conducted at

Aliskiren was studied for its ability to lower blood pressure and inhibit plasma renin activity (PRA) after oral or intravenous administration in severely sodium-depleted marmosets.

Marmosets (*Callithrix jacchus*) of both sexes weighing approximately 350 gm were maintained on a low-salt pellet diet for 1 week before and during the experiment. All animals received the diuretic, furosemide, in drinking water (6 mg/kg/day) 2 days before the start and a bolus dose (9 mg/kg, i.m.) 20 hr before drug treatment. Blood pressure and heart rate were measured by a telemetric system in unrestrained males cannulated 4 weeks before the start of the experiment. PRA and plasma concentrations of drug were measured in female marmosets. The hydrochloride salt of aliskiren was dissolved either in distilled water for oral administration or 5% dextrose for intravenous administration.

Aliskiren HCl was administered to the sodium-depleted marmosets orally by gavage as a single dose of 0.3, 1, 3, or 10 mg/kg, or intravenously as a single dose of 0.003, 0.01, 0.03 and 0.1 mg/kg. Animals were not fasted. Plasma concentrations of aliskiren were measured using an enzymatic assay. PRA was measured by the antibody trapping method.

Oral administration of a single dose of aliskiren caused dose-dependent reductions in mean arterial blood pressure (MAP). No significant change in MAP was observed after administration of either vehicle or 0.3 mg aliskiren/kg. The threshold dose of aliskiren required to lower MAP significantly ($p < 0.05$) was between 0.3 and 1 mg/kg; 1 mg aliskiren/kg lowered MAP by 14 ± 5 mmHg, while 3 mg/kg lowered MAP by 30 ± 3 mmHg ($p < 0.001$ relative to vehicle control). A

higher dose (10 mg/kg) did not further increase the magnitude of the MAP response, but the duration of the response increased (up to 16 hr, Figure 1.1.2.1). The threshold dose for i.v. administration was between 0.003 and 0.1 mg/kg and the maximum reduction in MAP of 25 mmHg was noted at a dose of 0.03 mg/kg (Figure 1.1.2.2). Higher doses increased the duration of the response (from 2 hr to 6 hr) without further increasing the hypotensive response. Aliskiren had no significant effect on heart rate after oral or i.v. administration.

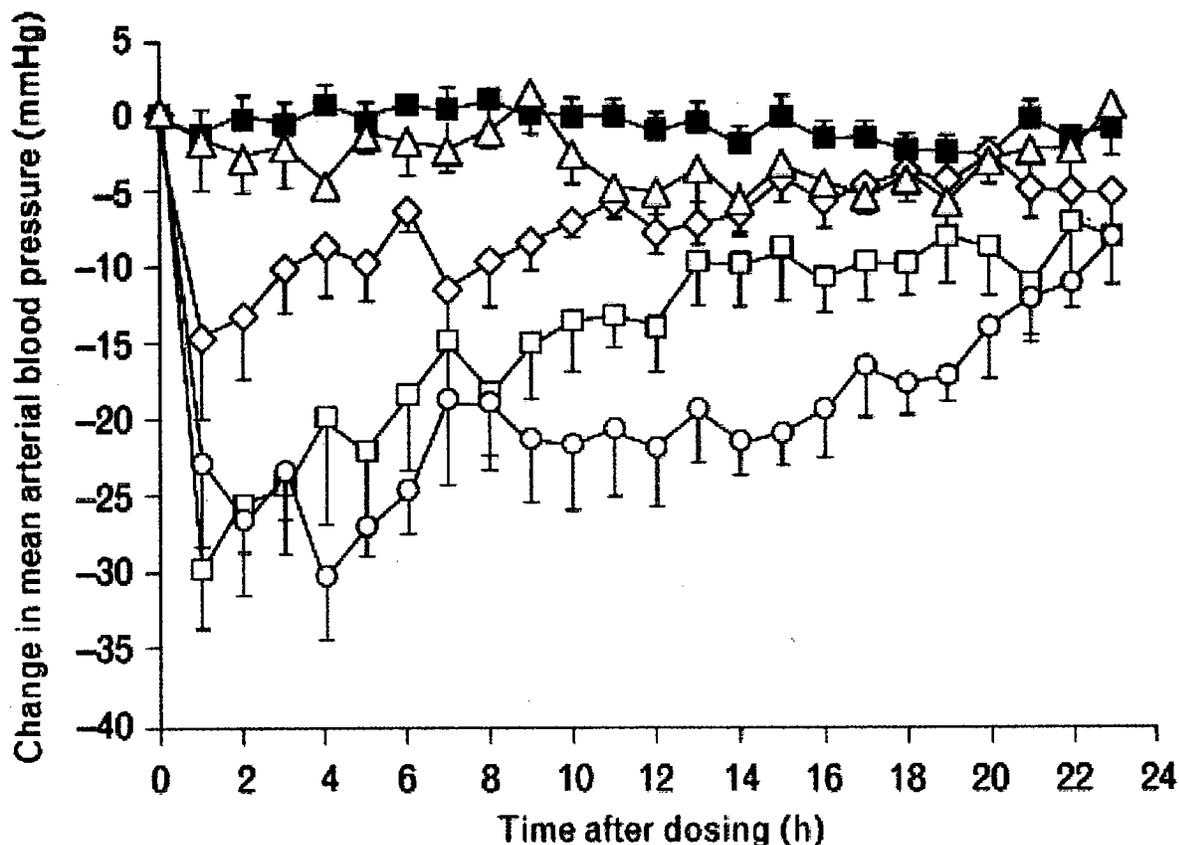


Fig. 1.1.2.1.: Changes from baseline in MAP following administration of a single oral dose of aliskiren HCl: 0.3 mg/kg (open triangles), 1 mg/kg (open diamonds), 3 mg/kg (open squares) or 10 mg/kg (open circles), or vehicle control (filled squares) to severely sodium-depleted marmosets. MAP was continuously measured by telemetry. Data presented as mean \pm SEM. Mean baseline MAP (mmHg) in each group: vehicle: 80 ± 2 ; 0.3 mg/kg aliskiren: 78 ± 4 ; 1 mg/kg aliskiren: 80 ± 4 ; 3 mg/kg aliskiren: 81 ± 6 ; and 10 mg/kg aliskiren: 73 ± 5 . N=6/group.

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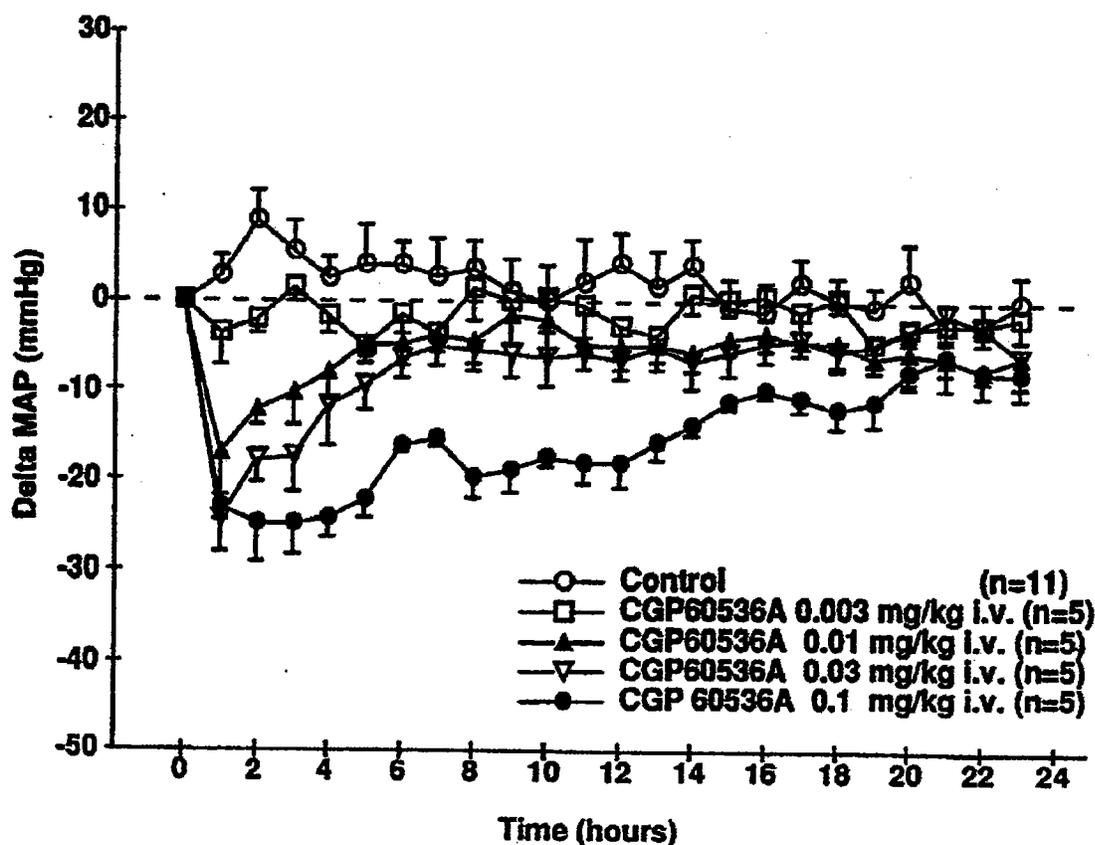


Fig. 1.1.2.2.: Changes in MAP after i.v. administration of aliskiren HCl to conscious sodium-depleted marmosets.

Plasma concentrations of aliskiren and PRA were measured in female marmosets after a single dose of aliskiren HCl. Plasma concentrations of aliskiren increased dose-dependently after both oral and i.v. administration of aliskiren HCl. PRA was completely inhibited up to 3 hrs after the lowest dose of 0.3 mg/kg, p.o. It was still inhibited by 87% after 6 hrs and returned to above baseline levels after 24 hrs. After i.v. dosing, PRA was almost completely inhibited 1.5 hrs after the lowest dose tested and had recovered to above baseline values by 24 hrs. The duration of complete inhibition increased with dose; PRA was completely inhibited for up to 6 hrs after the 0.1 mg/kg dose and was still inhibited by 61% at 24 hrs (Table 1.1.2.1). It may be noted that lower doses of aliskiren are required to inhibit PRA than to reduce MAP.

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TABLE 1.1.2.1
PLASMA ALISKIREN CONCENTRATIONS AND THEIR EFFECT ON PLASMA RENIN
ACTIVITY IN SEVERELY SODIUM DEPLETED MARMOSETS

Dose (mg/kg)	Plasma Parameter	Time (h)				N
		1.5	3	6	24	
0.3 p.o.	Conc. (µM)	0.12 ± 0.07	0.11 ± 0.07	0.04 ± 0.02	0.003 ± 0.003	4
	PRA inhib. (%)	100	99	87	-23	3
1.0 p.o.	Conc. (µM)	0.66 ± 0.3	0.44 ± 0.04	0.35 ± 0.36	0.028 ± 0.023	4
	PRA inhib. (%)	100	100	100	78	4
3.0 p.o.	Conc. (µM)	1.42 ± 1.29	0.90 ± 0.93	0.24 ± 0.17	0.06 ± 0.04	4
	PRA inhib. (%)	100	100	100	100	4
10.0 p.o.	Conc. (µM)	6.48 ± 2.22	3.13 ± 2.34	1.73 ± 0.92	0.33 ± 0.21	4
	PRA inhib. (%)	100	100	100	100	3
0.003 i.v.	Conc. (µM)	0.015 ± 0.006	0.006 ± 0.003	ND	ND	3
	PRA inhib. (%)	91	75	41	36	3
0.01 i.v.	Conc. (µM)	0.06 ± 0.02	0.02 ± 0.01	0.009 ± 0.007	0.001 ± 0.001	4
	PRA inhib. (%)	99	92	67	31	4
0.03 i.v.	Conc. (µM)	0.09 ± 0.022	0.046 ± 0.014	0.013 ± 0.002	0.004 ± 0.001	3
	PRA inhib. (%)	100	100	97	1	3
0.1 i.v.	Conc. (µM)	0.6 ± 0.17	0.21 ± 0.05	0.056 ± 0.008	0.008 ± 0.001	4
	PRA inhib. (%)	100	100	100	61	4

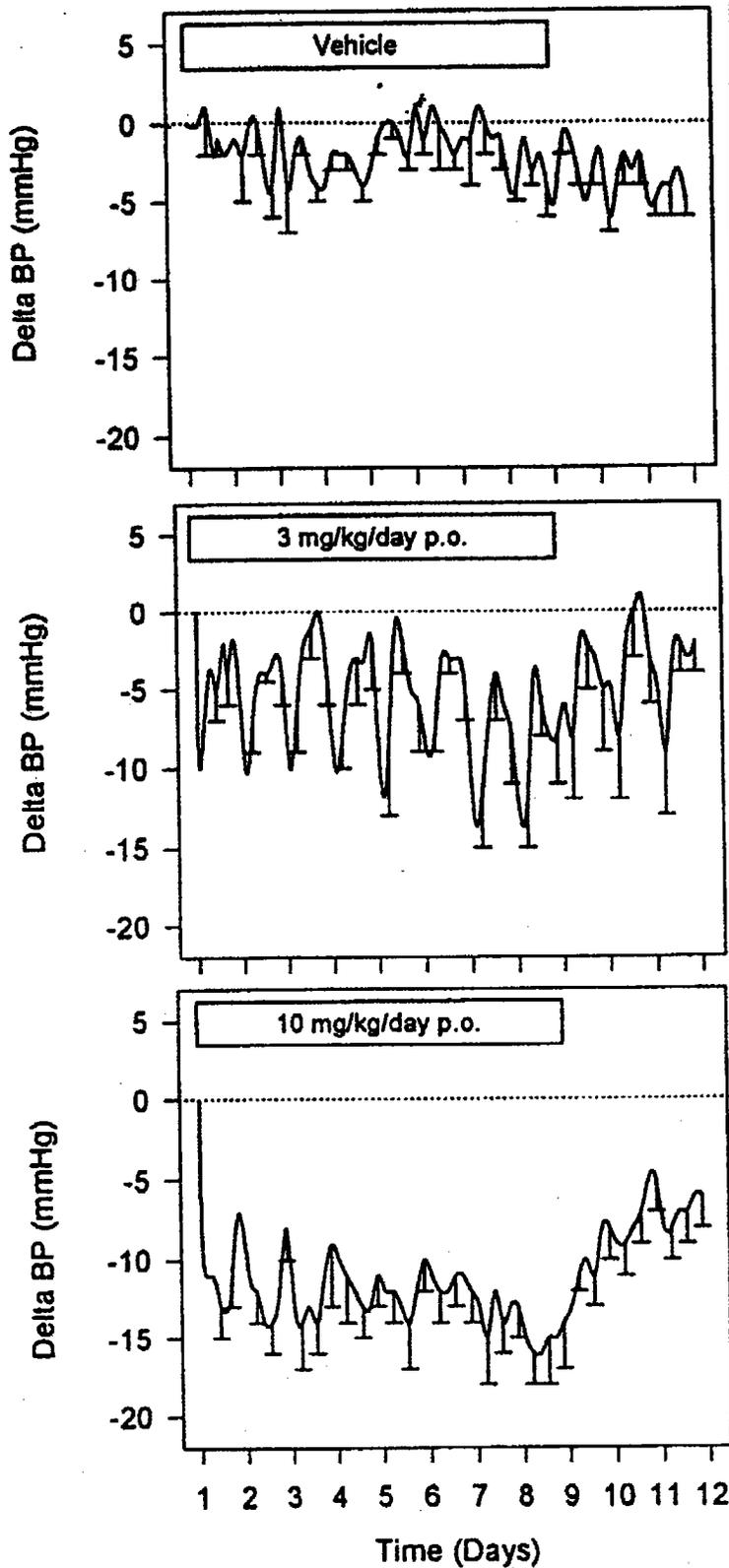
ND: not detectable; N: number of animals; Conc.: concentration of aliskiren; Inhib.: inhibition.

1.1.3. Effects of Repeated doses of Aliskiren in Mildly Sodium-Depleted Marmosets

This non-GLP study (study #BS 18/1995, report #RD-1995-02699, dated September 5, 1996) was conducted at

Aliskiren was studied for its ability to lower blood pressure and inhibit plasma renin activity (PRA) after repeated daily oral administration for 8 days in mildly sodium-depleted marmosets. The experimental procedure was similar to that described in the previous section except that furosemide was not administered to marmosets. Animals received vehicle (distilled water), 3 or 10 mg/kg aliskiren HCl orally by gavage once daily for 8 days in a non-fasted condition. As in the previous study, MAP and heart rate were evaluated in male marmosets implanted with telemetry transmitters. Plasma samples from mildly sodium depleted female marmosets were taken for the measurement of PRA, plasma concentrations of active and total renin, and plasma concentrations of aliskiren. The samples were taken before administration of the first dose, and 2 and 24 h after the last dose.

At a dose of 3 mg/kg, aliskiren HCl lowered MAP by approximately 10 mm Hg within 2 hr of administration on the first day of dosing. It remained lowered for about 4 hr and began to return to pretreatment values by 20 hr post dosing. A similar response was observed on subsequent days of dosing. In contrast, the high dose (10 mg/kg) lowered MAP maximally by approximately 13 mm Hg within 2 hr of administration. MAP did not return to baseline on the 2nd day of dosing as



it was still reduced by 10 mm Hg at the time of dosing. The recovery of MAP to base line at 24 hr was less complete on subsequent days of dosing. On the 8th (last) day of dosing, MAP was reduced to 15 mm Hg and returned to pretreatment values about 48 hr post dose (Fig. 1.1.3.1). There was no rebound increase in MAP after the end of treatment with either dose of aliskiren HCl. The maximum fall in MAP in mildly sodium depleted marmosets was 10-15 mmHg, which is less than that observed in severely sodium depleted marmosets (see above section 1.1.2) where a single dose of 3 mg aliskiren HCl/kg induced a 30 mm Hg drop in MAP. No significant changes in heart rate were seen with aliskiren HCl 3 mg/kg, and only transient increases in heart rate occurred within the first two hr after administration of aliskiren HCl 10 mg/kg.

Fig. 1.1.3.1.: Effect of multiple oral doses of aliskiren on mean arterial pressure and heart rate in mildly sodium depleted marmosets.

Plasma concentrations of active and total renin before treatment were similar in all treatment groups. In vehicle-treated marmosets, plasma concentrations of active and total renin tended to decrease with time. In contrast, plasma concentrations of total and active renin were significantly increased 2 hrs after the eighth and last dose in all of the aliskiren-treated marmosets. The increase was greatest in the marmosets treated with the 10 mg/kg dose. Plasma concentrations of renin were still significantly elevated 24 h after dosing either at 3 or 10 mg/kg (Fig. 1.1.3.2).

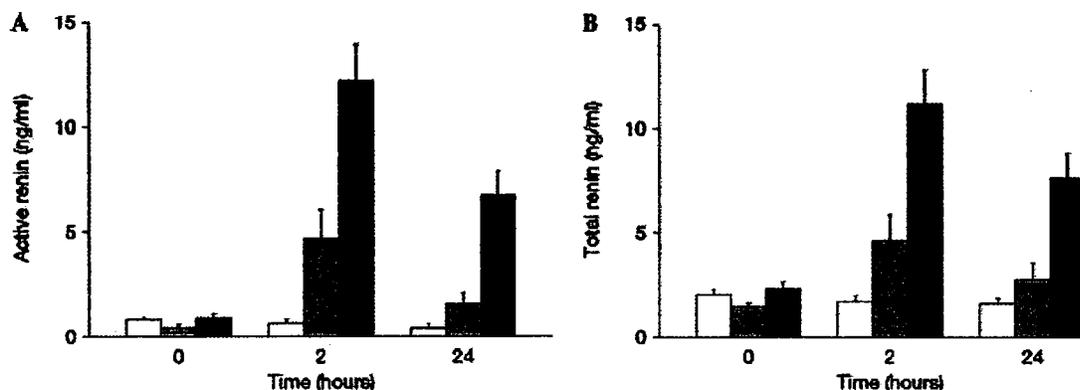


Fig. 1.1.3.2.: Effects of once daily oral doses of aliskiren 3 mg/kg (light bars, n = 5) or 10 mg/kg (dark bars, n = 10), or vehicle control (open bars, n = 7) on (A) plasma active renin levels and (B) plasma total renin levels in mildly sodium-depleted marmosets. Plasma samples were taken before administration of the first dose (baseline, time 0), and on day 8, 2 and 24 h after the last dose. Data are presented as means \pm SEM.

1.1.4. Effects of Aliskiren in Renin-Dependent and -Independent Hypertension in Mice

This non-GLP study (report #RD-2005-51368, dated December 19, 2005) was conducted at Novartis. Aliskiren was studied for its ability to lower blood pressure and inhibit plasma renin activity (PRA) after daily s.c. administration for 4 weeks in two models of hypertension in mice.

Male and female ApoE^{-/-} mice (C57/BL/6 background, apolipoprotein E gene knock out¹) were 15-16 weeks of age at the time of surgery. Mice were subjected to the 2K1C or 1K1C surgical procedure. Renin-dependent or renovascular hypertension was induced by placing a sliver clip on the left renal artery (2K1C or Goldblatt model) in bilaterally renal intact mice. In the case of the second model (1K1C model), volume overload hypertension was induced by uninephrectomy and clipping the remaining renal artery. The sham procedure consisted of performing the entire surgery with the exception of artery clippings. ApoE^{-/-} sham, 2K1C, and 1K1C mice were treated with aliskiren 50 mg/kg/day for four weeks, starting one week after surgery. Aliskiren (nature of the salt was not given) was dissolved in distilled water and administered *via* osmotic minipumps implanted subcutaneously between the scapulae. The minipumps used for control

¹ The mice used in this study were hypercholesterolemic (ApoE^{-/-}) because an additional objective of the study was to determine the effect of aliskiren on the development of atherosclerotic lesions in a high renin setting. The results from this portion of the work examining atherosclerosis were not yet complete at the time of the submission and therefore, were not reported here.

mice were filled with distilled water. MAP and heart rate were measured from the right carotid artery of conscious animals.

Clipping of the left renal artery (2K1C) in vehicle-treated mice resulted in hypertension (149 ± 1 mmHg) relative to the sham vehicle group (114 ± 3 mmHg $p < 0.001$). Aliskiren reduced this increase in MAP to a mean of 102 ± 2 mmHg ($p < 0.001$ vs 2K1C vehicle). In sham mice, aliskiren lowered MAP to 91 ± 2 mmHg, which is significantly below sham vehicle controls ($p = 0.009$). A similar increase in MAP was noted in 1K1C (151 ± 1 mmHg), a volume overload hypertension model, which is significantly different from that of sham vehicle animals (114 ± 3 mmHg; $p < 0.001$). Aliskiren significantly inhibited the rise in MAP in 1K1C mice ($p < 0.001$ vs 1K1C vehicle mice) (Fig. 1.1.4.1). Aliskiren had no significant effect of on heart rate in both hypertension models.

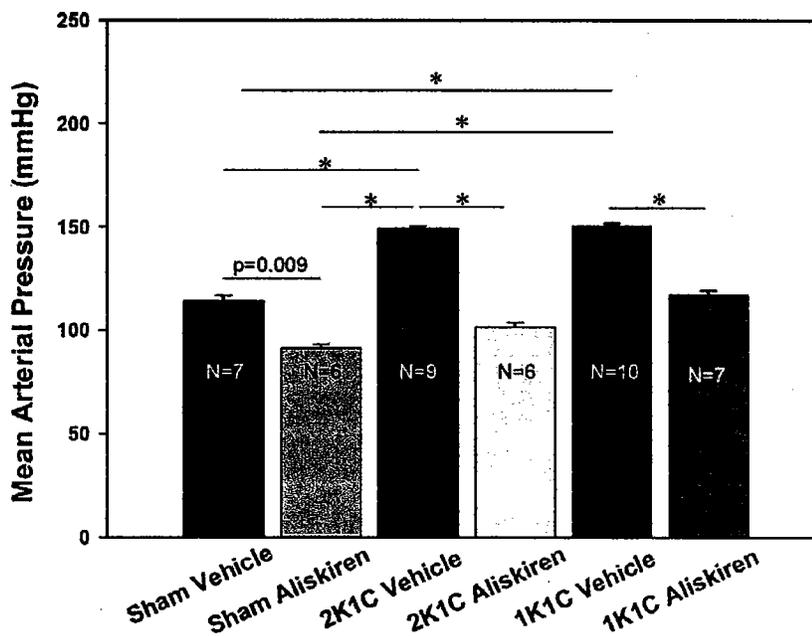


Fig. 1.1.4.1.: Effect of aliskiren treatment on blood pressure in hypercholesterolemic mice. Rats were subjected to renal artery clipping (2K1C) or uninephrectomy and renal artery clipping (1K1C) and treated with aliskiren 50 mg/kg/day or vehicle *via* osmotic minipumps for 4 weeks. MAP was measured directly from the carotid artery in conscious mice. * $p < 0.001$ (one-way ANOVA followed by Tukey's all pair wise multiple comparison test).

Plasma renin activity (PRA) in 2K1C mice was modestly increased from 5.7 ng/ml/hr (sham vehicle mice) to 9.9 ng/ml/hr; however, the difference was not statistically significant. Aliskiren inhibited PRA by 94% in 2K1C mice relative to vehicle control (0.6 ± 0.1 ng/ml/hr, $p < 0.05$). On the other hand, a small increase in PRA ($P > 0.05$) was noted in 1K1C vehicle-treated mice (Fig. 1.1.4.2). Though aliskiren inhibited PRA in 1K1C mice by 58% relative to vehicle-treated 1K1C mice, and by 68% in sham mice vs sham vehicle-treated mice, these differences are not statistically significant.

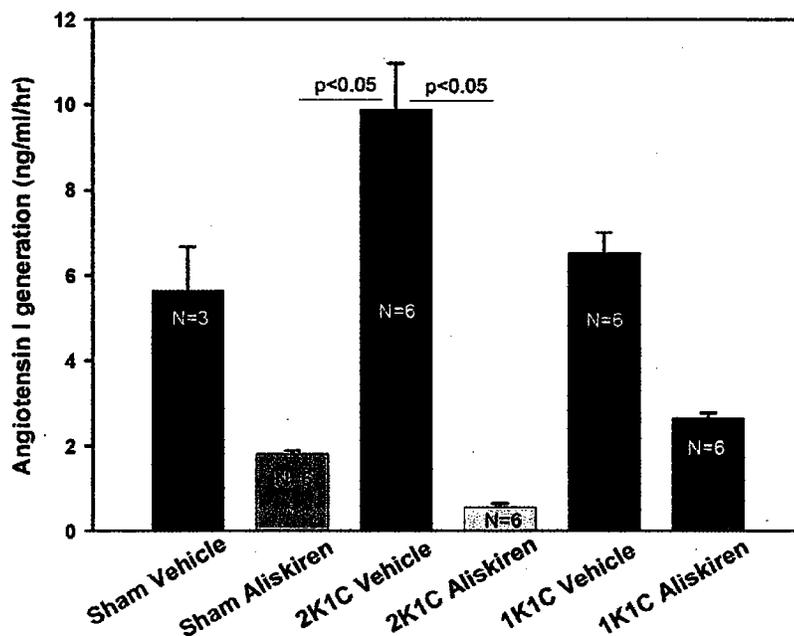


Fig. 1.1.4.2.: Effect of aliskiren treatment on plasma renin activity in hypercholesterolemic mice. P values are from Kruskal-Wallis one way ANOVA on ranks followed by Dunn's all pair wise multiple comparison test.

1.1.5. Effects of Oral Aliskiren in Double Transgenic Rats

This non-GLP study (report #RD-2005-51153, dated November 21, 2005) was conducted at Novartis. The antihypertensive effects of aliskiren were studied in double transgenic rats (dTGR) following either a single oral dose or after repeated dosing for 10 days.

Renin displays species specificity for its substrate. Thus, human renin inhibitors such as aliskiren cannot be tested efficiently in conventional hypertensive rat models. To circumvent this problem, transgenic rats and mice were developed harboring the human renin and the human angiotensinogen genes. Human renin does not effectively cleave rat angiotensinogen, and *vice versa*. The single transgenic rats and mice (i.e., transgenic for either human angiotensinogen or renin) are normotensive. However, when cross-bred, the dTGR offspring develop (at 3 weeks of age) severe and sustained hypertension (due to life-long over-expression of human renin and angiotensinogen) with severe organ damage and do not live beyond the seventh or eighth week of age.²

Male dTGR [(h-REN)L10J x (h-AOGEN)L1623] immediately after receipt (at 3 weeks of age) were given enalapril (10 mgEq/kg/day) in drinking water for five weeks. The enalapril regimen served to attenuate the rapid rise in blood pressure that occurs during the growth phase of the

² Pilz, B., *et al.* Aliskiren, a human renin inhibitor, ameliorates cardiac and renal damage in double-transgenic rats. *Hypertension* 46: 569-576, 2005.

dTGR. The average mean arterial pressure (MAP) of the dTGR was approximately 90 to 100 mmHg under this treatment regimen, and gradually rose to about 160 to 180 mmHg within two weeks after discontinuation of enalapril treatment. The average life span for dTGR treated with enalapril was about four to six months and thus, allowed animals, with a stabilized blood pressure to be studied in a chronic setting. Animals were given a minimum two week washout period following the enalapril pretreatment.

All dTGR were implanted with radiotransmitters (surgical implantation into the abdominal aorta for measurement of blood pressure and heart rate) between the ages of 8 to 12 weeks and were allowed to recover for 2 to 3 weeks following surgery and until body weight gain resumed. dTGRs were randomly assigned to receive a single oral dose of aliskiren at doses of 0.3, 1, 3, 10, 30 or 100 mg/kg (n = 8 rats/dose). Aliskiren HCl was administered in 0.9% saline and doses are expressed as free base. Separate groups of dTGR received aliskiren (1, 3 or 10 mg/kg/day), once daily, by oral gavage, for 10 days. Blood pressure (systolic, mean and diastolic blood pressure) and heart rate were continuously monitored and recorded in conscious, freely moving rats.

In the single dose study, aliskiren induced a rapid and dose-dependent reduction in MAP. The maximum change in MAP (peak response) following oral dosing with aliskiren was -13 ± 6 , -19 ± 4 , $-28 \pm 7^*$, $-45 \pm 5^*$, $-60 \pm 6^*$, and $-74 \pm 6^*$ mm Hg, respectively, at doses of 0.3, 1, 3, 10, 30, or 100 mg aliskiren/kg (*p < 0.05 vs vehicle). The highest dose of aliskiren resulted in a prolonged antihypertensive effect that lasted for more than 24 hours after dosing (Fig. 1.1.5.1). A small but significant (p < 0.05) increase in heart rate was noted in animals receiving aliskiren at 30 and 100 mg/kg which was probably due to the rapid and big drops in blood pressure at these dose levels.

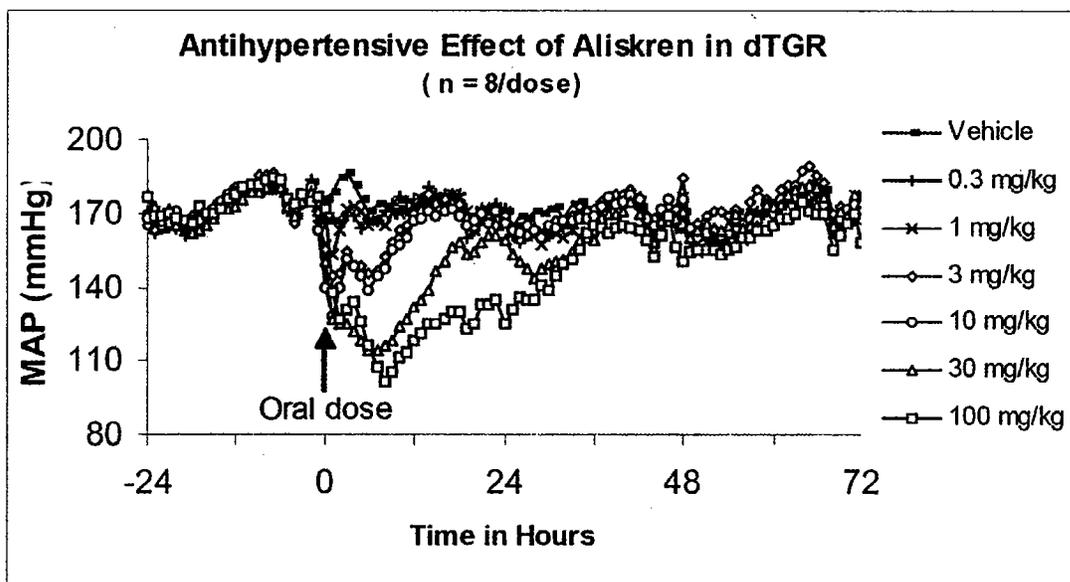


Fig. 1.1.5.1.: Time course of the blood pressure effects of aliskiren in dTGR. MAP was recorded in all rats receiving either a single oral dose of aliskiren (0.3 to 100 mg/kg) or vehicle (0.9% saline). Each point represents the group mean blood pressure \pm SEM and is calculated as an hourly average. The arrow at time 0 denotes the time at which drug was administered by oral gavage. Blood pressure recordings are illustrated over a four day period (one day prior to drug and 3 days following drug administration).

In the repeat dose study, aliskiren induced a dose-dependent reduction in MAP on each of 10 consecutive days. There was no evidence for attenuation or potentiation of the antihypertensive effect over time (Fig. 1.1.5.2). A small but significant ($p < 0.05$) increase in heart rate occurred following daily dosing at 10 mg/kg on days 1 and 10.

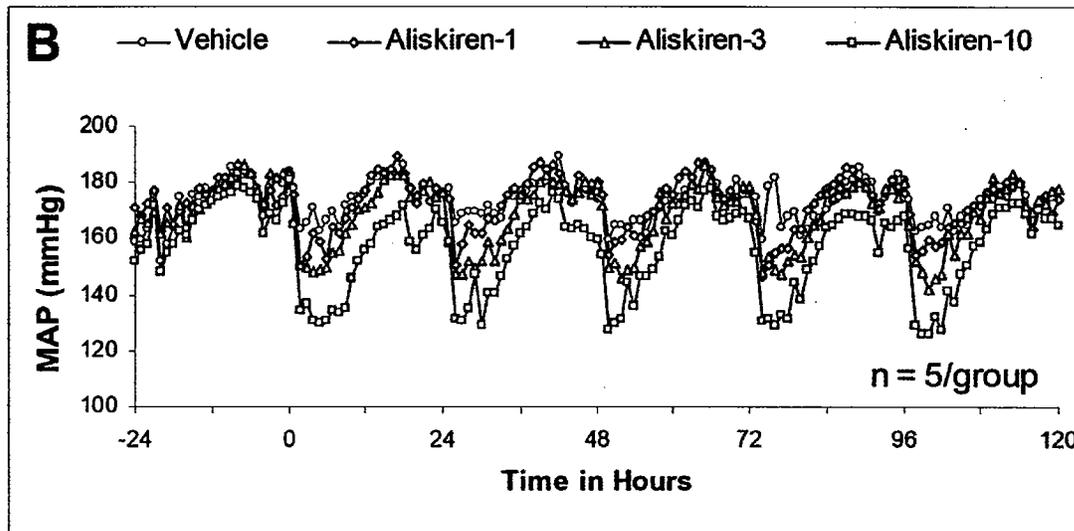


Fig. 1.1.5.2.: Time course of the blood pressure effects of repeat dosing of aliskiren in dTGR. MAP was recorded in all rats dosed daily with aliskiren at 1, 3 or 10 mg/kg or vehicle (0.9% saline). Each point represents the group MAP \pm SEM calculated as an hourly average (data illustrated here truncated at 120 hr).

1.1.6. PK/PD Evaluation of Aliskiren in Double Transgenic Rats

This non-GLP study (report #RD-2005-51355, dated December 16, 2005) was conducted at Novartis. The pharmacodynamic and pharmacokinetic relationships and PK parameters of aliskiren were studied in hemodynamically stabilized double transgenic rats (dTGR) following either a single oral or intravenous dose.

Male dTGR [(h-REN)L10J x (h-AOGEN)L1623] immediately after receipt (at 3 weeks of age) were given enalapril (10 mg/kg/day) in drinking water for five weeks in order to stabilize the arterial pressure and prolong their life span. This was followed by 3 weeks of washout from the enalapril pretreatment.

These animals were surgically instrumented to allow, in conscious rats, unrestrained direct measurement of arterial blood pressure (*via* femoral artery), i.v. administration of test agent and withdrawal of venous blood (*via* femoral vein), for measuring levels of compound. The rats were allowed to recover for a week. Aliskiren hemifumarate was dissolved in sterile saline and administered orally (3, 10 or 30 mg/kg) by gavage or intravenously (2 mg/kg) as a bolus ($n=3$ /dose). Doses are expressed as base. Arterial blood samples were collected at 5 min (i.v. only), 15 min, 30 min, 1, 2, 3, 4, 6, 8, 24, 48 and 72 hours post-dosing. Mean arterial pressure (MAP) and heart rate were measured continuously for 24 hours before and 72 hours after dosing.

A single i.v. dose of 2 mg aliskiren/kg caused a rapid decline in MAP. It decreased by 19 mm Hg at 5 min and reached peak (Δ -86 mm Hg) at 2 hr post dosing. MAP was sustained at <100 mm Hg for 8 hr and gradually returned to baseline by 48 hr post dosing (Fig. 1.1.1.6.1). Oral aliskiren dose- and time-dependently decreased MAP. The onset of the response was not as rapid as it was with the i.v., but the duration was similar. The peak effect (Δ -70 mm Hg at 30 mg/kg) was reached at 8 hr (Fig. 1.1.6.1). Variable minimal changes ($p > 0.05$) in heart rate were noted with both oral and i.v. administration of aliskiren hemifumarate.

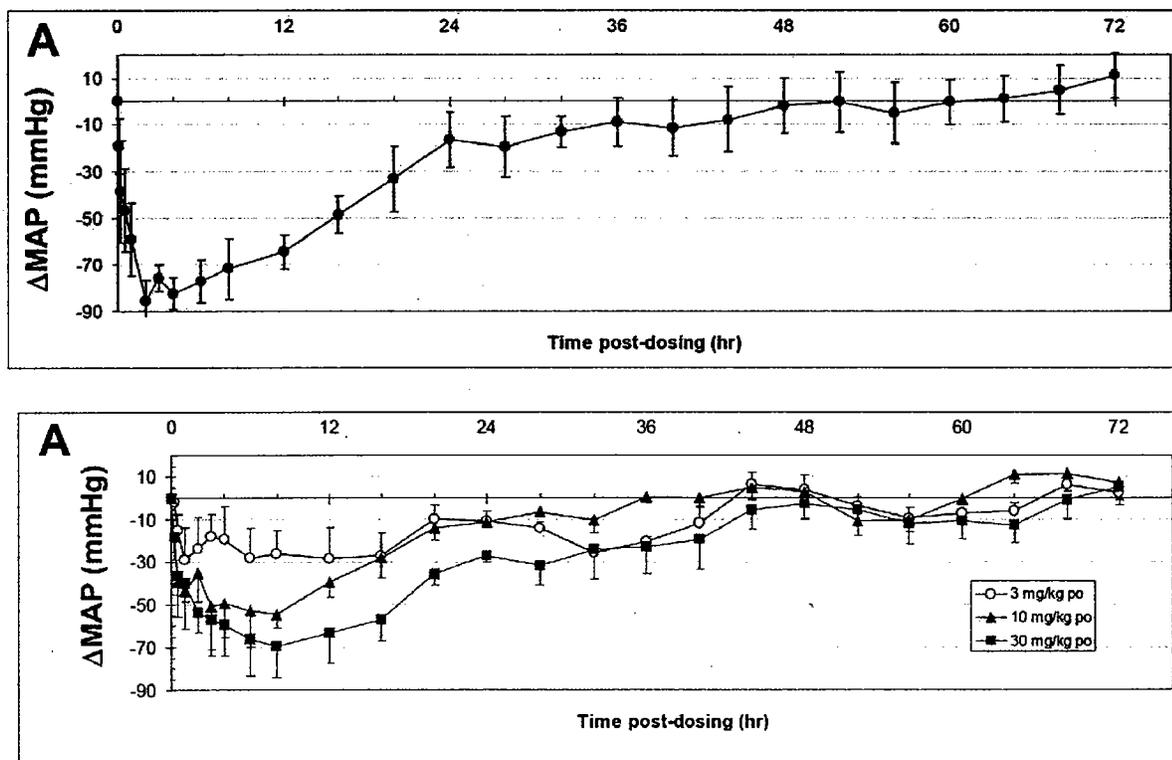


Fig. 1.1.6.1.: Time course of Δ MAP for aliskiren administered intravenously (2 mg/kg, upper panel) or orally (doses as indicated in the inset, lower panel) to dTGRs. Values are mean \pm SEM from baseline (pre-dosing).

Aliskiren plasma concentrations were dose- and time-dependently increased following i.v. (Fig. 1.1.6.2, upper panel) or p.o. (Fig. 1.1.6.2, lower panel) administration. Aliskiren plasma concentrations exhibited a secondary peak at the two highest oral doses, reflecting the apparent enterohepatic recirculation of the compound. The plasma exposure to aliskiren was long-lasting by both routes; the mean plasma concentrations of aliskiren were sustained in the low nanomolar range until at least 72 hours post-dosing (Table 1.1.6.1).

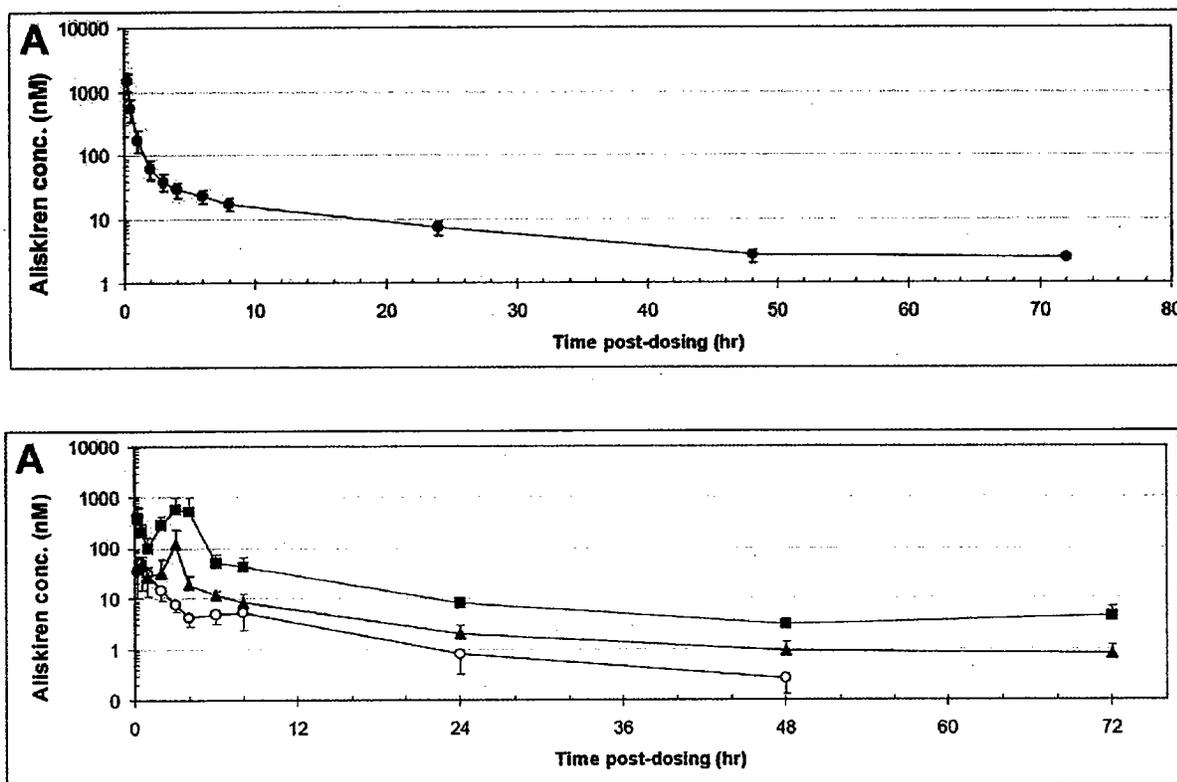


Fig. 1.1.6.2.: Upper panel: Time course of plasma aliskiren concentration for aliskiren administered intravenously to dTGRs (2 mg/kg). Lower panel: Time course of plasma aliskiren concentration for aliskiren administered orally to dTGRs (circle: 3 mg/kg, triangle: 10 mg/kg, square: 30 mg/kg). Values are mean \pm SEM.

Systemic exposure (C_{max} or AUC) to aliskiren following i.v. dosing (2 mg/kg) was higher than that following the highest oral dose (30 mg/kg). The time to C_{max} (0.083 hr for i.v. dosing, 0.42 to 2.42 hr for oral dosing, Fig. 1.1.6.2) was much shorter than the time to maximum decrease in MAP (see Fig. 1.1.6.1), suggesting some temporal dissociation between the PK and PD responses. The estimated plasma clearance was moderate (1.11 L/h/kg) compared to the liver blood flow in normal rats (\sim 3.3 L/h/kg). Compared to the total body water volume (\sim 0.67 L/kg), the estimated volume of distribution at steady state was high (12.2 L/kg), which suggests that the aliskiren was distributed to the tissues. Based on the dose-normalized C_{max} and AUC, the pharmacokinetic parameters were proportional between 3 and 10 mg/kg, p.o. but slightly over-proportional at the highest dose, 30 mg/kg, p.o. The long PK half-life translated into a prolonged duration of antihypertensive effect in the dTGRs. At the highest oral doses, recovery of MAP to baseline required up to 48 hours. The oral bioavailability was similar at the two lowest oral doses (2.9 and 2.2%) but nearly two-fold higher at the highest dose (4.8%). The overall bioavailability (mean of all nine values) was low (3.3%) and variable (0.6-8.6%, Table 1.1.6.1) and is similar to that reported in humans ($2.6 \pm 0.8\%$)

TABLE 1.1.6.1
PHARMACOKINETIC PARAMETERS FOR ALISKIREN ADMINISTERED I.V. OR P.O.
TO CONSCIOUS dTGRs

Parameter	i.v. 2 mg/kg	p.o. 3 mg/kg	p.o. 10 mg/kg	p.o. 30 mg/kg
C _{max} (nM)	8705 ± 2040	49 ± 33	128 ± 101	1014 ± 408
C _{max} /dose (nM/mg/kg)	4353 ± 1020	16 ± 11	13 ± 10	34 ± 14
T _{max} (h)	0.083 ± 0.0	0.42 ± 0.08	1.25 ± 0.88	2.42 ± 1.12
T _{1/2} (h)	21.6 ± 2.4	---	---	---
AUC _(0-72h) (nM x h)	3674 ± 951	158 ± 58	400 ± 230	2668 ± 1216
AUC _(0-72h) /dose (nM x h/mg/kg)	1837 ± 475	53 ± 19	40 ± 23	89 ± 41
CL (L/h/kg)	1.11 ± 0.32	---	---	---
V _{ss} (L/kg)	12.2 ± 5.9	---	---	---
F (%)	---	2.9 ± 1.1	2.2 ± 1.3	4.8 ± 2.2

Results are mean ± SEM of PK parameters derived (using WinNonlin) after dosing with aliskiren (2 mg/kg, i.v.; 3, 10 or 30 mg/kg, p.o.). Lower limit of quantification was 0.2 ng/ml (0.36 nM). For T_{max}, first sampling time was 5 min (0.083 hr) after i.v. dosing and 15 min (0.25 hr) after oral dosing.

$$F = \frac{AUC_{(0-72h)} \text{ p.o.}}{AUC_{(0-72h)} \text{ i.v.}} \times \frac{\text{Dose i.v.}}{\text{Dose p.o.}}$$

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ON ORIGINAL**

1.2. Safety Pharmacology

The following table summarizes most of the Safety Pharmacology studies discussed in this section.

Summary of safety/general pharmacology studies with SPP 100			
System Tested	Species and Doses	Active / Inactive	Comments
Central nervous system			
Affinity to (16) neurotransmitter receptors, <i>in vitro</i>	Rat tissue preparations	Inactive	At 10µM lack of interaction with alpha-1, alpha-2, beta-, 5-HTA2, muscarinic, AMPA, Kainate and NMDA--receptors Weak interactions (13-33%) at 10µM with 5HT-1, opiate µ-type, benzodiazepine, adenosine-1, glycine binding and channel sites of NMDA receptors, considered to be irrelevant in view of the therapeutic dose <i>in vivo</i>
Single i.v. dosing in animals	Mouse 0.3, 1 or 3 mg/kg		
- General observation		Inactive	
- Ethanol-induced sleeping time		Inactive	
- Step-through passive avoidance		Inactive	
- Rotarod	Rats	Inactive	
- Body temperature	0.3, 1, or 3, 5 and 10 mg/kg	Inactive	
- Locomotor activity		Inactive	
Cardiovascular system			
- Isolated atria, <i>in vitro</i>	Guinea pig, 1, 3, 10µM	Inactive	No significant effects up to 10µM
- Systemic and cardiac effects	Anaesthetized rats, 0.3, 1 and 3 mg/kg (iv, single dose)	Active	Dose-dependent, transient reduction of systolic and diastolic blood pressure and slight, transient decrease of heart rate. No effect on electrocardiogram
Respiratory system			
	Anaesthetized rats, 0.3, 1 and 3 mg/kg (iv, single dose)	Inactive	No significant effects on respiratory rate, tidal and minute volumes
Renal effects			
	Conscious rats, 0.3, 1, and 3 mg/kg (iv, single dose)	Inactive	Marginal, non-significant decrease in potassium excretion at 1 and 3 mg/kg

1.2.1. Interactions of Aliskiren with Neurotransmitter Receptors *in vitro*

This non-GLP study (report #BS 7, 1996, date of issue: January 19, 1996) was conducted at Aliskiren was evaluated for its interactions with 16 different neurotransmitter receptors *in vitro* to assess its selectivity.

Male rats [Tif: RAI f(SPF)] weighing about 200 gm were used in the study. Radioligand binding assays were performed to measure the ability of aliskiren hemifumarate to compete with labeled ligands for binding at their respective receptor sites. Aliskiren showed no affinity for the following receptors: alpha₁, alpha₂ or beta-adrenergic receptors, 5-HT₂ serotonergic receptors, muscarinic agonist binding sites, AMPA, kainate and NMDA glutamatergic receptors. Weak binding was measured at a concentration of 10 µM for the following receptor sites: 5-HT₁ (13%), 5-HT₃ (23%), histamine-1 (33%), opiate µ-type (22%), benzodiazepine (17%), adenosine-1 (18%), the glycine binding site of the NMDA receptor (27%), and at the [³H]-MK-801 channel binding site of the NMDA receptor (13%).

1.2.2. Effect of Aliskiren on CNS (*in vivo*)

This non-GLP study (report #BS 03, 1996, date of issue: March 14, 1996) was conducted at _____ Aliskiren was evaluated for its possible side effects on the CNS in mice and rats.

The experiments were performed in male mice [Tif: MAG f(SPF)] weighing 22-26 gm and in male rats [Tif: RAI f(SPF)] weighing 110-140 gm. Aliskiren hemifumarate was dissolved in distilled water and given intravenously (10 ml/kg for mice, 2 ml/kg for rats). The following tests were performed:

- Global behavioral assessment in mice (0.3 to 3 mg/kg)
- Rotarod-test in rats (0.3 to 3 mg/kg)
- Body-temperature in rats (0.3 to 3 mg/kg)
- Ethanol-induced sleeping-time in mice (0.3 to 10 mg/kg)
- Passive avoidance in mice (0.3 to 3 mg/kg)
- Motility in rats (0.3 to 3 mg/kg)

No significant effects were observed on global behavior, ethanol-induced sleeping time or passive avoidance in mice, nor on the motor coordination, horizontal and vertical locomotor activity, or body temperature in rats. A single intravenous administration of aliskiren to mice and rats caused no relevant effects on the CNS.

1.2.3. Effects of Aliskiren on CV, Respiratory and Renal Systems in Rats and Guinea Pigs

This GLP study (Novartis Study #604642) was conducted by _____

_____ between September 11 and October 3, 1995.

Methods

Three separate studies were performed. The effects of aliskiren on the cardiovascular and respiratory systems were investigated in anesthetized male rats. Changes in urine and electrolyte excretion were studied in conscious female rats. Finally, effects of aliskiren on heart rate and force of contraction were evaluated in isolated atria of male guinea pigs. Aliskiren hemifumarate (batch 0/800195, purity — , was solubilized in water and saline.

Male rats (Hans Wistar outbred, SPF, 220-251 g and 8 weeks old at treatment) were anesthetized with urethane (1.2 to 1.5 g/kg, i.p.). The trachea was intubated and connected to a pneumotachograph to measure respiratory rate, tidal volume and minute volume. The left carotid artery was cannulated to a pressure transducer to record systolic and diastolic blood pressure and, indirectly, heart rate. Aliskiren hemifumarate (0.3, 1 and 3 mg aliskiren/kg) or vehicle (0.9% saline) was administered cumulatively (30 min interval between doses) into the jugular vein.

Conscious female rats (Hans Wistar outbred, SPF, 150-190 g and 8 weeks old at treatment) were intravenously administered aliskiren hemifumarate or vehicle (0.9% saline) *via* the caudal vein,

followed by 20 ml/kg water p.o. The animals were housed individually in metabolism cages. No food was given but water was given *ad libitum* for the next 2 hr, the duration for urine collection. Sodium, potassium and chloride were analyzed from the collected urine. Aliskiren hemifumarate was tested at 3 dose levels: 0.3, 1 and 3 mg aliskiren/kg (n=6 rats/dose level).

Male guinea pigs (Ibm: GOHI albio, BRL; 342-514 g and 5 to 6 weeks old at treatment) were killed under urethane anesthesia. The heart was rapidly excised and rinsed in Krebs-Henseleit solution. The right and left atria were separated and mounted in a tissue bath containing Krebs-Henseleit solution at 32°C. The right, spontaneously beating atrium was used to determine the effects of test substance on the frequency of beating and the quiescent left atrium was electrically stimulated and used to determine the strength of contraction and its response to test substance. Four paired organs were exposed to vehicle or aliskiren hemifumarate (1, 3 and 10 µM aliskiren), added cumulatively to the bath, every 10 min.

Results

The intravenous cumulative administration of aliskiren hemifumarate at 0.3, 1 and 3 mg aliskiren/kg produced a dose dependent, statistically significant decrease in both systolic (9.7%, 13.7%, 13.8%) and diastolic (24.1%, 24.1%, 38.6%) blood pressures from baseline. Recovery was complete within 10 min following a dose of 0.3 mg/kg but was slower (20 min) at 1 and 3 mg/kg. A small but statistically significant fall in heart rate accompanied the reduction in blood pressure at doses of 1 and 3 mg/kg. Aliskiren had no effect on the ECG of the anesthetized rat at all three dose levels. The cumulative administration of aliskiren hemifumarate had no effect on the respiratory parameters.

The volume of urine excreted, together with its sodium, potassium and chloride content, 2 hr after treatment with aliskiren hemifumarate at 0.3, 1 or 3 mg aliskiren/kg remained unchanged in comparison with control rats.

In the isolated atria from guinea pigs, aliskiren (up to 10 µM) had no effect either on the force of contraction or on the spontaneous heart beat.

1.2.4. Electrophysiological Investigations of Aliskiren in the Isolated Rabbit Heart

This non-GLP study (Novartis Study #0350334) was conducted at _____ for Preclinical Safety Europe, Novartis Pharma AG, Basle, Switzerland. No study dates are given; however, the report is dated March 8, 2004.

Methods

Three female rabbits (2.5 to 3.0 kg) were killed by cervical dislocation and the hearts were quickly removed, cannulated and perfused using the Langendorff method with carbogenated Krebs solution (pH 7.35 at 34°C) of composition (mM): NaCl 118, KCl 4, NaHCO₃ 22, MgCl₂ 1.1, NaH₂PO₄ 0.4, CaCl₂ 1.8, dextrose 5, pyruvate 2 and creatine 0.038. The His bundle was cut

and stimulating electrodes were sutured on either side. In addition, recording electrodes were placed, one subendocardially and the second, epicardially. The heart was stimulated until a stable APD₆₀ (action potential duration at 60% of repolarization) was recorded. The following parameters were measured: AP duration at 30% (APD₃₀), 60%, and 90% of repolarization from monophasic action potentials (MAP), reverse rate-dependency of APD, instability (variability in APD), triangulation (difference in milliseconds between APD₃₀ and APD₉₀), proarrhythmia index, coronary perfusion rate, interventricular conduction, variability in pacemaker activity and amplitude of the threshold stimulation current. The association of the three experimental parameters (triangulation, reverse rate-dependency and instability) is used for the assessment of the test substance's proarrhythmic potential. Aliskiren (as aliskiren hemifumarate, batch #0323004) was tested at concentrations of 1, 3, 10, 30 and 100 μ M (solvent not described).

Results

Aliskiren did not exert any electrophysiological effects on the measured parameters in any of the rabbit hearts up to a concentration of 100 μ M.

1.2.5. Effects of Aliskiren on Cloned hERG Channels Expressed in Mammalian Cells

This non-GLP study (Novartis Study #0380193, — study #031002.OPW) was conducted by — a CRO, for Novartis Pharma, USA. The study was conducted between October 13 and October 20, 2003.

Methods

Human embryonic kidney cells (HEK293) that lack endogenous hERG channels were stably transfected with cDNA for hERG. The current mediated through the expressed channel protein was measured using standard voltage clamp techniques at 35°C. The concentrations of aliskiren hemifumarate (lot #062K1198) were chosen to achieve a range of approximately 10% to 90% block of channels within the limit of solubility in DMSO. For the positive control, terfenadine was used based on previous results from the same lab (60 nM terfenadine blocked 80% of the hERG current). Stock solutions of both aliskiren hemifumarate and terfenadine were prepared in DMSO and diluted further in HEPES-buffered physiological saline.

Results

Based on the initial testing at 10 μ M, two additional concentrations of aliskiren (100 and 1000 μ M) were tested for their effects on hERG current. Aliskiren produced concentration-dependent inhibition of hERG current. On average, aliskiren inhibited (mean \pm SEM) hERG current by 1.4 \pm 0.6%, 6.0 \pm 1.4%, and 32.0 \pm 6.7% (n = 3 to 5) at 10, 100 and 1000 μ M, respectively. An IC₅₀ was not calculated since the inhibition did not reach the 50% level. The calculated IC₂₅ was 670.9 μ M. hERG block observed at 100 and 1000 μ M was statistically significant when compared to vehicle control values (0.2 \pm 0.1%). Under the same experimental conditions, 60 nM terfenadine, the positive control, inhibited hERG current by 88.2 \pm 1.4%. The sponsor concludes that aliskiren revealed a very shallow dose-response over a 100-fold concentration range that did not achieve 50% inhibition.

2.0. DRUG DISPOSITION (ADME)

2.1.1. Absorption, Metabolism and Excretion of Aliskiren in Mice After Single Oral and I.V. Administration of [¹⁴C]aliskiren Hemifumarate

This nonGLP study (report #PCS(EU)R0301336) was conducted at Bioanalytics and Pharmacokinetics, Novartis Pharma AG, Basel, Switzerland, between April 16 and July 25, 2004.

Male mice (TgRasH₂ “wild type”) weighed 21.7 to 30.1 gm (age not specified) at initiation of drug administration. Animals were not fasted. A single oral dose of 553 mg [¹⁴C]aliskiren hemifumarate/kg (batch #E-3277-147-39, 3.62 MBq³/mg; 500 mg free base/kg) was administered orally by a stomach tube to 3 mice. Another group of mice received a single i.v. bolus dose of 11.1 mg [¹⁴C]aliskiren hemifumarate/kg (10 mg free base/kg) by a tail vein. A solution of labeled and unlabeled test substance (batch #0323010) was prepared in water for oral administration and in 0.9% saline for intravenous administration. Pharmacokinetics, metabolism and excretion were investigated in plasma, urine and feces after oral and i.v. dosing. Urine (on ice) and feces were collected quantitatively at daily intervals up to 72 hr post dose and were stored at -20°C. Blood samples were collected at 0.083 (i.v. group only), 0.5, 1, 2, 4, 8, 24 and 48 hr after dosing by sacrificing the animal (n=3 animals/time point). Radioactivity in the biological samples (blood, plasma, urine and feces) was measured by liquid scintillation counting.

Results

Following oral administration, the test substance was rapidly absorbed (t_{max} , 1hr). Plateau mean blood [¹⁴C] levels were noted between 30 and 120 min. Thereafter, the [¹⁴C] concentrations steadily declined to a barely detectable level at 48 hr post dose, the last measuring time point. The concentration of unlabeled test substance in plasma was distinctly lower than the total radioactivity (AUC: 7.04 vs. 18.3) suggesting the presence of metabolites in plasma after oral administration (Table 2.2.1.1). After intravenous administration, the [¹⁴C] concentrations in blood declined rapidly and multi-exponentially: 10% and 1% of the initial concentrations were reached at 2 and 8 hr, respectively.

TABLE 2.1.1.1
PHARMACOKINETIC PARAMETERS IN PLASMA OF TOTAL RADIOACTIVITY AND UNLABELED ALISKIREN AFTER ORAL / IV ADMINISTRATION OF [¹⁴C] ALISKIREN HEMIFUMARATE TO MICE.
(MEAN VALUES)

Parameter	Oral, 500 mg/kg		Intravenous, 10 mg/kg	
	Unlabeled	[¹⁴ C]	Unlabeled	[¹⁴ C]
AUC (μmol.h/L)	7.04	18.3	9.03	5.01
T _{max} (hr)	1	1	0.083	0.083
C _{max} (μmol/L)	1.45	2.17	19.8	14.6

³ The becquerel (Bq) is the SI derived unit of radioactivity. The older unit of radioactivity was the Curie (1 Ci = 37 GBq).