

The absolute oral bioavailability of aliskiren, assessed on dose-normalized values for aliskiren AUC after oral *versus* intravenous administration was 1.5%.

Unchanged parent compound accounted for the main proportion of radioactivity in plasma after both intravenous (94% in first 4 hr) and oral (56% in first 24 hr) administration. Minor metabolite peaks (M2, M5 and M6) were noted in plasma extracts with both routes of administration. Elimination of aliskiren in mice occurred mainly *via* the biliary/fecal route. As in plasma, parent compound was the major radioactive compound in urine and feces after oral and i.v. dosing. The main metabolite in fecal extracts was M2, though traces of M1 and M2 were found after i.v. dosing. The proportion of metabolites in all biological samples (plasma, urine and feces) was higher after oral than after i.v. dosing. Based on LC-MS and LC-NMR analysis, the proposed main biotransformation pathways were demethylation on the methoxypropoxy side chain followed by C-oxidation, yielding the carboxylic acid M2 as a main metabolite. The phenolic metabolites (M1 and M4) were observed as glucuronic acid conjugates M5 and M6. The proposed metabolic pathways of aliskiren in mice for the metabolites numbered M1 through M6 are shown in Fig 2.1.1.1.

Most of the radioactivity administered was excreted during the first 24 hr after administration and the main route of excretion (about 90%) was the fecal pathway. About 94% of the dose was excreted within 3 days, the total number of measuring days. The excretion was not yet finished by then, with 9.3% of the dose still present in the 48-72 hr urine + feces fractions (Table 2.1.1.2).

TABLE 2.1.1.2
EXCRETION OF RADIOACTIVITY IN URINE AND FECES AFTER ORAL / IV ADMINISTRATION OF [¹⁴C]
ALISKIREN HEMIFUMARATE TO MICE.
MEAN VALUES FOR EXCRETION OF RADIOACTIVITY (% OF DOSE)

Time Period	Oral			IV		
	Urine	Feces	Urine & feces, mean	Urine	Feces	Urine & feces, mean
0-24 hr	0.47	69.49	69.96	NA	84.68	84.68
24-48 hr	1.02	9.49	10.51	1.82	5.69	7.51
48-72 hr	0.34	8.99	9.33	0.16	0.74	0.90
0-72 hr	1.84	87.94	89.80	1.98	91.10	93.08
Total (including cage wash)			94.44			93.38

NA: data not available

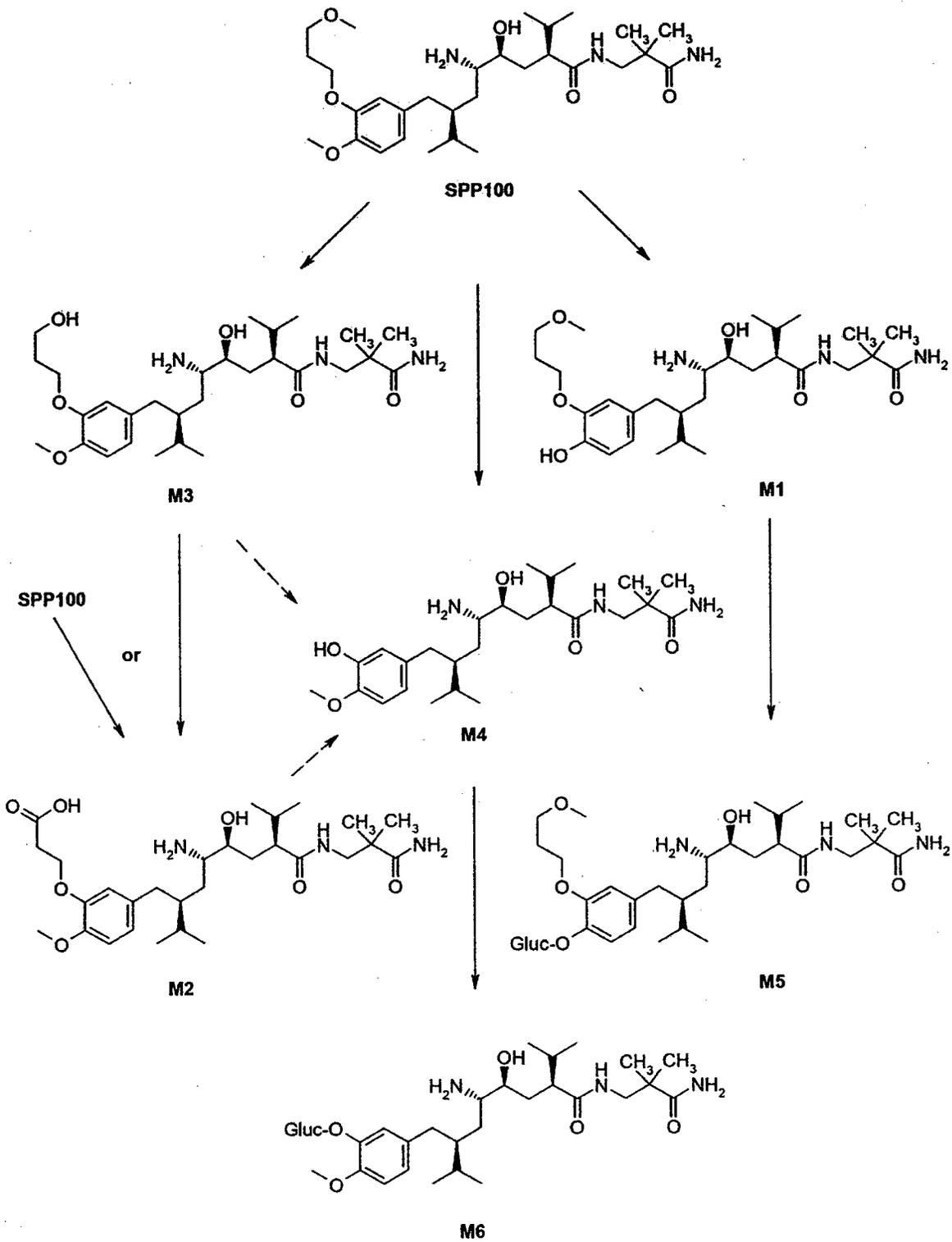


Fig. 2.1.1.1.: Proposed main biotransformation pathway of aliskiren (SPP100) in mice (and rats) showing only the metabolites observed without probable intermediates.

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

This nonGLP study (report #PCS(EU)R0300781) was conducted at Bioanalytics and Pharmacokinetics, Novartis Pharma AG, Basel, Switzerland, between April 16 and July 25, 2004.

Male albino rats [HAN:WIST(SPF)] weighed 236 to 251 gm (age not specified) at initiation of drug administration. Animals were not fasted. A single oral dose of 110.5 mg [¹⁴C]aliskiren hemifumarate/kg (batch #E-3277-147-39, 3.62 MBq/mg; =100 mg free base/kg) was administered orally by a stomach tube to 3 mice. Another group of 3 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 96 hr post dose and were stored at -20°C. Blood samples were collected sublingually at 0.083 (i.v. group only), 0.25, 0.5, 1, 2, 4, 8, 24, 48, 72 and 96 hr after dosing (n=3 animals/time point). Radioactivity in the biological samples (blood, plasma, urine and feces) was measured by liquid scintillation counting. The structures of the metabolites were characterized by LC-MS with the HPLC-method and mass spectrometric conditions.

Results

After i.v. administration, the radioactivity in blood declined rapidly and multi-exponentially, reaching 8% and 1% of the initial concentration ($C_{5 \text{ min}} = 19.1 \mu\text{mol/L}$) at 1 hr and 48 hr, respectively. The concentration of unlabeled aliskiren followed a similar course as that of the [¹⁴C] concentration. With oral administration, a peak in radioactivity was detectable in plasma as early as 15 min, the first measuring time point (Fig. 2.1.2.1). It was immediately followed by a decline but reached a second peak at 2 hr and remained more or less at a plateau up to 4 hr post-dose before declining to reach 10% of the second peak at 48 hr after dosing. After p.o. administration, though the course of the concentrations of the unlabeled aliskiren paralleled the course of the concentrations of radioactivity, the aliskiren AUC was lower than the radioactivity AUC by a factor of 1.4 to 3.7 (Table 2.1.2.1). This suggests a greater proportion of metabolites are present in plasma after oral administration, suggesting first pass metabolism. The absolute oral bioavailability of aliskiren, assessed on dose normalized values for AUC of aliskiren after oral vs. i.v. administration was 2.4%.

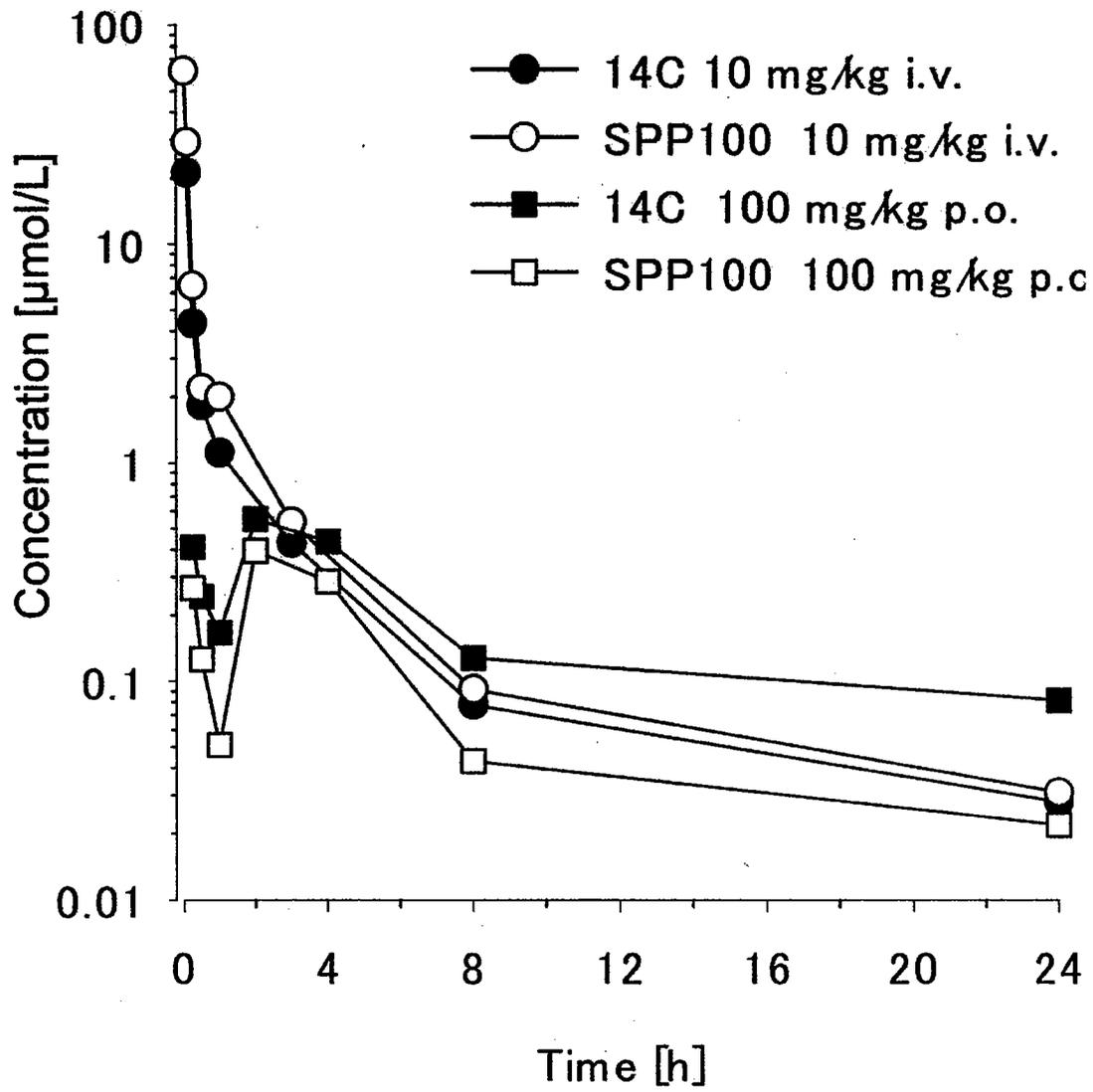


Fig. 2.1.2.1.: Total radioactivity and concentrations of aliskiren in plasma after intravenous and after oral administration of [¹⁴C]aliskiren (SPP100) hemifumarate to male rats

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TABLE 2.1.2.1
PHARMACOKINETIC PARAMETERS FOR TOTAL RADIOACTIVITY AND UNLABELED ALISKIREN IN
PLASMA AFTER ORAL / IV ADMINISTRATION OF [¹⁴C] ALISKIREN HEMIFUMARATE TO RATS.
(MEAN VALUES)

PK parameters	Oral, 100 mg/kg		Intravenous, 10 mg/kg	
	Unlabeled	[¹⁴ C]	Unlabeled	[¹⁴ C]
t _{max} (hr)	0.25	0.25	0.083	0.083
C _{max} (μmol/L)	0.27	0.416	29.20	21.20
C _{max} /dose (μmol/L)/(mg/kg)	0.0027 ^b	0.0042 ^b	2.92	2.12
AUC (μmol·h/L)	3.01	5.38	12.60	8.08
AUC / dose (μmol·h/L)/(mg/kg)	0.03 ^b	0.054 ^b	1.26 ^{b,c}	0.81
CL (L/h/kg)	n.a	n.a	1.2 ^{b,c}	1.28
V _{ss} (L/kg)	n.a	n.a	7.8 ^{b,c}	n.a
Apparent terminal t _{1/2} (hr)	n.a.	n.a.	23.1 ^{b,c}	n.a
Absorption (% of dose)	≥ 3.4	n.a	n.a	n.a
Bioavailability (%)	2.4	n.a	n.a	n.a

a: median value

b: calculated value

c: calculated between 8 and 96 h based on the experimental value reported for 1-24 hr

n.a.: not applicable

Parent compound accounted for a large percentage total radioactivity in the plasma at early time points after intravenous (92.5% in first 8 hrs) and oral (51% in first 4 hrs) administration. Besides unchanged compound, minor metabolite peaks were detected in plasma extracts. Elimination of radioactivity in rats was mainly *via* the biliary route. Unchanged parent drug was the major radioactive compound detected in urine and fecal extracts after i.v. (3.6% and 55% in urine and feces) and oral (0.24% and 72% in urine and feces) dosing. Only minor proportions of metabolites were detected in excreta (Table 2.1.2.2). The biotransformation pathway in rats was comparable to that observed in mice (section 2.1.1). One of the primary biotransformation reactions of aliskiren in rats was the demethylation on the methoxypropoxy side chain, generating the intermediate alcohol derivative M3, which was essentially further oxidized to the carboxylic acid M2. In parallel, the parent drug underwent additional oxidative dealkylations giving rise to the minor phenolic metabolites M1 and M4. Subsequently, these intermediate phenols were conjugated with glucuronic acid yielding M5 and M6, respectively. Figure 2.1.1.1 (in section 2.1.1 for mice) shows the main metabolites of the [¹⁴C]aliskiren biotransformation, observed without probable intermediates. Through another pathway (not shown here) metabolites M9 and M10 were formed, which were probably the product of [¹⁴C]aliskiren hydrolysis followed by lactonization of the intermediate carboxylic acid. M10 was further metabolized to metabolite, M11.

TABLE 2.1.2.2
PARENT COMPOUND AND METABOLITES OBSERVED *IN VIVO* IN PLASMA AND EXCRETA AFTER ORAL / IV ADMINISTRATION OF [¹⁴C] ALISKIREN HEMIFUMARATE TO RATS.

Aliskiren/Metabolite	Oral dosing			IV dosing		
	Plasma	Urine	Feces	Plasma	Urine	Feces
Aliskiren	+++	t	+++			
M1	-	t	t			
M2	t	t	t			
M3	t	t	t			
M4	t	t	t			
M5/M6	+	t	-			
M7	-	-	-			
M8	-	t	-			
M9	-	b	-			
M10/ M11 ^a	++	t	t			
M12	-	-	t			
M13/M14	-	-	t (M13)			

+++: prominent component : >40-99% of total ¹⁴C in urine and feces or total ¹⁴C-AUC in plasma.
 ++: substantial component : >10-40% of total ¹⁴C in urine and feces or total ¹⁴C-AUC in plasma.
 +: minor component : >2-10% of total ¹⁴C in urine and feces, bile or total ¹⁴C-AUC in plasma.
 t: trace component : ≤ 2% of total ¹⁴C in urine and feces, bile, total ¹⁴C-AUC in plasma or detected only by LC-MS.
 -: not detected or not investigated.
 a: Front peak was assigned to the metabolites M10 and/or M11
 b: Non-radiolabeled metabolite was observed by LC-MS.

The radioactivity was excreted predominantly by the fecal route. The excretion of radioactivity in the urine was minimal (4.2% of the dose after intravenous and 0.7% after oral administration). A large amount of the radioactivity administered was excreted within 48 hr. About 96% of the dose was excreted within 4 days, the total number of measuring days. (Table 2.1.2.3). Unchanged aliskiren in feces accounted for 72-83% of the dose.

TABLE 2.1.2.3
EXCRETION OF RADIOACTIVITY IN URINE AND FECES AFTER ORAL / IV ADMINISTRATION OF [¹⁴C] ALISKIREN HEMIFUMARATE TO RATS.
MEAN VALUES FOR EXCRETION OF RADIOACTIVITY (% OF DOSE)

Time Period	Oral			IV		
	Urine	Feces	Urine & feces, mean	Urine	Feces	Urine & feces, mean
0-24 hr	0.47	35.60	36.10	2.96	55.70	58.60
24-48 hr	0.17	40.00	40.20	0.68	28.20	28.90
48-72 hr	0.04	16.20	16.30	0.36	4.69	5.05
72-96 hr	0.01	2.50	2.54	0.17	2.07	2.24
0-96 hr	0.69	94.40	95.10	4.2	90.00	94.80
Total (including cage wash)			NA			96.20

NA: data not available

2.1.3. Disposition in Rats After Repeated Oral Administration of [¹⁴C]aliskiren Hemifumarate

This nonGLP study (report #DMPK R0300779) was conducted at Novartis Pharma AG, Basel, Switzerland (report release date: October 26, 2005).

Male albino rats (HAN:WIST) were 9-11 weeks of age and weighed 239 to 304 gm at initiation of drug administration. Animals were not fasted on the day of dosing. An oral dose of 110.5 mg [¹⁴C]aliskiren hemifumarate/kg (2.904 MBq/mg, 100 mg free base/kg) was given each day for 10 days (by gavage) to 4 groups of rats. The study was divided into four experiments, the details of which are listed in Table 2.1.3.1. A solution of labeled and unlabeled test substance was prepared in water. The batch numbers for labeled and non-labeled substances were, respectively, E-30585-11-36 and 0323010. Radioactivity in blood, plasma, urine and feces was measured by liquid scintillation spectrometry. The structures of the metabolites were characterized by LC-MS with the HPLC-method and mass spectrometry.

TABLE 2.13.1
EXPERIMENTAL DESIGN

Group	Objective	Methods
1.	Determination of radioactivity concentrations in blood and plasma	Blood samples were taken at 2, 4, 8 hours after the first dose; sampled before dosing on 2 nd , 3 rd , 7 th and 9 th day; and at 2, 4, 8, 24, 48, 96, 120 and 168 hour (16 th day) after the last dose (n=4/time point). Same animals were used at all time points.
2.	Determination of metabolism & excretion of radioactivity in blood, plasma, urine and feces	Blood and plasma, as in group 1. Urine and feces were collected 0 to 24 h after daily dosing and every 24 h up to the last blood sample collection (n=4).
3.	Distribution of radioactivity concentrations in tissues (quantitative whole body autoradiography, QWBAL)	One rat each was sacrificed at 2 and 24 h after the first dose. Immediately after sacrifice, the rats were deep frozen and sagittal sections were prepared.
4.	QWBAL	One rat each was sacrificed at 72 ^a , 168 ^a (a: before dosing) 218, 240 and 384 h after the 1 st dosing. Frozen sections were made as in group 3.

Results

Test substance-related clinical signs were not noted for any of the animals that received all 10 doses. A slight gain in body weight was recorded during the course of the study.

The radioactivity or unchanged aliskiren concentration in plasma reached maximum at 2 hr, the earliest time of measurement, on day 1 ($t_{\max(1)}$) and day 10 ($t_{\max(10)}$) (Fig. 2.1.3.1). C_{\max} of total radioactivity or parent substance was higher following the first dosing ($C_{\max(1)}$) than following the last dosing ($C_{\max(10)}$). This suggests no accumulation of radioactive compounds or unchanged parent compound following 10 repeated oral administrations of radiolabeled test substance. The concentration ratio between aliskiren and radioactive compound amounted to about 75% at both $t_{\max(1)}$ and $t_{\max(10)}$ showing that at both t_{\max} values the parent compound accounted predominately for the radioactivity determined in plasma (Table 2.1.3.2). At 24 hr following first or last dosing, the mean aliskiren concentration was only approximately 1.7% of $C_{\max(1)}$ or 1.5% of $C_{\max(10)}$. This suggests the rate of elimination was independent of the number of doses administered. The concentration-time course of aliskiren in plasma after $C_{\max(10)}$ followed a multiexponential

decline. The concentration declined rapidly, reaching 1.5% of the initial concentration (at $t_{\max(10)}$) at 240 hr. The decline during this first period faded into a slower decline, which followed apparently first-order kinetics (Fig. 2.1.3.1). The mean $t_{1/2}$ for the terminal elimination of aliskiren in plasma was 39.5 hr (Table 2.1.3.2). At 384 hr following first dosing, the mean aliskiren concentration in plasma was less than 0.15% of $C_{\max(10)}$. The accumulation of parent substance was calculated by 3 PK parameters: $C_{\min(ss)}/C_{\min(1)} = 1.3$; $C_{\max(10)}/C_{\max(1)} = 0.56$; $AUC_{ss(\tau)}/AUC_{(0-24)} = 0.58$. Based on these values, the sponsor concludes that no apparent accumulation of aliskiren was found after 10 repetitive administrations of 110.5 mg [^{14}C]aliskiren hemifumarate/kg.

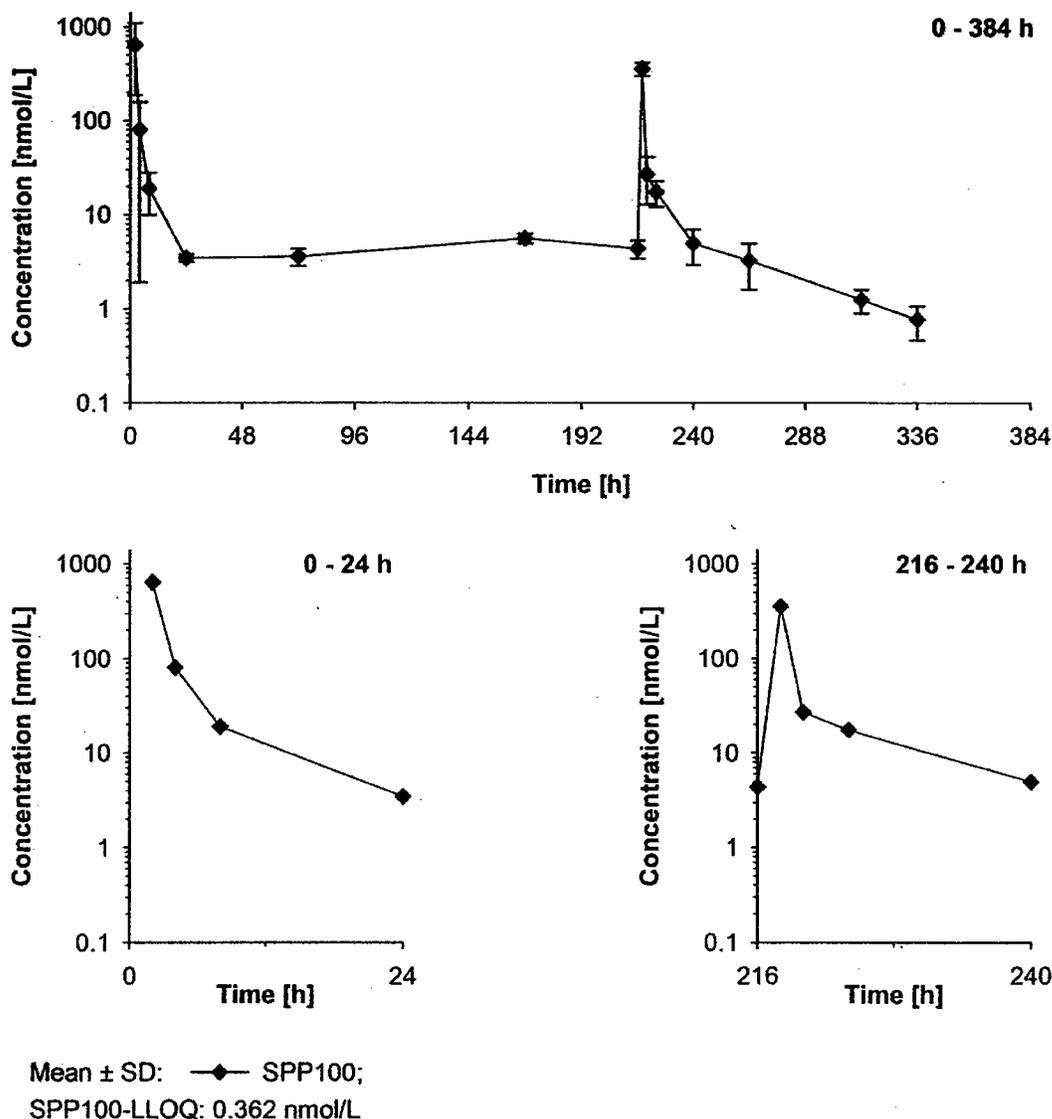


Fig. 2.1.3.1.: Mean plasma concentration-time courses of unchanged aliskiren (SPP100) after oral administration of [^{14}C]aliskiren hemifumarate to rats for 10 days. Mean concentrations of total radioactivity is not shown in the figure. LLOQ: lower limit of quantification.

TABLE 2.1.3.2
PHARMACOKINETIC PARAMETERS FOR PLASMA TOTAL RADIOACTIVITY AND ALISKIREN AFTER
SINGLE OR REPEATED ORAL ADMINISTRATION OF [¹⁴C] ALISKIREN HEMIFUMARATE TO RATS
(MEAN VALUES)

PK parameters	¹⁴ C radioactivity	SPP100
T _{max(1)} (first time point after dosing) [h] ^a	2 ^c	2
T _{max(10)} (first time point after dosing) [h] ^a	218	218
C _{max(1)} [nmol/L] ^b	1086 ^c	639
C _{max(10)} [nmol/L] ^b	473	357
C _{min(1)} [nmol/L] ^d	-- ^e	3.47
C _{min(ss)} [nmol/L] ^d	-- ^e	4.54
C _{av(ss)} [nmol/L]	-- ^e	42.3
AUC(0-24)/dose [(nmol*h/L)/(mg/kg)]	-- ^e	17.2
AUC _{ss(τ)} /dose [(nmol*h/L)/(mg/kg)]	-- ^e	10.0
t _{1/2} [h]	-- ^e	39.5 ^f

a) T_{max} after the 1st (T_{max(1)}) or 10th (T_{max(10)}) dosing

b) C_{max} after the 1st (C_{max(1)}) or 10th (C_{max(10)}) dosing

c) mean of 3 rats out of 4

d) lowest concentration observed after the first dosing (C_{min(1)}) or at steady state during a dosing interval (C_{min(ss)})

e) values could not be given or calculated due to the limited number of measured concentrations

f) range used for calculation: 240 to 384 h following 1st dosing or 24 to 168 h after the 10th dosing.

Measurements of the concentrations of the radioactivity in the tissues by whole-body autoradioluminograms showed that the overall exposure to test substance was marginal either after a single oral dose or after once a day dosing for 3, 7 or 10 days. The distribution patterns at 24 hr after 3, 7 and 10 doses were comparable to that at 2 and 24 hr after a single dose. The highest radioactivity concentration was observed in the colon wall (with both 2 hr and 24 hr samples on all measurement days). Radioactivity was also detected in liver, brown fat and kidney medulla. The levels in other tissues were below the limit of detection. No significant accumulation occurred in any tissue after repeated dosing. Since the number of animals exposed at each interval was one, a firm conclusion cannot be drawn from the limited data on distribution of radioactive test substance in a variety of tissues.

Parent compound was the main radioactive material detected in plasma, urine and feces extracts. Minor amounts of metabolites (M2, M6 and M9) previously identified in rats after a single oral dose were also detected. Fecal extracts contained two distinct metabolites in measurable quantities, M12 and M13, which were only identified in trace amounts in a single dose study (see Table 2.1.2.2). The biotransformation of radioactive aliskiren in rats after repeated oral dosing was comparable to that observed after a single oral dose (see Fig. 2.1.2.2).

Radioactivity was mainly eliminated into the feces. Urine and fecal recovery of radioactivity were approximately 0.3% and 102%, respectively (0-384 hr). In chromatograms (0-24 hr fraction), unchanged aliskiren in feces and urine accounted for approximately 80% and 43% of radioactivity, respectively.

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

This nonGLP study (report #DMPK R0400300) was conducted at Preclinical Safety / Drug Metabolism and Pharmacokinetics, Absorption, Distribution, Metabolism and Excretion Section, Novartis Pharmaceuticals Corp., East Hanover, NJ. (Report release date: December 16, 2005.)

Male marmosets weighed 402 to 486 gm (age not specified) at initiation of drug administration. Animals were not fasted before dosing. A single oral dose of 3.3 mg [¹⁴C]aliskiren hemifumarate/kg (97.7 μ Ci/mg, 3 mg free base/kg) was administered orally (5 ml/kg) by gavage to 3 marmosets. Another group of 3 marmosets received a single i.v. bolus dose (1 ml/kg) of 1.1 mg [¹⁴C]aliskiren hemifumarate/kg (1.0 mg free base/kg) by a tail vein. A solution of labeled and unlabeled test substance was prepared in a 5% dextrose solution for oral administration and in 0.9% saline for intravenous administration. The batch number for the tagged drug substance was E-3277-147-39. The batch number for the unlabeled substance was not provided.

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 168 hr post dose and were stored at -20°C. Blood samples were collected from the femoral vein at 0.083 (i.v. group only), 0.25 (oral only), 0.5, 1, 2, 4, 8, 24, 48, 72, 96 and 168 hr after dosing (n=3 animals/time point). Radioactivity in the biological samples (blood, plasma, urine and feces) was measured by liquid scintillation counting. The structures of the metabolites were characterized by LC-MS with the HPLC-method and mass spectrometric conditions.

Results

Plasma radioactivity determined after i.v. administration showed peak levels at the first time point (5 min), decreasing to approximately 51% at 1 hr and 4% at 24 hr. The concentration of unlabeled aliskiren declined biphasically with a long terminal elimination half-life (36 hr). The steady state volume of distribution of the unchanged compound ($V_{ss} = 0.58$ L/kg) was similar to that of total body water, suggesting that aliskiren is distributed to tissues. The systemic plasma and blood clearances (0.036 and 0.055L/hr/kg, respectively) were lower than the hepatic blood flow (2.8-3 L/hr/Kg), indicating a very low total systemic clearance. The C_{max} of plasma radioactivity from the oral dose was attained at approximately 2 hr (Table 2.1.4.1). The terminal elimination of radioactivity after both i.v. and oral doses was long (29.5 to 45.5 hr). After both doses, the radioactivity in plasma was much higher than in blood, indicating that the compound and its metabolites were distributed more into plasma than to red blood cells. The concentration of unlabeled aliskiren followed a similar course as that of the [¹⁴C] concentration following a single i.v. or oral dosing. As in mice and rats, aliskiren bioavailability in marmosets was very low (3%).

TABLE 2.1.4.1
PHARMACOKINETIC PARAMETERS FOR TOTAL RADIOACTIVITY AND UNLABELED ALISKIREN IN PLASMA AFTER ORAL / IV ADMINISTRATION OF [¹⁴C] ALISKIREN HEMIFUMARATE TO MARMOSETS (MEAN VALUES, N=3)

PK parameters	Oral, 3 mg/kg		Intravenous, 1 mg/kg	
	Unlabeled	[¹⁴ C]	Unlabeled	[¹⁴ C]
t _{max} (hr)	2	2	0.083	0.083
C _{max} (μmol/L)	0.395	0.359	14.24	11.42
C _{max} /dose (μmol/L)/(mg/kg)	0.132 ^b	0.12 ^b	14.24	11.42
AUC (μmol·h/L) ^a	4.55	4.84	49	52.92
AUC / dose (μmol·h/L)/(mg/kg)	1.52 ^b	1.61 ^b	49 ^b	52.92
CL (L/h/kg)	na	na	0.036	na
V _{ss} (L/kg)	na	na	0.58	na
Apparent terminal t _{1/2} (hr)	na	45.5 ^c	36 ^c	39.5
Absorption (% of dose)	na	25 ^d	na	na
Bioavailability (%)	3	na	na	na

a: 0 - 96 or 0 - 168 hr post dose

b: calculated value

c: calculated between 48 and 168 hr post dose

d: absorption was estimated from excreta recording, where the radioactivity recovered in urine was 4.5% of the oral dose and metabolites detected in feces accounted for 20% of the oral dose

n.a.: not applicable/not determined

Unchanged parent compound was the major circulating component in the plasma. It accounted for 92% of the AUC up to 72 hr after intravenous and 91% of the AUC up to 8 hr after oral administration. Minor metabolite peaks detected in the plasma extracts were M3 and M4 (7.7% and 0.7% of the AUC, respectively) after the intravenous and M3, M4 and M10/11 (6%, 1.2% and 2.2% of the AUC, respectively) after the oral dose (Table 2.1.4.2). Aliskiren was eliminated in the urine mainly as unchanged drug accounting for ≤3% of the dose after each route of administration. Small amounts of metabolites, M2, M3, M4 (absent with oral dosing) and M10/M11, each representing 0.1 to 0.4% of dose were observed on the chromatograms (Table 2.1.4.2). Parent compound accounted for a greater proportion in the fecal extract (36% and 17% of the dose, respectively, after oral and intravenous dosing) than in the urine. Small amounts of metabolites, M2, M3, M4, M10/M11, M13/M14, were present as well (Table 2.1.4.2). The biotransformation pathway in marmosets was comparable to that observed in mice (section 2.1.1) and rats (section 2.1.2). The primary metabolic reaction involved *O*-demethylation on the methoxypropoxy side chain to form M3. Further oxidation of M3 to form the carboxylic acid metabolite M2 and its dealkylation to form metabolite M4 were minor pathways. M3 and M4 are the *O*-dealkylated products. Hydrolysis of the central amide bond of aliskiren resulted in formation of polar metabolites M10 and M11, whereas the lactone M9 metabolite probably was formed via cyclization of the carboxylic acid intermediate (not detected) with loss of water (Figure 2.1.4.1).

TABLE 2.1.4.2
PARENT COMPOUND AND METABOLITES DETECTED *IN VIVO* IN PLASMA AND EXCRETA AFTER ORAL / IV ADMINISTRATION OF [¹⁴C] ALISKIREN HEMIFUMARATE TO MARMOSETS.

Plasma exposure				
1 mg/kg intravenous dose		3 mg/kg oral dose		
Plasma component	AUC _{0-72h} (ngEq-h/mL or ng-h/mL)	Plasma component	AUC _{0-24h} (ngEq-h/mL or ng-h/mL)	
M3	2008	M3	58	
M4	185	M4	12	
M10/M11	-	M10/M11	21	
SPP100	23806	SPP100	868	

Metabolites	Urinary metabolites (Percent of dose) ^a		Fecal metabolites (Percent of dose) ^b	
	Intravenous	Oral	Intravenous	Oral
M2	0.1	0.1	6	2
M3	0.4	0.1	17	2
M4	0.1	0.0	2	1
M10/M11 ^d	0.2	1.8	3	8
M13/M14	- ^c	-	2	5
SPP100	3	2.3	36	78
Others ^f	0	0.2	6	2
Total ¹⁴ C (0-72 h) ^e	4	4.5	72	98

^a Relative proportions of metabolites were based on metabolite profiles from pooled samples 0-168 h after the intravenous or the oral doses. These pools represented 90±5% of the total urinary radioactivity excretion.

^b Relative proportions of metabolites were based on metabolite profiles from pooled samples 0-72 h (intravenous and oral gavage) post dose. These pooled samples accounted for 90±5% of the total fecal excretion after intravenous and oral (gavage) doses, respectively.

^c Not detected or not quantifiable

^d M10/M11 may comprise more than one poorly resolved components

^e determined by LSC, amount expressed as % of dose

^f Summation of minor metabolites not identified

Fecal recovery accounted for 72% and 97.5% of administered radioactivity in the intravenous and oral dose groups. These results suggest that the biliary route of excretion was the major excretory route for total radioactivity. The excretion of radioactivity in the urine was minimal, regardless of the dose route (Table 2.1.4.3).

TABLE 2.1.4.3
MEAN EXCRETION OF RADIOACTIVITY (% OF DOSE) IN URINE AND FECES AFTER ORAL / IV ADMINISTRATION OF [¹⁴C] ALISKIREN HEMIFUMARATE TO MARMOSETS.

		Intravenous	Oral
Excretion in urine (% dose)			
Radioactivity	0-24 h	0.61 ± 0.61	2.22 ± 3.14
	0-168 h	4.04 ± 2.51	4.50 ± 2.70
Aliskiren	0-168 h	3	2.3
Excretion in feces (% dose)			
Radioactivity	0-24 h	51.5 ± 16.88	84.0 ± 17.5
	0-168 h	72.0 ± 5.67	97.5 ± 4.95
Aliskiren	0-72 h	36	78
Cage wash (% dose)		7.08 ± 2.29	1.91 ± 0.80
Total radioactivity recovery (% dose)		83.1 ± 5.77	104 ± 2.70

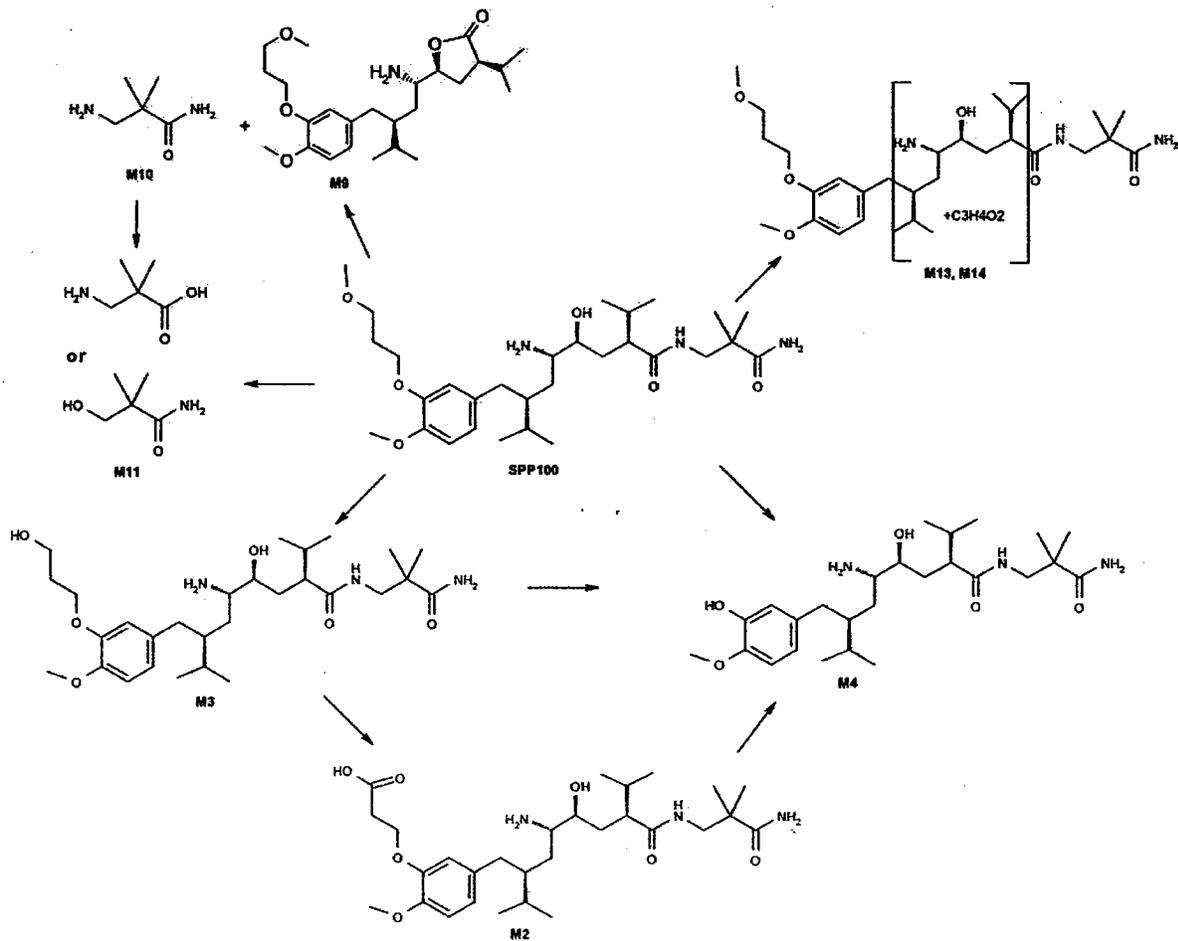


Fig. 2.1.4.1.: Proposed metabolic pathways of aliskiren (SPP100) in marmosets

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2.1.5. Absorption, Distribution and Excretion of Aliskiren in Pregnant Rabbits After Single Oral Administration of [¹⁴C]aliskiren

This nonGLP study (report #PCS(EU) R0300778-01) was conducted at Novartis Pharma AG, Basel, Switzerland (report release date: November 30, 2004).

Three non-fasted, mated and presumed pregnant female New Zealand rabbits [ESD:KBL(NZW)BR] (7 months old and weighing 3.5 to 4.0 kg) each received a single oral dose of 221 mg [¹⁴C]aliskiren hemifumarate/kg (3620 KBq/mg, 200 mg free base/kg) orally, by gavage, on day 17 of gestation. A solution of labeled and unlabeled test substance was prepared in water. The batch number for the tagged drug was E-3277-147-39. The batch number for the non-labeled substance was 0323010. Pharmacokinetics and excretion were investigated in blood, plasma, urine (feces not collected), fetus, placenta and amniotic fluid. Urine (on ice) was collected quantitatively in one 0-24 hr fraction/animal and was stored at -20°C. Blood samples were collected at 0.5, 1, 2, 4, 8 and 24 hr after dosing by ear vein. Animals were sacrificed at 24 hr after dosing. Blood from dams, whole fetuses, placentas and amniotic fluid was sampled for determination of radioactivity by liquid scintillation counting. The tissue distribution of radioactivity in the fetuses at 24 hr post dose was investigated using quantitative whole-body autoradioluminography.

Results

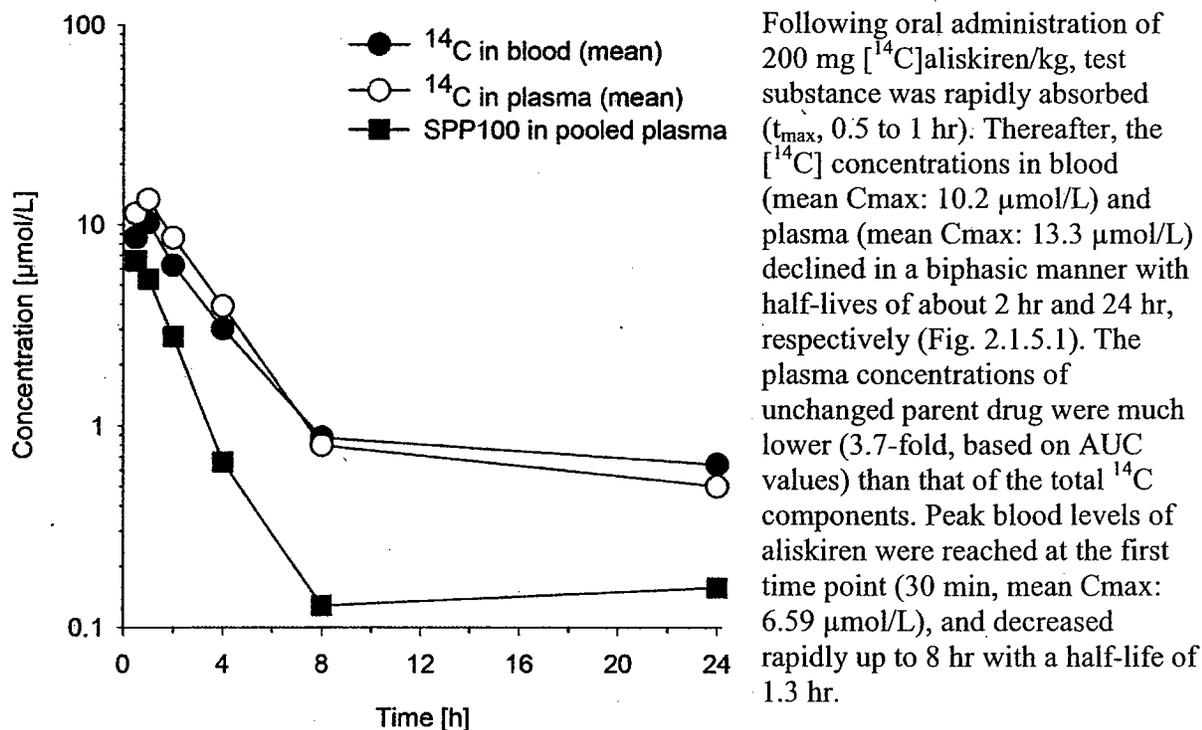


Fig. 2.1.5.1.: Blood and plasma concentrations of radioactivity and unchanged aliskiren (SPP100,) after oral administration of [¹⁴C]aliskiren hemifumarate to pregnant rabbits.

Six fetuses, 3 each from two rabbits were collected 24 hr after dosing for determination of tissue concentrations of radioactivity. The mean concentration of radioactivity in these six fetuses was 0.67 nmol/g (Table 2.1.5.1), while the mean ^{14}C concentration in maternal blood collected at the same time point was 0.64 $\mu\text{mol/L}$ (6% of C_{max} at 1 hr post dose). Radioactivity was also found in placenta (mean 1.05 nmol/g) and amniotic fluid (mean 0.2 nmol/g) 24 hr post dose, indicating free passage of parent compound and metabolites. Thus, fetuses of the embryo-fetal development study in the rabbit were significantly exposed to aliskiren and /or its metabolites during the entire duration of the study. One fetus from the third rabbit was used for quantitative whole-body autoradioluminography. No specific uptake into any fetal tissue was noted, as all concentrations were below the limit of detection (3.3 nmo/g). This part of the study should be considered incomplete because of the use of only one fetus and the lack of detailed results.

TABLE 2.1.5.1
CONCENTRATIONS OF RADIOACTIVITY IN FETUSES, PLACENTA AND AMNIOTIC FLUID 24 HR
AFTER ORAL ADMINISTRATION OF [^{14}C] ALISKIREN HEMIFUMARATE TO RABBITS

Sample	^{14}C concentrations [nmol/g]		
	RB1	RB2	RB3
fetus 1	/	/	M
fetus 2	/	/	M
fetus 3	/	/	M
fetus mean	0.824	0.508	M
CV%	0.2	6	M
placenta 1	/	/	/
placenta 2	/	/	M
placenta 3	/	/	M
placenta mean	1.326	0.769	0.917
CV%	12	8	NC
amniotic fluid 1	BLOD	/	/
amniotic fluid 2	BLOD	/	M
amniotic fluid 3	BLOD	/	M
amniotic fluid mean	BLOD	0.189	0.231
CV%	NC	26	NC

LOD: for fetus 0.086 $\mu\text{mol/L}$, for placenta 0.098 $\mu\text{mol/L}$, for amniotic fluid 0.057 nmol/g

M: missing sample

NC: not calculated

BLOD: below limit of detection; LOD: limit of detection

A mean of 1.48% of the dose was excreted in the 0-24 hr urine collection, which is similar to that noted for mice and rats.

2.1.6. Distribution and Excretion of Aliskiren in Rats After Oral and I.V. Administration of [¹⁴C]aliskiren Hemifumarate

This GLP study (report #1940/11-D1145) was conducted at the _____ between July 11 and August 29, 2001.

Male pigmented rats (Lister-Hooded strain from _____), were 6-7 weeks of age and weighed 186 to 241 gm at initiation of drug administration. Animals were fasted overnight prior to oral dosing (fed state in case of i.v. dosing) and fed 4 hr after dosing. Animals had free access to water at all times. A single oral dose of 100 mg [¹⁴C]aliskiren hemifumarate/kg (3.87 MBq/mg or 104.6 µCi/mg, 90.5 mg free base/kg) was administered orally by gavage to 4 rats. Another group of 6 rats received a single i.v. bolus dose of 10 mg [¹⁴C]aliskiren hemifumarate/kg (9.05 mg free base/kg) *via* a tail vein. Mean doses administered *via* the oral (n=4) or intravenous (n=6) route were 91 mg aliskiren/kg or 8.69 mg aliskiren/kg, respectively. A solution of labeled and unlabeled test substance was prepared in deionized water for oral administration and in 0.9% isotonic saline for intravenous administration, respectively. The batch number for tagged substance was CFQ12601. The batch number for the non-labeled substance was S100B2001002. Rats in the oral dose group were sacrificed at 2 hr and 1, 7 and 14 days after dosing; those in the intravenous group were sacrificed at 5 min, 120 min and 1, 3, 7 and 14 days after dosing (one animal per time point). Prior to sacrifice, a blood sample was taken from a caudal vein of each animal. Carcasses were rapidly deep frozen and subjected to quantitative whole body autoradiography (QWBAL). Sagittal sections were made at 5 levels through the carcass. Animals from oral or intravenous dose groups designated for sacrifice at 7 and 14 days were placed in all glass metabolism cages for separate collection of urine and feces. Radioactivity in plasma, urine and feces was measured by liquid scintillation spectrometry.

Results

Test substance-related clinical signs were not noted for any of the animals during the course of the study. The whole-body autoradiograms analyzed 2 hr following oral dosing showed a moderate amount of radioactivity in cecum (277.2 µg equiv/g), small intestine (173.0 µg equiv/g), the mucosa of the stomach (52.00 µg equiv/g) and large intestine (13.30 µg equiv/g). Quantifiable levels of radioactivity were noted in a few tissues: adrenal (7.51 µg equiv/g), the liver (6.83 µg equiv/g), seminal vesicles (5.72 µg equiv/g) prostate (2.64 µg equiv/g) and plasma (0.32 µg equiv/g). At 24 hr, the radioactivity was no longer detected in any of the tissues except in cecum and large intestine (2.98 and 3.28 µg equiv/g, respectively). The radioactivity was not detectable in these tissues 7 or 14 days after dosing. Following intravenous administration, peak concentrations of radioactivity were noted in the majority of tissues at the first sampling time (5 min); the highest concentrations were in kidney medulla and cortex followed by liver, plasma, salivary glands, pancreas and pineal body. Moderate to detectable levels of radioactivity were noted in most of the other tissues, including gastrointestinal tract and brain. At 2 hr, the radioactivity concentrations had declined in most tissues and were absent in the brain. On the other hand, concentrations started rising in the choroid plexus, preputial gland, seminal vesicles, small and large intestine mucosa and brown fat. Concentrations further declined at 24 hr in all tissues and were below limit of detection in most tissues. Moderate levels of radioactivity were

still present in the bile ducts, brown tissue, pancreas, pituitary, choroid plexus, kidney pyramid, stomach, small and large intestine and cecum. The overall distribution of radioactivity in all the above tissues was further decreased at day 3 post dose compared to the results obtained at day 1. On day 7 post dose, quantifiable levels were still noted in pituitary, the choroid plexus, uveal tract and stomach.

After oral or i.v. administration, the radioactivity was excreted predominantly by the fecal route. The excretion in the urine and feces for 24 hr after oral dose was 73% and 86%, respectively. The recovery of radioactivity in urine (0.576%) and feces (95.25%) was complete by 48 hr after oral dosing. Following i.v. administration, 10% and 66% of the radioactivity was excreted in urine and feces, respectively, in the first 24 hr post dose. Recovery of radioactivity was not complete by 168 hr (urine 10.4%, feces 75% of total radioactivity), suggesting residues of radioactivity remaining in the carcass at 7 days.

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2.1.7. In vitro Blood Distribution and Plasma Protein Binding of ¹⁴C-aliskiren in Mouse, Rat, Marmoset, Rabbit and Human

This non-GLP study (report #DMPK(CH) R0100669) was conducted at Preclinical Safety, Drug Metabolism and Pharmacokinetics, Novartis Pharma AG, Basel, Switzerland, between July and October 2001.

Methods

Test substance: [¹⁴C]aliskiren hemifumarate (batch #CFQ12601, purity 3.87 MBq/mg) — specific activity

Species/strain: Male mice — CD-1(ICR)BR

Male rats — VI(Glx/BRL/Han)BR

Male and female marmosets, *Callithrix jacchus*

Male rabbits, — NZW/Kbl BR (New Zealand White Rabbit)

Male Humans (healthy Caucasian volunteers)

Plasma and serum samples were prepared from pooled blood samples (at least 3 of each animal species, n = 3 for human). For the *in vitro* blood distribution study, [¹⁴C]aliskiren hemifumarate was incubated with fresh blood (triplicates) at 37°C for 1 hr at final concentrations of 10, 50, 250, 500, 5000 or 10,000 ng free base/ml (in case of human 10 to 500 ng/ml). After the incubation, whole blood was centrifuged for plasma separation. Total ¹⁴C radioactivity was measured by liquid scintillation counting in whole blood before centrifugation and in plasma after centrifugation. For the *in vitro* plasma/serum protein binding study, [¹⁴C]aliskiren hemifumarate was incubated with plasma (triplicates except for marmosets in duplicates) or serum (only for human in triplicates) at 37°C for 1 hr at final concentrations as described above for blood distribution. After the incubation, plasma/serum samples were centrifuged, followed by ultrafiltration to separate the free (unbound) fraction of [¹⁴C]aliskiren in plasma or serum from the bound. The binding ratio was calculated as percent of total radioactivity. The radioactivity in all samples was measured by liquid scintillation counting.

Results

Distribution equilibrium of [¹⁴C]aliskiren between human blood cells and plasma was reached within 1 hr. [¹⁴C]aliskiren distributed fairly well into blood cells for all species although it was >50% in plasma. The distribution into blood cells was species dependent, marmoset being the lowest followed by human (Table 2.1.7.1).

The mean extent of binding of aliskiren to plasma protein in all species was moderate, species dependent (marmoset >> mouse > rat > rabbit > human) and concentration independent except in the mouse and the marmoset in which the plasma free fraction (unbound form) increased with increasing concentrations (Fig. 2.1.7.1). The plasma free fraction in marmoset was 4 to 6 times lower than in other species studied (Table 2.1.7.2).

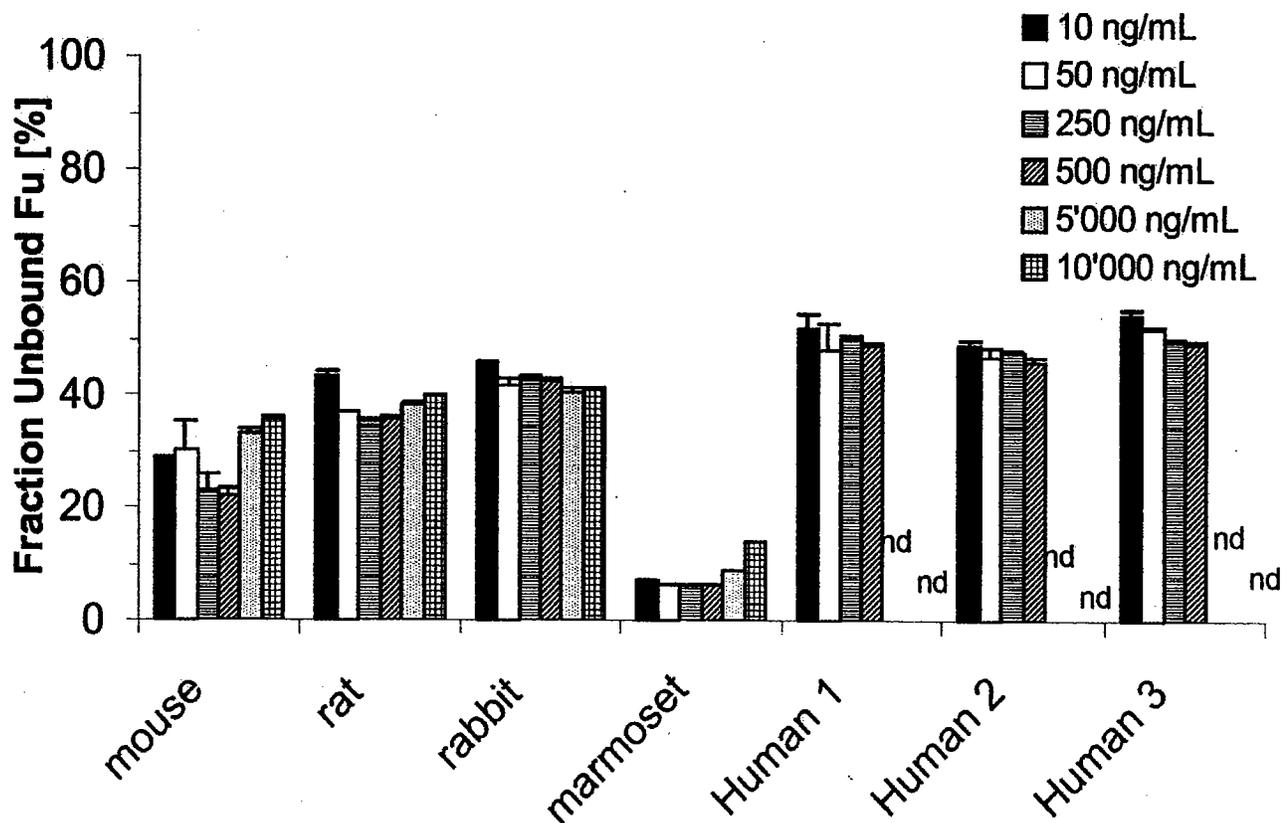


Fig. 2.1.7.1: *In vitro* plasma protein binding of [¹⁴C]aliskiren hemifumarate in mice, rats, marmosets, rabbits and the human. Animal plasma was pooled, whereas human plasma was from three individuals. Values are means ±SD of triplicates and means of duplicates for marmosets.

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TABLE 2.1.7.1
IN VITRO BLOOD DISTRIBUTION OF [¹⁴C]ALISKIREN IN THE MOUSE, RAT,
 MARMOSET, RABBIT AND HUMAN

Concentration range	Species	F _p [%]
10-10'000 ng/mL	Mouse	65.0 ± 5.6
	Rat	54.3 ± 1.1
	Rabbit	59.1 ± 2.0
	Marmoset	83.5 ± 10.8
10-500 ng/mL	Human 1	69.6 ± 0.9
	Human 2	74.7 ± 0.8
	Human 3	70.1 ± 2.3

F_p: fraction in plasma

TABLE 2.1.7.2
IN VITRO PLASMA PROTEIN BINDING OF [¹⁴C]ALISKIREN IN THE MOUSE, RAT,
 MARMOSET, RABBIT AND HUMAN

Concentration range	Species	F _u [%]
10-10'000 ng/mL	Mouse	28.8 ± 5.4
	Rat	38.3 ± 3.1
	Rabbit	42.5 ± 1.9
	Marmoset	8.1 ± 3.1
10-500 ng/mL	Human 1	49.8 ± 1.7
	Human 2	47.3 ± 1.3
	Human 3	51.4 ± 2.4

F_u: plasma free fraction or unbound fraction

2.1.8. Inhibition of P450 Enzymes by Aliskiren

This non-GLP study (Report #000202a) was conducted by a CRO,
The report is dated July 5, 2000.

Methods

Inhibition of the enzymatic activity of the major human cytochrome P450 (CYP450) isoforms by aliskiren was studied with cDNA-derived enzymes in microsomes prepared from a human lymphoblastoid cell line or baculovirus-infected insect cells. The study determined the degree of inhibition of each enzyme (CYP1A2, CYP2A6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4) for two concentrations (20 and 200 μ M, each tested in triplicate) of test substance. A known inhibitor (positive control) for each specific enzyme was also tested in triplicate (Table 2.1.8.1). All these inhibitors were dissolved in acetonitrile. Aliskiren base used in this study (lot # or batch # not given) was dissolved in water to a final concentration of 10 mM and further diluted to working concentrations.

TABLE 2.1.8.1
EXPERIMENTAL DESIGN

P450 isoform	Source of Enzyme	Substrate and Conc., μ M	Positive control and Conc. μ M
CYP1A2	cDNA-expressed	Phenacetin 50	7,8-benzoflavone 0.3
CYP2A6	cDNA-expressed	Coumarin 3	Tranlycypromine 100
CYP2C8	cDNA-expressed	Paclitaxel 10	Quercetin 30
CYP2C9	cDNA-expressed	Diclofenac 6	Sulfaphenazole 3
CYP2C19	cDNA-expressed	(S)-mephenytoin 50	Tranlycypromine 100
CYP2D6	cDNA-expressed	Bufuralol 10	Quinidine 1
CYP2E1	cDNA-expressed	P-nitrophenol 100	4-methylpyrazole 50
CYP3A4	cDNA-expressed	Testosterone 120	Ketoconazole 1
Control Microsomes	Lymphoblastoid cell line	Added to standardize protein concentration	- -

Results

As shown in Table 2.1.8.2, aliskiren exhibited inhibition of CYP2C9 (15-23% at 200 μ M), CYP2D6 (37-43% at 200 μ M) and CYP3A4 (11-23% at 200 μ M), whereas there was no inhibition of the other cytochrome P450 isoforms examined at the highest concentration tested (200 μ M). Based on these findings, the report concludes that the possibility of clinically significant metabolic drug interactions of aliskiren with co-administered drugs cleared by CYP2C9, CYP2D6 and CYP3A4 is considered unlikely.

TABLE 2.1.8.2

Inhibition of cDNA-expressed CYP1A2

Concentration (µM)	Pmoles / Incubation	Percent Inhibition
0	519, 504, 521	—
20	502, 545, 489	2.5, -5.8, 5.0
200	484, 548, 578	6.0, -6.4, -12.4
Positive Control - 7,8 Benzoflavone		
0	545, 524, 563	—
0.3	12.4, 56, 69	98, 90, 87

Inhibition of cDNA-expressed CYP2A6

Concentration (µM)	Pmoles / Incubation	Percent Inhibition
0	36, 40, 38	—
20	39, 37, 37	-2.1, 2.6, 2.6
200	37, 37, 34	2.6, 2.6, 10.5
Positive Control- Tranylcypromine		
0	35, 34, 34	—
100	1.2, 0.6, 0.3	97, 98, 99

Inhibition of cDNA-expressed CYP2C8

Concentration (µM)	Pmoles / Incubation	Percent Inhibition
0	220, 247, 227	—
20	Interference	Coelution of test sample
200	Interference	
Positive Control - Quercetin		
0	171, 162, 167	—
30	41, 44, 37	76, 74, 78

Inhibition of cDNA-expressed CYP2C9

Concentration (µM)	Pmoles / Incubation	Percent Inhibition
0	256, 269, 258	—
20	227, 259, 258	13.1, 0.9, 1.3
200	222, 201, 220	14.9, 23, 15.8
Positive Control- Sulfaphenazole		
0	328, 305, 328	—
3	56, 59, 80	83, 82, 75

Inhibition of cDNA-expressed CYP2C19

Concentration (µM)	Pmoles / Incubation	Percent Inhibition
0	1146, 1247, 1183	—
20	1174, 1131, 1214	-1.5, 5.1, -1.9
200	1375, 1156, 1077	-15.4, 3.0, 9.6
Positive Control- Tranylcypromine		
0	1100, 1223, 1394	—
100	49, 15, 34	96, 99, 97

2.1.9. Inhibition of Specific P450 Enzyme Activities by Aliskiren in Human Liver Microsomes

This non-GLP study (Report #PCS(EU) RO101128) was conducted at Novartis Pharma AG, Basel, Switzerland. The study dates are not given but the report is dated September 15, 2003.

Methods

Working concentrations of aliskiren — batch #NE-5810-Batch-02-01, lot #NE-5810-B-11-10, purity — were prepared from a 5 mM stock solution of aliskiren in distilled water. Inhibition of the enzymatic activity of the human cytochrome P450 (CYP450) isoforms by aliskiren was studied on a pool of liver microsomes prepared from ten healthy human donors (preparation obtained from — Catalytic activities of the microsomes for different P450 isoforms (CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4/5) were tested using substrates specific to an enzyme. Aliskiren was tested at concentrations ranging from 0 to 200 μM .

Results

Significant and distinct inhibition of the respective marker enzyme activities was only observed for CYP2C9, CYP2C19 and CYP2D6 and only at the highest concentration of 200 μM . Aliskiren did not significantly inhibit CYP1A2, CYP2C8, CYP2E1 or CYP3A4/5 (Table 2.1.9.1). At the lower, more *in vivo* relevant concentrations (20 or 25 μM), none of the investigated P450 enzymes were substantially inhibited by aliskiren. Thus, the report concludes that metabolic interactions of aliskiren with co-administered drugs metabolically cleared by CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4/5 appear to be unlikely, based on the low levels of aliskiren observed in plasma of human subjects treated with aliskiren hemifumarate (C_{max} 0.03 μM).

TABLE 2.1.9.1
INHIBITION OF HUMAN P450 ISOFORMS BY ALISKIREN

Human P450 enzyme	Specific Enzymatic Activity	Inhibition Constant IC_{50} for Aliskiren
CYP1A2	Phenacetin <i>O</i> -deethylation	84.8% rel. activity at 200 μM
CYP2C8	Paclitaxel 6 α -hydroxylation	95.5% rel. activity at 200 μM
CYP2C9	S-warfarin 7-hydroxylation	67.9% rel. activity at 200 μM
CYP2C19	S-mephenytoin 4'-hydroxylation	57.5% rel. activity at 200 μM
CYP2D6	Bufuralolol 1'-hydroxylation	67.1 % rel. activity at 200 μM
CYP2E1	Chlorzoxazone 6- hydroxylation	112.8% rel. activity at 200 μM
CYP3A4/5	Midazolam 1'-hydroxylation	116.6% rel. activity at 10 μM

2.1.10. Identification of Human Cytochrome P450 Enzymes in the Metabolism of Aliskiren

This non-GLP study (Report #DMPK R0101129) was conducted at Novartis Pharma AG, Basel, Switzerland between July and September 2004.

Methods

A pool of liver microsomes (obtained from _____) was prepared from 22 individual donors. Biotransformation was carried out by incubating microsomes with the substrate, 1 or 20 μM [^{14}C]aliskiren hemifumarate (batch #E-13669-128-33 and E-30585-11-46, specific activity 2.904 MBq/mg), and with or without isoenzyme specific inhibitors for varying times (up to 60 min) at 37°C. Enzymatic reaction was initiated with the addition of NADPH and the reaction was stopped by the addition of acetonitrile. Inhibition of the enzymatic activity of human cytochrome P450 isoforms by aliskiren (1 to 700 μM [^{14}C]aliskiren) was studied under similar conditions with recombinant human cytochrome P450 isoenzymes expressed in microsomes prepared from baculovirus infected insect cells (membrane preparations were obtained from _____). This study determined the degree of inhibition of each enzyme (22 cDNA recombinant CYPs: CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2B6, CYP2C8, CYP2C9*1, CYP2C18, CYP2C19, CYP2D6*1, CYP2D6*10, CYP2E1, CYP2J2, CYP3A4, CYP3A5, CYP3A7, CYP4A11, CYP4F2, CYP4F3A, CYP4F3B, CYP4F12, CYP19) for different concentrations of test substance. Mean specific CYP enzyme concentrations in human liver microsomes were obtained from literature values.

Results

In pooled human liver microsomes, the metabolism of aliskiren followed apparent single enzyme Michaelis-Menten kinetics. The apparent K_m and V_{max} values were 43.8 μM and 1807.6 pmol/min/mg, respectively.

The recombinant human CYP450 enzyme study showed that aliskiren was mainly metabolized by CYP3A4 (with some contribution of 3A5 in individuals expressing this isoform) and CYP2D6 with significant turnover. The metabolism of aliskiren in human liver microsomes was almost completely inhibited by the 3A4/5 selective chemical inhibitor ketoconazole and significantly by troleandomycin (Fig. 2.3.3.1). A monoclonal antibody specific to CYP3A4 produced 90 % inhibition of the human liver microsomal metabolism of aliskiren. Thus, based on kinetic results with recombinant human CYP450s and the relative abundance values of the currently known CYP isoforms, the report estimated that CYP3A4/5 was contributing predominantly (99.6 %) to the oxidative metabolism of aliskiren in human liver microsomes. Other CYP enzymes (e.g., CYP2D6) may also contribute to a minor extent.

In vitro metabolism of radiolabeled aliskiren in human liver microsomes in the presence of recombinant human CYPs identified four metabolites: M1, M3, M4 and M8. Additionally, several oxidative metabolites were formed under the experimental conditions. These oxidation processes were found to be catalyzed largely by CYP3A4/5 enzymes. Two phenolic metabolites were generated from oxidative demethylation (M1) and dealkylation (M4). Demethylation on the methoxypropoxy side chain formed the alcohol derivative, M3.

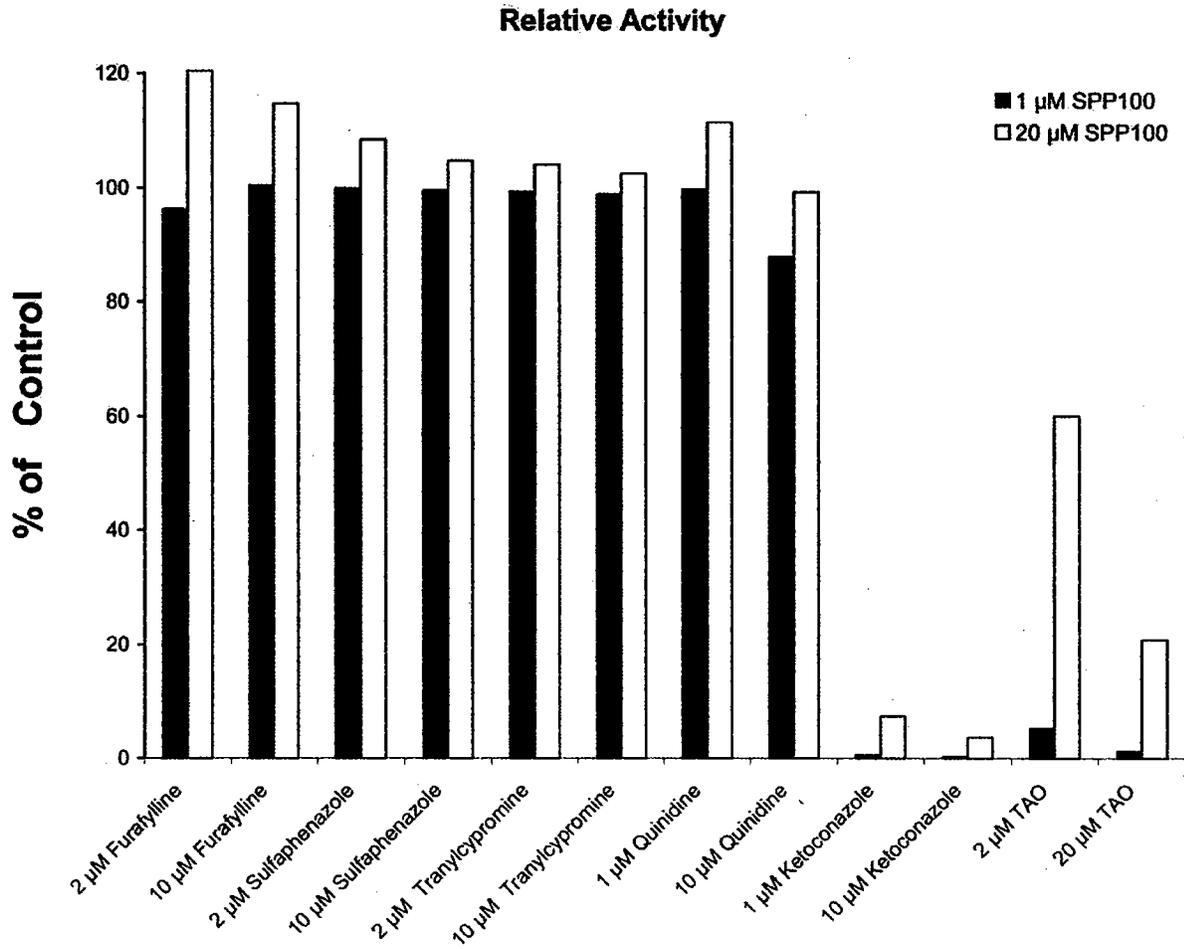


Fig. 2.1.10.1.: Inhibition of aliskiren (SPP100) metabolism in human liver microsomes by chemical inhibitors. TAO: troleandomycin.

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2.1.11. Enterohepatic Circulation in Rats After I.V. Administration of [¹⁴C]aliskiren Hemifumarate

This non-GLP study (Report #PCS(EU) R0301337) was conducted at Novartis Pharma AG, Basel, Switzerland between April 26 and September 29, 2004.

Methods

A group of 4 non-fasted, bile duct-cannulated male rats (HAN:WIST(SPF)) weighing 255 to 348 gm and 10 to 12 weeks old received single intravenous bolus doses of 11.1 mg [¹⁴C]aliskiren hemifumarate/kg (specific activity 3.62 MBq/mg, 10 mg free base/kg) *via* a tail vein. A solution of labeled and unlabeled test substance was prepared in 0.9% saline. The batch number for the tagged substance was E-32775-147-39. The batch number for the non-labeled substance was 0323010. For bile duct cannulation, animals were anesthetized with isoflurane and allowed 45 hr for recovery. One single biliary fraction was collected (on an ice/salt mixture) individually in the 0-24 hr period following dosing. Synthetic bile was continuously substituted *via* the cannula leading into the duodenum with a flow rate of 1 ml/hr. Urine and feces were also collected at the same time (0-24 hr fraction). Liquid scintillation counting was performed on a small aliquot of the individual 0-24 hr bile fraction. Thereafter, the individual fractions were pooled (n=4) and biliary metabolic profiles were carried out on a small aliquot. The remaining large amount of pooled bile (from donor rats) was divided into 3 equal parts and infused (0.92 ml/h for 24 hr) into the duodenum of 3 bile duct-cannulated rats (receiver rats) in group 2 for the first 24 hr. At the end of 24 hr and up to 48 hr, the receiver rats received synthetic bile at a rate of 1 ml/hr. Bile, urine and feces were collected from group 2 rats in 0-24 hr and 24-48 hr fractions. Radioactivity in the biological samples (bile, urine and feces) was measured by liquid scintillation counting. The structures of the metabolites were characterized for bile and urine (donor rats only) by LC-MS with the HPLC-method and mass spectrometry.

Results

After intravenous administration of 10 mg [¹⁴C]aliskiren/kg to male rats (donor rats), 83% of the radioactivity was recovered within 24 hr in the donor rats (70% in bile, 6.2% in urine, 4.6% in feces). The radioactivity infused into the duodenum of receiver rats was considered as the dose and set at 100%. Within 48 hr, 93% of this dose was recovered in the excreta: 59.8% in the gastrointestinal tract, 26.6% in feces, 2.2% in bile, 0.6% in urine and 4% in cage wash. The sum of radioactivity in feces and GI tract (86.4%) represents unabsorbed test substance. The combined amount of radioactivity found in bile and urine (2.8% of the infused radioactivity) was absorbed from the duodenum and was available for enterohepatic circulation, a minor pathway. The 2.8% represents 2% of the original radioactivity administered intravenously to the donor rats. Since the bioavailability of aliskiren in rats is very low (<3%), the report concludes that the enterohepatic circulation after oral administration is negligible.

Bile of donor rats was analyzed for the presence of metabolites. Unchanged parent compound accounted for the main component of the radioactivity in the bile. Previously characterized metabolites M1, M2, M3, M4, M5 and M6 were detected in small quantities. Since bile of receiver rats contained only a low amount of radioactivity (2%), only trace amounts of

metabolites M2, M3 and a peak of M5/M6 were detectable in addition to the predominant parent compound. Urine from the donor rats showed a pattern of metabolites comparable to that of the bile sample (Table 2.1.11.1).

TABLE 2.1.11.1
THE PATTERNS OF METABOLITES AND PARENT COMPOUND IN BILE AND URINE OF RATS
FOLLOWING I.V. ADMINISTRATION OF [¹⁴C]ALISKIREN HEMIFUMARATE

Metabolite	Proportion of radioactivity [%]			
	donor rats (0-24 h) Bile untreated	donor rats (0-24 h) Bile hydrolyzed ^a	donor rats (0-24 h) Urine untreated	receiver rats (0-48 h) Bile untreated
M5+M6	5.0	ND	3.2	8.9
M4	1.4	4.5	0.9	ND
M3	15.9	15.5	4.7	15.2
M2	7.4	10.2	2.9	15.4
M1	1.3	2.8	0.8	ND
SPP100	64.3	61.8	79.8	60.4
Other peaks	4.7	5.2	12.9	0.1

a) hydrolyzed using β -glucuronidase from *E. coli*; ND) not detected

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2.1.12. Galactogenic Transfer, Kinetics and Metabolism in Milk and Plasma After Oral Administration of [¹⁴C]aliskiren Hemifumarate

This nonGLP study (report #PCS(EU) R0300782) was conducted at Novartis Pharma AG, Basel, Switzerland (report release date: December 15, 2004).

Lactating albino rats (HAN:WIST — , weighed 264 to 318 gm (age not specified) at initiation of drug administration. The rats were dosed on day 11 after parturition. Each rat had a reduced litter of 5 pups and was housed individually. Animals were not fasted. A single oral dose of 110.5 mg [¹⁴C]aliskiren hemifumarate/kg (3.62 MBq/mg, 100 mg free base/kg) was administered orally by gavage to 8 rats. A solution of labeled and unlabeled test substance was prepared in water. The batch number for the tagged substance was E-3277-147-39. The batch number for the non-labeled substance was 0323010. The pups were separated from their dams two hours before sample collection. In order to stimulate milk secretion, oxytocin (4 IU/kg, i.p.) was administered 3 to 5 min before sample collection. Blood and milk were collected at 0.25, 1, 3, 8, 24, 48 and 72 hr after dosing (4 animal/time point). Radioactivity in the biological samples (blood, plasma and milk) was measured by liquid scintillation counting.

Results

No test substance-related clinical signs were noted for any of the animals. Following oral administration of 100 mg [¹⁴C]aliskiren/kg, peak plasma levels of radioactivity (1.61 $\mu\text{mol/L}$) were noted at 0.25 hr (t_{max}). This was followed by a fall and then a second peak (1.17 $\mu\text{mol/L}$) at 3 hr ($t_{\text{max}} = 2\text{hr}$) (see Table 2.1.12.1). The pharmacokinetic results obtained in this study were comparable to those obtained in an earlier study (see section 2.1.2). Elimination kinetics of radioactivity was not studied. Radioactivity was below the level of detection for most of the rats at 48 hr and all rats at 72 hr.

Total radioactivity in milk increased slowly and reached a maximum at 3 hr, then slowly declined and was still detectable at 72 hr, the time of the last measurement. The concentration-time course of total radioactivity in milk was distinctly different from that in plasma. At the two earliest time points, 0.25 and 0.5 hr, the concentrations of radioactivity were higher in plasma than in milk, resulting in milk to plasma ratios of 0.12 and 0.57, respectively. This ratio was reversed at 3, 8 and 24 hr post dose, when the concentration in milk was distinctly higher than in plasma (milk: plasma ratios of 2.8, 13 and 2.7, respectively) (Table 2.1.12.1). Radioactivity in milk up to 72 hr after dosing accounted for about 0.08% of the administered dose. Assuming a systemic bioavailability of 6.7% after oral administration for total radioactivity (value estimated in section 2.1.2), approximately 1.1% of the systemically available radioactivity was eliminated *via* milk (assuming that the production of milk was constant). The sponsor did not provide the basis for this number.

TABLE 2.1.12.1
MILK TO PLASMA RATIO OF RADIOACTIVITY IN LACTATING RATS AFTER ORAL
ADMINISTRATION OF [¹⁴C] ALISKIREN HEMIFUMARATE

Time [h]	Mean ¹⁴ C concentration [μmol/L]		Ratio Milk:Plasma
	Plasma ^a	Milk ^b	
0.25	1.614	0.197	0.12
1	0.650	0.369	0.57
3	1.173	3.306	2.8
8	0.119	1.531 ^a	13
24	0.065	0.178 ^c	2.7
48	BLOQ	0.072	-
72	BLOQ	0.045	-

a: mean of four concentrations

b: if not indicated otherwise, mean of 3 values

c: mean of two concentrations

BLOQ: below limit of detection, LOQ for ¹⁴C = 0.03 μmol/L

The parent compound accounted for the main portion of radioactivity in plasma (61% of total based on AUC_{0.25-24 h}). The known metabolites M2, M5 and M6 were also detected in plasma extracts. In milk samples, parent compound was detectable up to 72 hours after administration (27% of the total radioactivity, based on AUC_{0.25-72 h}). The pattern of metabolites in milk varied with time though M2, M5 and M6 were detected in samples up to 3 hr.

**APPEARS THIS WAY
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3.0 TOXICOLOGY

3.1. Single-dose toxicity

3.1.1. Oral Toxicity Study in Female Rats

This non-GLP study (Test #94-6209) was conducted by _____ between December 12 and 29, 1994.

Albino female rats (Tif:RAIf (SPF)) were 4 to 5 weeks of age and weighed 100 to 116.3 gm on the day of dosing. Aliskiren hemifumarate (batch #6) was administered once by gavage at a dose of 1000 or 2000 mg/kg to a single rat. Since the highest dose, 2000 mg/kg, was tolerated, it was given to another 2 female rats. The test substance was dissolved in 5% mannitol and administered in a dose volume of 10 or 20 ml/kg. The study did not involve a control group. All animals were observed for approximately 14 days after dosing for any potential toxic effects. Body weights were recorded two times a week and at study termination. Necropsies were performed on day 15. No histopathological examinations were performed.

No animals died. No remarkable test substance-related clinical signs of toxicity were observed. No changes in body weights were observed. No abnormal macroscopic observations were noted in any of the treated animals.

**APPEARS THIS WAY
ON ORIGINAL**

3.2. Repeat-dose Toxicity

3.1.1. Four-Week Oral (Dietary) Dose Range-Finding Study in CB6F1 WT Mice

Key Study Findings: Dietary administration of aliskiren hemifumarate at increasing doses resulted in body weight and body weight gain reductions that were more severe for males (overall reduction in gain 19 to 107% of control) than females (overall reduction in gain 14 to 82% of control) at doses ≥ 500 and ≥ 1000 mg aliskiren/kg/day for males and females, respectively.

Study No: 470069

Location of Report: EDR

Conducting Laboratory and Location: Novartis Pharmaceuticals Corporation, East Hanover, NJ

Dates of Study: The animals were dosed from March 2 to March 31, 2004 and necropsied on March 31 and April 1, 2004.

GLP Compliance: Yes

QA'd Report: yes (X) no ()

Drug, Lot #: Aliskiren hemifumarate, batch #0323010

Formulation: Aliskiren hemifumarate was admixed with the diet. Concentration, homogeneity and stability of each drug-diet formulation was determined at the end of study weeks 1 and 4.

Animals

Species/Strain: (CB6F1/Jic-TgrasH2@Tac wild-type) mice

#/Sex/Group: 10/sex/group for toxicology; 12(4 allocated for control group 1)/sex/group for toxicokinetics

Age: 7-8 weeks at the time of dosing

Weight: males: 20.8-27.1 gm, females: 17.0-22.0 gm

Husbandry: Animals were housed singly and received standard rodent diet and water *ad libitum*.

Dosing

Doses: Four groups of mice were given aliskiren hemifumarate admixed with the diet at concentrations calculated to result in mean daily target doses of 500, 1000, 1500 and 2000 mg/kg (dose levels are expressed as base) for 4 weeks. The control group (n=10/sex) received untreated feed. Additional (satellite) animals were treated with aliskiren hemifumarate in a similar way for toxicokinetic analyses (Table 3.2.1.1). The study was designed to provide data for dose selection for a future carcinogenicity study in transgenic CB6F1-TgrasH2 mice.

The doses were selected on the basis of a 2 week oral dosing study in which gavage administration of aliskiren hemifumarate in CD-1 mice produced serious morphological alterations to the respiratory epithelium of the nasal cavity at all dose levels of 350, 800 and 1200 mg aliskiren/kg/day. In a second 2 week study in CD-1 mice, aliskiren hemifumarate was administered either by gavage (1000 mg/kg/day) or diet (150, 600 or 1000 mg/kg/day). Nasal cavity lesions severe enough to result in the deaths of 2 mice were noted in the group of 12 mice that had received 1000 mg/kg/day by gavage. However, there were no test article-related findings in the respiratory tracts of mice

which had received the same dose *via* the diet. Dietary doses were well tolerated. In a 13 week dietary study in CD-1 mice, doses up to 2000 mg/kg/day did not result in mortality or clinical signs. Mean body weight gain was significantly decreased ($p < 0.05$) at doses ≥ 1000 mg aliskiren/kg/day in the first 4 weeks of the study, relative to control weight gain, and group mean body weights remained lower than control ($p > 0.05$) at these doses for the entire treatment period.

TABLE 3.2.1.1
STUDY DESIGN

Allocation of animals	Sex	Control	Target Dose level, mg/kg/day			
			500	1000	1500	2000
		Group 1	Group 2	Group 3	Group 4	Group 5
Toxicology	M	10	10	10	10	10
	F	10	10	10	10	10
Toxicokinetics	M	4	12	12	12	12
	F	4	12	12	12	12

All of the above doses are expressed in terms of the aliskiren base (salt/base ratio is 1.105)

Observations and Measurements

Clinical Signs: All animals were observed twice daily for mortality and clinical signs.

Body Weights: Recorded for all animals once during acclimatization and then once weekly during the dosing period. Terminal body weights were determined for toxicology group animals at scheduled sacrifice.

Food Consumption: Measured on study days 1, 8, 15, 22, and 29 (toxicology study animals only). However, due to excessive spillage, food consumption could not be accurately determined and, thus, was not analyzed or reported.

Ophthalmoscopy: Conducted on control and high dose toxicity animals during study week 4.

Hematology and Clinical Biochemistry: Blood was collected from the abdominal aorta/vena cava for clinical biochemistry (first 5 animals in each group) and hematology (remaining 5 animals in each group) and the animals then killed by exsanguination.

Gross Pathology: All surviving toxicology animals were fasted overnight (18 hr) prior to necropsy. The animals were first weighed and then anesthetized using isoflurane/O₂. The necropsy included detailed internal and external examinations on all toxicology group animals.

Organs Weighed: All main study animals at the scheduled necropsy.

Histopathology: Histopathological examination was performed on tissues listed in Table 3.2.1.2 for all animals in the control and high dose groups. Target organs (thymus, ovaries and uterus) and macroscopic lesions were processed for all animals in the remaining groups.

TABLE 3.2.1.2
TISSUES/ORGANS SAMPLED FOR HISTOPATHOLOGICAL EXAMINATION

Adrenal glands*	Kidney*	Skeletal muscle
Aorta	Lacrimal gland	Skin
Bone marrow	Liver*	Spinal cord
Bone marrow (in bone)	Lung	Spleen*
Brain*	Lymph node (bronchial, mandibular, mesenteric)	Sternum
Cecum	Mammary gland	Stomach
Cervix	Nasal passage [§]	Testes*
Colon	Ovary*	Thymus*
Duodenum	Pancreas	Thyroid
Epididymides	Parathyroid	Tongue
Esophagus	Pituitary	Trachea
Eye	Prostate*	Urinary bladder
Femur/tibia	Rectum	Uterus*
Harderian gland	Salivary gland	Vagina
Heart*	Sciatic nerve	Zymbal glands
Ileum	Seminal vesicles	Gross lesions
Jejunum		Animal identification [§]

*: Organ weighed; [§]: tissue collected but not processed

Toxicokinetics: Blood samples for test substance determination were collected from the retro-orbital venous plexus of animals under isoflurane anesthesia in study week 4. Control animals were bled at 7 and 9 AM (2 animals/sex/time point) and animals from the drug-treated groups were bled at 6, 6:30, 7 and 9 AM, and 4 PM (n= 2/sex/time point). All animals were killed following blood collection.

Results

Analysis of Formulations: Aliskiren hemifumarate rodent feed admixtures at concentrations of 2 mg/g to 55.25 mg/g were found to be stable for at least 21 days at room temperature. Concentrations of the drug in the week 1 and week 4 admixtures ranged from _____ of target concentrations. Analysis of control samples revealed no test substance. Test substance intake values for this study could not be calculated due to the inability to accurately assess food consumption because of excessive feed spillage. Achieved doses were estimated throughout the study by utilizing a standard food consumption value of 5.5 g/day for males and 5.0 g/day for females.

Mortality: With the exception of one toxicokinetics animal (found dead due to a mechanical injury), all animals survived until scheduled necropsy.

Clinical Signs: There were no test substance-related clinical signs noted in the study.

Body Weights: Significant (p <0.05) reductions in mean body weight values relative to control were observed for males treated with aliskiren hemifumarate on days 8 (9-13%), 15 (3-11%) 22 (8-11%) and 29 (7-8%) at doses ≥1000 mg/kg/day. The mean body weight reductions on study day 29 were approximately 7%, 8%, and 8% of control at doses of 1000, 1500 and 2000 mg/kg/day, respectively (Table 3.2.1.3, Fig. 3.2.1.1 top panel). Mean absolute body weight gains were significantly less than control weight gain (p <0.05) for all drug-treated groups on study day 22 and at doses ≥1000 mg/kg/day for the remaining days of the study (Table 3.2.1.4). The overall reduction (day 1 to day 29) in

mean body weight gain was approximately 19%, 41%, 78% and 107% of control at 500, 1000, 1500 and 2000 mg/kg/day, respectively ($p < 0.05$ at doses ≥ 1000 mg/kg/day). The difference from control in absolute body weight at doses ≥ 1500 mg/kg/day had decreased to 8% by the end of the study (day 29) from a peak of 13% (day 8), a consequence of group mean body weight gains for these drug-treated groups during the last week of treatment being 550 and 350% of control (Table 3.2.1.5).

Statistically significant reductions ($p < 0.05$) in mean body weight relative to control were noted for females at 2000 mg/kg/day on day 8 (10%) and at 1500 and 2000 mg/kg/day on days 22 (7% and 8%) and 29 (5% and 10%, Table 3.2.1.3, Fig 3.2.1.1 bottom panel).

Mean reductions ($p < 0.05$) in body weight gain relative to control were noted at doses ≥ 1000 mg/kg/day on day 22 and at 1500 and 2000 mg/kg/day on day 29 (Table 3.2.1.4). The overall reduction (days 1 to 29) in female body weight gain was approximately 14%, 27% and 82% of control at 1000, 1500 and 2000 mg/kg/day, respectively, with statistical significance at doses ≥ 1500 mg/kg/day. Although there was a notable recovery of body weights for the 500, 1000 and 1500 mg/kg/day groups (167 to 333% of control during the last week of treatment), the high dose group failed to gain weight during the final treatment week (Table 3.2.1.5).

TABLE 3.2.1.3
GROUP MEAN BODY WEIGHTS (in g) FOR MALES AND FEMALES

Group (s)		Day of Phase					
		1†	1"	8	15	22	29
Male Animals							
1	(N)	14	10	10	10	10	10
	Means	23.5	23.7	24.4	25.3	26.2	26.4
	Sdevs	1.73	2.03	1.73	2.00	1.86	1.93
2	(N)	22	10	10	10	10	10
	Means	23.4	23.8	23.6	24.9	24.9	25.9
	Sdevs	1.64	1.43	1.93	1.38	1.32	1.42
3	(N)	22	10	10	10	10	10
	Means	23.4	22.9	21.8*	23.9	24.2*	24.5*
	Sdevs	1.71	1.14	1.20	1.22	1.55	1.59
4	(N)	22	10	10	10	10	10
	Means	23.4	23.7	21.2*	22.4*	23.2*	24.3*
	Sdevs	1.54	1.49	2.01	1.47	2.00	1.51
5	(N)	22	10	10	10	10	10
	Means	23.4	24.5	22.2*	24.6	23.6*	24.3*
	Sdevs	1.65	1.51	2.03	1.13	1.43	1.33
Female Animals							
1	(N)	14	10	10	10	10	10
	Means	19.0	19.6	20.0	20.9	21.5	21.8
	Sdevs	1.05	0.75	0.93	0.83	0.73	0.64
2	(N)	22	10	10	10	10	10
	Means	19.0	19.5	18.2	21.2	21.1	21.6
	Sdevs	1.07	1.22	2.11	1.11	1.15	1.31
3	(N)	22	10	10	10	10	10
	Means	19.0	19.9	19.8	20.4	20.9	21.9
	Sdevs	1.09	1.23	1.46	1.03	1.28	1.10
4	(N)	22	10	10	10	10	10
	Means	19.0	19.1	18.4	20.2	19.9*	20.8†
	Sdevs	1.10	1.01	1.76	1.02	0.98	1.15
5	(N)	22	10	10	10	10	10
	Means	19.0	19.3	18.1*	19.8	19.7*	19.7†
	Sdevs	1.09	1.10	1.95	1.58	1.63	2.34

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

Group:	1	2	3	4	5
Dose level (mg/kg/day)	0	500	1000	1500	2000

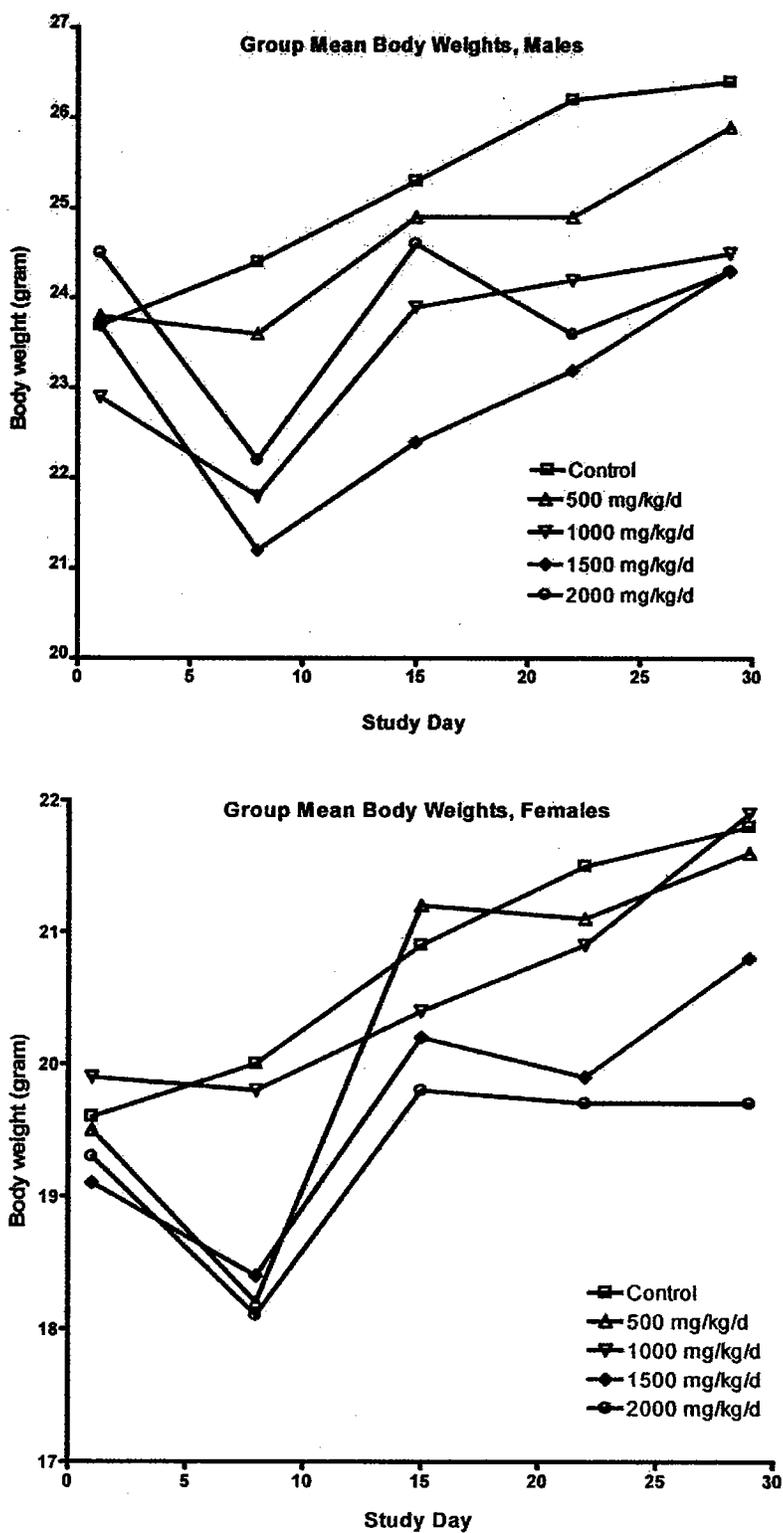


Fig. 3.2.1.1.: Group Mean Body Weights for Males and Females

TABLE 3.2.1.4
GROUP MEAN BODY GAINS (in g) FOR MALES AND FEMALES

Absolute weight gains referenced to treatment phase (Day 1)
 Study start date: 24-Feb-04

Group (s)		Day of Phase			
		8	15	22	29
Male Animals					
1	(N)	10	10	10	10
	Means	0.7	1.6	2.5	2.7
	Sdevs	1.00	0.64	0.89	1.05
2	(N)	10	10	10	10
	Means	-0.2	1.1	1.2*	2.2
	Sdevs	0.75	0.49	0.53	0.53
3	(N)	10	10	10	10
	Means	-1.1+	1.0	1.3*	1.6*
	Sdevs	1.02	0.91	1.07	1.25
4	(N)	10	10	10	10
	Means	-2.5+	-1.2+	-0.4+	0.6+
	Sdevs	0.89	1.00	1.16	0.74
5	(N)	10	10	10	10
	Means	-2.2+	0.1+	-0.8+	-0.2+
	Sdevs	1.17	0.60	1.23	0.69
Female Animals					
1	(N)	10	10	10	10
	Means	0.4	1.3	1.9	2.2
	Sdevs	0.98	0.87	0.59	0.40
2	(N)	10	10	10	10
	Means	-1.3*	1.7	1.7	2.1
	Sdevs	1.48	0.79	0.59	0.59
3	(N)	10	10	10	10
	Means	-0.1	0.5+	1.0*	1.9
	Sdevs	1.01	0.60	0.77	0.51
4	(N)	10	10	10	10
	Means	-0.8	1.0	0.7+	1.6†
	Sdevs	1.58	0.49	0.49	0.52
5	(N)	10	10	10	10
	Means	-1.2*	0.5	0.4+	0.4§
	Sdevs	1.40	0.91	0.90	1.65

Note: Data for 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

Group:	1	2	3	4	5
Dose level (mg/kg/day)	0	500	1000	1500	2000

TABLE 3.2.1.5
GROUP MEAN BODY WEIGHT GAINS (G) AND GROUP MEAN BODY WEIGHTS (G) ON DAY 29 FOR THE 4-WEEK DIETARY STUDY IN CB6F1 WT MICE

Dose, mg/kg/day	Control		500		1000		1500		2000	
	M	F	M	F	M	F	M	F	M	F
Weeks 0 to 1	0.7	0.4	-0.2	-1.3	-1.1	-0.1	-2.5	-0.7	-2.3	-1.2
Weeks 1 to 2	0.9	0.9	1.3	3.0	2.1	0.6	1.2	1.8	2.4	1.7
Weeks 2 to 3	0.9	0.6	0.0	-0.1	0.3	0.5	0.8	-0.3	-1.0	-0.1
Weeks 3 to 4	0.2	0.3	1.0	0.5	0.3	1.0	1.1	0.9	0.7	0.0
			(500)	(167)	(150)	(333)	(550)	(300)	(350)	(0)
Weeks 0 to 4	2.7	2.2	2.2	2.1	1.6	1.9	0.6	1.6	-0.2	0.4
			(81)	(95)	(59)	(86)	(22)	(73)	(-107)	(18)
Gp mean body wt.(g) on day 29	26.4	21.8	25.9	21.6	24.5*	21.9	24.3*	20.8§	24.3*	19.7§

Numbers in parentheses indicate group mean body weight gain (%) relative to control
 * : mean value of group was significantly different from control at P = 0.05 with Dunnett's test of significance
 § : mean value of group was significantly different from control at P = 0.05 with modified T test of significance

Ophthalmoscopy: No drug-related ophthalmoscopic changes across groups.

Hematology and Clinical chemistry: No drug-related findings for any dose group.

Organ Weights: Significant decreases ($p < 0.05$) in absolute and relative-to-body weights of kidneys, spleens, and hearts relative to concurrent control were noted for both high dose males and females at 2000 mg/kg/day. Absolute and relative thymus weights were significantly lower ($p < 0.05$) than control only for aliskiren-treated males at 2000 mg/kg/day (Table 3.2.1.6). None of these organ weight decreases had correlating macroscopic or microscopic changes. The decreases were considered by the sponsor to reflect decreased body weight mean values and thus were not considered a direct effect of the test substance. (This interpretation may not be valid for those organs where both absolute and relative to body weights were decreased.) However, small decreases in absolute and relative ovarian ($p < 0.05$) and uterine ($p > 0.05$) weights at 2000 and ≥ 1500 mg/kg/day, respectively, had correlating microscopic observations.

TABLE 3.2.1.6
MEAN ORGAN WEIGHTS AFTER 4 WEEKS OF DOSING IN RASH2 MICE

Group	Kidney		Spleen		Thymus		Heart		Uterus		Ovary		
	Abs	%BW	Abs	%BW	Abs	%BW	Abs	%BW	Abs	%BW	Abs	%BW	
Control	M	0.399	1.779	0.050	0.224	0.030	0.133	0.136	0.606				
	F	0.295	1.633	0.053	0.290	0.032	0.173	0.114	0.637	0.098	0.538	0.011	0.061
500	M	0.390	1.794	0.050	0.231	0.031	0.145	0.128	0.591				
	F	0.285	1.558	0.053	0.288	0.032	0.175	0.114	0.622	0.097	0.537	0.011	0.058
1000	M	0.351*	1.740	0.043*	0.214	0.031	0.153	0.118*	0.585				
	F	0.283	1.565	0.054	0.296	0.030	0.168	0.107	0.593*	0.096	0.525	0.011	0.064
1500	M	0.348*	1.718	0.044*	0.217	0.033	0.161	0.115*	0.569*				
	F	0.265*	1.544	0.046	0.271	0.035	0.202	0.098*	0.571*	0.081	0.466	0.011	0.064
2000	M	0.339*	1.653*	0.040*	0.195*	0.022*	0.108*	0.115*	0.564*				
	F	0.255*	1.516*	0.042*	0.245*	0.027	0.158	0.098*	0.583*	0.074	0.433	0.007 [§]	0.039 [§]

Data were NOT analyzed by the sponsor. This reviewer analyzed differences between groups using ANOVA followed by Dunnett's T-test (Sigma Stat). *: $p < 0.05$.

Difference between the control and the high dose groups was analyzed by t-test. [§]: $p < 0.05$

Histopathology: Treatment-related microscopic observations were restricted to ovary and uterus.

Increased ovarian follicular atresia and absence of corpora lutea at 2000 mg/kg/day and decreased density of uterine glands at doses ≥ 1500 mg/kg/day were noted. According to the sponsor there could be a compromise in hormonal support for these organs. Microscopic evaluation showed no abnormalities of spermatogenesis.

Toxicokinetics: Measurable concentrations (— ng/ml) of aliskiren were detected in samples of blood taken from 2 males and 1 female control at 7 AM. The values corresponded to 7.4 and 10.8% of C_{max} in the low dose male animals and 27.7% of C_{max} in the low dose female animals. No aliskiren above the limit of quantitation was measured in any of the control samples taken at 9 AM. It may be noted that feed admixtures analyzed during week 4 of the study showed absence of aliskiren in the control feed. The sponsor notes that control animals were not exposed directly to test substance and the presence of test article is believed to be due to sample contamination from an unknown source.

Inter-animal variability was moderate to high, and greater for males than females. The standard errors computed for AUC values are very high since only 2 animals/sex/group/time point were used. Plasma concentrations of aliskiren after repeated daily administration of test substance in the feed increased with increasing dose levels but were non-dose proportional, especially for males in the mid-high dose group. Based on $AUC_{(0-24h)}/dose$ values, exposures in males and females were of the same order of magnitude. Time to reach maximum concentration (t_{max}) ranged from 0 to 3 hr (Table 3.2.1.7).

TABLE 3.2.1.7
TOXICOKINETICS FOR ALISKIREN IN MICE AFTER DIETARY ADMINISTRATION FOR 4 WEEKS
GROUP MEAN VALUES

Dose group	2-Low		3-Mid		4-Mid-High		5-High	
Dose (mg/kg/day)	500		1000		1500		2000	
Parameters	Male	Female	Male	Female	Male	Female	Male	Female
t_{max}	3	0	3	3	3	0.5	1	0.5
C_{max}	312	63.8	879	167	363	1020	439	1230
$C_{max}/Dose$	0.624	0.128	0.879	0.167	0.242	0.68	0.22	0.615
$AUC_{(0-24h)}$	1700	857	4820	2280	4140	5470	7720	11800
	(1040)*	(351)*	(2390)*	(275)*	(1010)*	(810)*	(1570)*	(1830)*
$AUC_{(0-24h)}/Dose$	3.41	1.71	4.82	2.28	2.76	3.65	3.86	5.88
	(2.08)*	(0.70)*	(2.39)*	(0.28)*	(0.68)*	(0.54)*	(0.79)*	(0.91)*

t_{max} in hours; C_{max} in ng/mL; $C_{max}/dose$ in (ng/mL)/(mg/kg/day); $AUC_{(0-24h)}$ in (ng/ml) x hours and $AUC_{(0-24)}/dose$ in ((ng/mL)x hours)/(mg/kg/day)

For $AUC_{(0-24h)}$ calculation, concentration at time 24 h was considered equal to concentration at 0 h (assuming steady state was reached on week 4).

*Standard error (SE) on $AUC_{(0-24h)}$ or $AUC_{(0-24h)}/dose$

In summary, aliskiren hemifumarate administered in the feed to CB6F1 wild type mice for 4 weeks was tolerated at target doses up to 2000 mg aliskiren/kg/day. Doses ≥ 500 mg/kg/day in males and ≥ 1000 mg/kg/day in females resulted in decrements in body weight and body weight gain relative to control that were more severe for males than females.

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3.2.2 Thirteen-Week Oral (Dietary) Study in CD Mice

Key Study Findings: Dietary administration of aliskiren hemifumarate resulted in decrements in body weight and body weight gain relative to control at doses as low as 1000 mg aliskiren/kg/day (statistically significant in the first 4 weeks of study). Males were more affected than females. The NOAEL for this study was less than 1000 mg/kg/day.

Study No: 940/19

Location of Report: EDR

Conducting Laboratory and Location:

Dates of Study: Dosing was initiated on November 26, 2001 and terminal necropsies were begun on February 26, 2002.

GLP Compliance: Yes

QA'd Report: yes (X) no ()

Drug, Lot #: Aliskiren hemifumarate, batch #S100B-2001002, S100B-2001004, S100B-2001005, S100B-2001006

Formulation: Aliskiren hemifumarate was admixed with the diet. The formulation (in triplicate) was analyzed for test article concentration immediately after preparation and after 7 days to determine stability. Analysis of the formulations for achieved concentration was performed in week 1 and week 13 of treatment for each treated group.

Animals

Species/Strain: (CD-1(ICR)BR) Wistar mice

#/Sex/Group: 20/sex/group for toxicology; 9/sex/group for toxicokinetics

Age: 7 weeks at the time of dosing

Weight: males: 27.6-38.4 gm, females: 21.8-32.1 gm

Husbandry: Animals were housed in groups of 3 and received standard mouse maintenance diet and water *ad libitum* through out the study period unless otherwise specified.

Dosing

Two groups of 12 male and 12 female mice were given aliskiren hemifumarate admixed with the diet at doses of 0 and 1000 mg/kg/day (dose levels are given as base), respectively, for 13 weeks. Additionally, two groups of 12 male and 12 female mice were given test article mixed in the diet for one week at doses of 2500 or 5000 mg/kg/day, respectively. The dosing of these animals was stopped after a week due to adverse effects on food consumption and body weight. Dosing was resumed two weeks later at levels of 1500 and 2000 mg/kg/day, respectively, for an additional 13 weeks. The control animals received diet without the test drug. Additional groups of aliskiren-treated mice, each composed of 18 mice per sex, were used for toxicokinetic study (Table 3.2.2.1).

TABLE 3.2.2.1
STUDY DESIGN

Allocation of animals	Sex	Control	Dose level, mg/kg/day		
			1000	2500/1500 [§]	5000/2000 [§]
		Group 1	Group 2	Group 3	Group 4
Toxicology	M	12	12	12	12
	F	12	12	12	12
Toxicokinetics	M	18	18	18	18
	F	18	18	18	18

[§] : Due to adverse effects, dosing of animals in groups 3 and 4 was suspended from day 8 to day 21. Treatment resumed at reduced dose levels from day 22 (for the next 13 weeks). All of the above doses are expressed in terms of the aliskiren base.

The doses were selected on the basis of a 2 week oral study in which gavage administration of aliskiren hemifumarate produced serious morphological alterations to the respiratory epithelium of the nasal cavity at all dose levels: 350, 800 or 1250 mg aliskiren/kg/day. In a second 2 week study, the drug was administered either by gavage or diet. Nasal cavity lesions severe enough to result in the deaths of 2 mice were noted in the group that had received 1000 mg aliskiren/kg/day by gavage. However, there were no test article-related findings in the respiratory tract of mice which had received the same dose in the diet. Dietary doses were well tolerated.

Observations and Measurements

Clinical Signs: All animals were observed twice daily for mortality and clinical signs.

Body Weights: Recorded before treatment on the first day of dosing and then at weekly intervals and before necropsy for all animals.

Food Consumption: Determined weekly.

Hematology and Clinical Chemistry: Blood samples for hematology (first 6 animals in each group) and clinical biochemistry (remaining 6 animals in each group) were collected from the toxicology group in week 13. Blood was drawn from the retro-orbital plexus under halothane anesthesia in non-fasted animals.

Urinalysis: Urine samples were collected overnight from 6 animals/sex from groups 1 and 2 (main study only) but were not analyzed.

Gross Pathology: All surviving and moribund toxicology animals were anesthetized by i.p. injection of pentobarbital and killed by exsanguination. The necropsy included detailed internal and external examinations, weighing of selected organs and sampling of tissues for histopathological examination (Table 3.2.2.2).

Histopathology: All tissues from all animals of the control and the high dose groups, all animals that died or were sacrificed moribund, and all gross lesions from all animals were examined histopathologically. Histopathological examination of organs/tissues in the remaining animals from the main study was limited to nasal cavities, kidneys, liver, and spleen.

TABLE 3.2.2.2
TISSUES/ORGANS SAMPLED FOR HISTOPATHOLOGICAL EXAMINATION

Adrenal glands*	Liver*	Spinal cord - cervical, thoracic, lumbar
Aorta	Lungs with mainstream bronchi	Spleen*
Bone marrow (sternum, femur)	Lymph nodes - mesenteric, mandibular	Stomach
Bone marrow smear (femur)	Mammary (females only)	Testes + Epididymides*
Brain*	Muscle (quadriceps)	Thymus
Cecum	Nasal turbinates	Thyroid + parathyroids*
Colon	Nasopharynx	Tongue
Duodenum	Optic nerves	Trachea
Esophagus	Ovaries*	Trachea bifurcation
Eyes	Pancreas	Urinary bladder
Harderian gland	Pituitary gland*	Uterus
Head	Prostate gland	Vagina
Heart*	Rectum	Zymbal glands
Ileum	Salivary glands	Gross lesions
Jejunum	Sciatic nerves	
Kidney*	Seminal vesicles	
Larynx	Skin	
Lachrymal glands		

*: Organ weighed

Toxicokinetics: Blood samples were collected from the retro-orbital plexus of the toxicokinetics animals under halothane anesthesia on day 1 and during study week 13. Blood sampling time points were as follows.

Blood sampling schedule for Week 2

Group/sex	Sample time (hours post-dose)					
	1	2	4#	8	16	24
	Animal number					
1M	97-99	100-102	103-105	97-99	100-102	103-105
2M	115-117	118-120	121-123	115-117	118-120	121-123
3M	133-135	136-138	139-141	133-135	136-138	139-141
4M	151-153	154-156	157-159	151-153	154-156	157-159
1F	169-171	172-174	175-177	169-171	172-174	175-177
2F	187-189	190-192	193-195	187-189	190-192	193-195
3F	205-207	208-210	211-213	205-207	208-210	211-213
4F	223-225	226-228	229-231	223-225	226-228	229-231

food hoppers removed from cages as soon as lights switched on, then replaced following the 4 hour bleed time point.

Blood sampling schedule for Week 13

Group/sex	Sample time (hours post-dose)					
	1	2	4	8	16	24
	Animal number					
1M	106-108	109-111	112-114	106-108	109-111	112-114
2M	124-126	127-129	130-132	124-126	127-129	130-132
3M	142-144	145-147	148-150	142-144	145-147	148-150
4M	160-162	163-165	166-168	160-162	163-165	166-168
1F	178-180	181-183	184-186	178-180	181-183	184-186
2F	196-198	199-201	202-204	196-198	199-201	202-204
3F	214-216	217-219	220-222	214-216	217-219	220-222
4F	232-234	235-237	238-240	232-234	235-237	238-240

Food hoppers were not removed during the Week 13 bleed

Results

Analysis of Formulations: Dietary formulations were found to be homogeneous. The achieved concentration of test substance in the dietary formulation was 85 to 110% of the targeted concentration.

Mortality: One low dose male died on study day 62. The animal had ulceration and inflammation of the skin of the hind limb and genital areas together with renal tubular dilatation. As there were no deaths at higher dose levels, this death was not considered to be treatment-related.

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

Body Weights: Group mean body weights remained low in all treated groups relative to control for the entire dosing period (Fig. 3.2.2.1 and 3.2.2.2, Table 3.2.2.3). Mid and high dose groups lost weight during the first week of the study. Mean body weight gain was statistically significantly decreased ($p < 0.05$) for all dose groups in the first 4 weeks of the study relative to control values (dosing had been suspended for mid and high dose groups after the first week). Males were more affected than females (Table 3.2.2.4). Body weights tended to recover 4 weeks into the study. Reductions in body weight gain relative to control for weeks 4 to 13 (for males and females) were not statistically significant ($p > 0.05$) except for the low dose male group (Table 3.2.2.4).

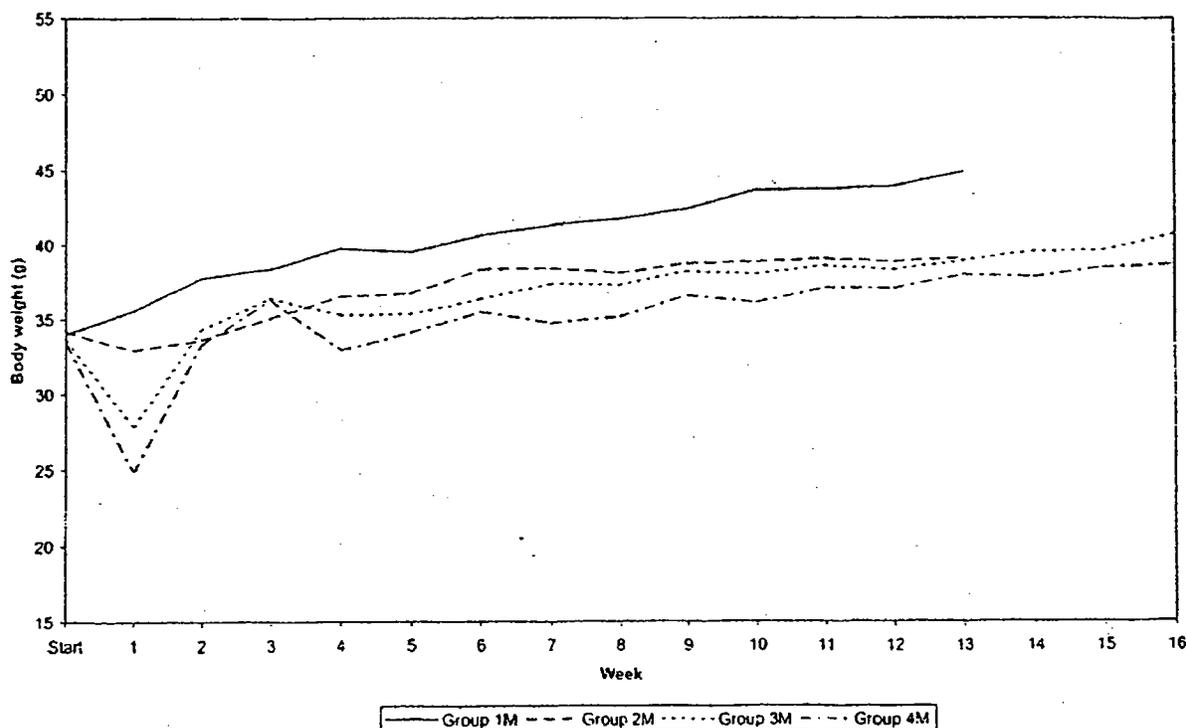


Fig. 3.2.2.1.: Group Mean Body Weights, Males

Group:	1	2	3	4
Dose level (mg/kg/day)	0	1000	2500/1500 [§]	5000/2000 [§]

[§]: Dose levels for groups 3 and 4 were reduced to zero after 1st week. Dosing was resumed at 1500 and 2000 mg/kg/day, respectively, on study day 22.

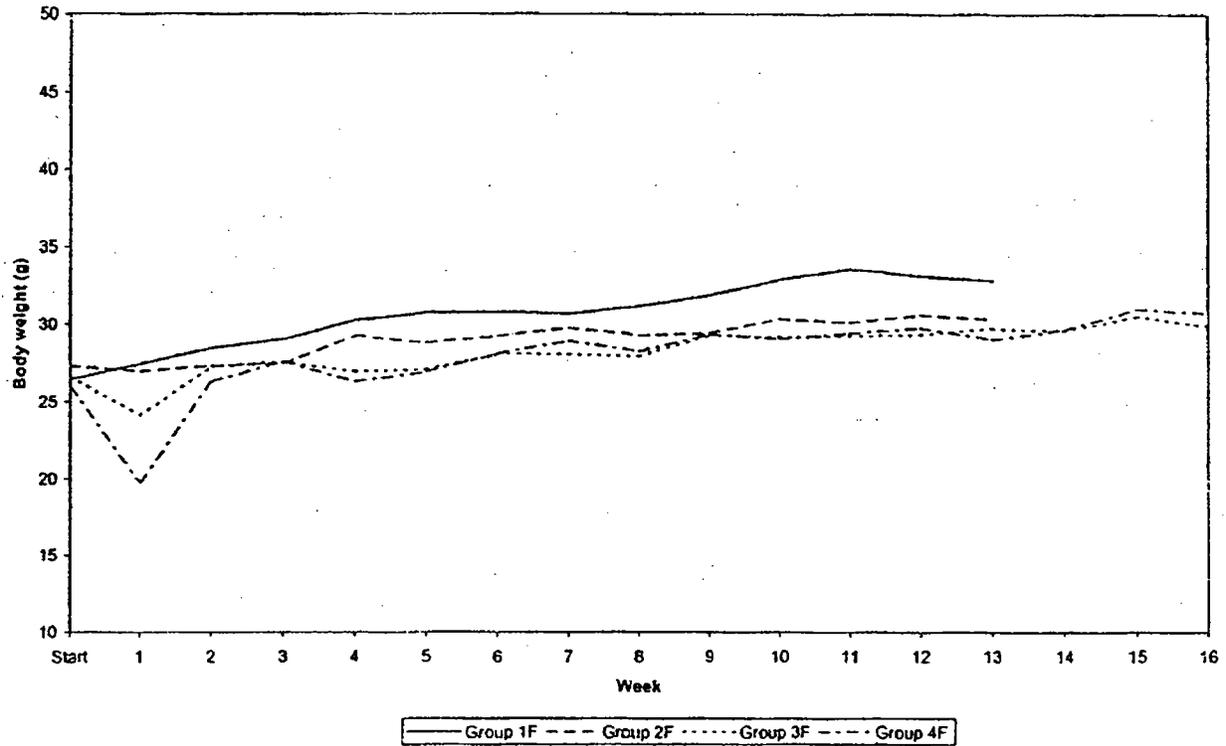


Fig. 3.2.2.2.: Group Mean Body Weights, Females

Group:	1	2	3	4
Dose level (mg/kg/day)	0	1000	2500/1500 [§]	5000/2000 [§]

[§]: Dose levels for groups 3 and 4 were reduced to zero after 1st week. Dosing was resumed at 1500 and 2000 mg/kg/day, respectively, on study day 22.

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TABLE 3.2.2.3
GROUP MEAN BODY WEIGHTS

Week of study		Mean body weights (g) for Group:				Week of study		Mean body weights (g) for Group:			
		1M	2M	3M	4M			1F	2F	3F	4F
Start	Mean	34.0	34.2	33.6	33.3	Start	Mean	26.5	27.3	26.6	25.9
	SD	2.76	3.14	2.45	2.58		SD	1.95	2.70	1.99	2.67
1	Mean	35.6	32.9	27.9	24.9	1	Mean	27.4	27.0	24.1	19.8
	SD	2.77	3.17	3.09	3.17		SD	2.23	2.73	2.12	1.94
2	Mean	37.7	33.6	34.3	33.3	2	Mean	28.4	27.4	27.3	26.4
	SD	2.68	3.07	2.44	2.37		SD	2.65	3.45	2.14	2.76
3	Mean	38.4	35.1	36.4	36.3	3	Mean	29.0	27.5	27.6	27.6
	SD	2.53	3.27	2.65	2.65		SD	3.18	2.88	2.50	2.75
4	Mean	39.8	36.6	35.3	33.0	4	Mean	30.3	29.3	27.0	26.3
	SD	2.92	3.30	2.93	2.85		SD	2.77	2.99	2.11	2.88
5	Mean	39.5	36.7	35.4	34.1	5	Mean	30.8	28.8	27.1	27.0
	SD	3.10	3.22	2.98	2.42		SD	3.38	3.53	2.27	3.28
6	Mean	40.6	38.4	36.4	35.5	6	Mean	30.8	29.2	28.1	28.1
	SD	3.22	2.63	3.51	2.78		SD	3.60	3.51	2.37	3.06
7	Mean	41.3	38.4	37.4	34.7	7	Mean	30.7	29.8	28.1	28.9
	SD	3.33	2.72	2.68	3.12		SD	3.01	3.36	2.27	3.06
8	Mean	41.8	38.1	37.3	35.2	8	Mean	31.2	29.3	28.0	28.3
	SD	3.75	2.72	3.00	3.07		SD	3.34	3.52	2.35	2.44
9	Mean	42.4	38.8	38.2	36.6	9	Mean	31.9	29.4	29.3	29.4
	SD	3.74	2.65	3.53	2.89		SD	3.67	3.54	2.56	3.74
10	Mean	43.7	38.9	38.0	36.1	10	Mean	32.9	30.4	29.2	29.1
	SD	4.16	2.54	2.61	3.30		SD	3.35	3.97	2.55	3.48
11	Mean	43.7	39.1	38.6	37.1	11	Mean	33.6	30.1	29.3	29.4
	SD	4.14	2.46	2.53	3.11		SD	4.58	3.27	2.49	2.95
12	Mean	43.9	38.9	38.3	37.1	12	Mean	33.1	30.6	29.4	29.8
	SD	4.09	2.24	2.50	2.84		SD	4.48	4.13	2.01	2.69
13	Mean	44.9	39.1	38.9	38.0	13	Mean	32.9	30.4	29.8	29.1
	SD	4.42	2.22	2.75	2.20		SD	4.20	3.06	2.19	3.03
14	Mean	-	-	39.6	37.8	14	Mean	-	-	29.6	29.7
	SD	-	-	2.84	2.55		SD	-	-	2.76	2.53
15	Mean	-	-	39.6	38.5	15	Mean	-	-	30.6	31.0
	SD	-	-	2.87	2.68		SD	-	-	2.58	3.05
16	Mean	-	-	40.7	38.7	16	Mean	-	-	30.0	30.8
	SD	-	-	2.88	3.04		SD	-	-	2.41	3.10

Group: 1 2 3 4
Dose level (mg/kg/day) 0 1000 2500/1500[§] 5000/2000[§]

[§]: Dose levels for groups 3 and 4 were reduced to zero after 1st week. Dosing was resumed at 1500 and 2000 mg/kg/day, respectively, on study day 22.

TABLE 3.2.2.4
GROUP MEAN BODY WEIGHT GAINS

Week to Week of study		Mean body weight gains (g) for Group:			
		1M	2M	3M	4M
Start to 4	Mean	5.8	2.4***	1.8***	-0.4***
	SD	1.28	1.94	1.35	1.60
Start to 13	Mean	10.9	4.8***	5.4***	4.7***
	SD	2.48	2.56	1.31	2.49
4 to 13	Mean	5.1	2.1***	3.6	5.0
	SD	1.91	1.54	1.17	1.64

		1F	2F	3F	4F
Start to 4	Mean	3.8	2.0*	0.4***	0.4***
	SD	1.28	1.23	1.91	1.79
Start to 13	Mean	6.4	3.0**	3.1**	3.1**
	SD	3.11	1.80	1.86	1.71
4 to 13	Mean	2.6	1.1	2.8	2.7
	SD	2.28	2.00	1.93	1.14

* P<0.05

** P<0.01

*** P<0.001

Statistics: ANOVA, regression and Dunnett's.

Group:	1	2	3	4
Dose level (mg/kg/day)	0	1000	2500/1500 [§]	5000/2000 [§]

[§]: Dose levels for groups 3 and 4 were reduced to zero after 1st week. Dosing was resumed at 1500 and 2000 mg/kg/day, respectively, on study day 22.

Food Consumption: Food intake was significantly lower than control (P <0.05) in the first week of dosing for the mid and high dose males and high dose females. This coupled with loss of body weight resulted in temporary stoppage of dosing of these two groups at the end of the first week. The food consumption recovered in the 2nd week and posted gains in subsequent weeks. There were no treatment-related effects on food consumption for the remainder of the study period.

Hematology and Clinical Chemistry: The only hematology parameters that appeared to be affected by aliskiren were those related to platelets: mean platelet crit (MPV x platelets / 10,000), mean platelet volume, and mean platelet distribution width. Statistically significant reductions (53 to 60% of control, p <0.001) in all the above

parameters were noted in mid and high dose males and females. Mean AST values were elevated for the mid and high dose animals. However, the values were significantly different from control ($p < 0.01$) only for females receiving 1500 mg/kg/day (170% of control). Slight and non dose-dependent but statistically significant decreases ($p < 0.05$) in total bilirubin levels (58 to 64% of control) and glucose levels (75 to 84% of control) were noted for males and females at all dose levels. Additionally, a significant increase in group mean urea (138% of control) was noted for high dose males.

Organ Weights, Gross Pathology and Histopathology: No treatment-related organ weights, macroscopic or microscopic findings were observed at any dose level at the scheduled necropsy.

Toxicokinetics: Small concentrations of aliskiren were detected in the blood of control animals analyzed at week 2. From week 8 of the study, the control animals were housed in a block of cages with several rows of empty cages separating them from the treated animals. In spite of these measures, test substance was still detected at week 13 at a mean concentration equal to or higher than that found at week 2. Measurable concentrations of aliskiren were found in all control animals at both study weeks (Table 3.2.2.5). C_{max} values for control males and females at week 13 were 39% and 26%, while AUC_{0-24h} values were 23% and 24% of low dose group values, respectively. According to the sponsor, exposure of control animals to aliskiren was due to air-borne contamination of control feed within the animal room.

Plasma concentrations of aliskiren after repeated daily administration of test substance in the feed at escalating doses increased with the increasing dose levels but were non-dose proportional. At all doses, animals showed significant systemic exposure to aliskiren. The AUC values for males were higher (1.4 to 2.9 times) than for females at all dose levels except for 1000 mg/kg/day at week 13. There was no uniformity in the time at which maximum concentration (t_{max}) was observed (ranged from 1 to 24 hr in both weeks of measurement, Table 3.2.2.5).

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TABLE 3.2.2.5
Toxicokinetic Summary

SPP100B (Aliskiren hemifumarate): 13 Week Oral (Dietary Administration) Study in the Mouse

Week 2					
Dose Level (mg/kg/day)	Group	Sex	C _{max} (ng/mL)	AUC ₍₀₋₂₄₎ (h*ng/mL)	t _{max} (h)
0	1	Male	61.2605	770.8030	2
0	1	Female	44.0249	648.5554	16
1000	2	Male	795.2762	9511.3217	8
1000	2	Female	258.9133	4636.2979	16
2500	3	Male	841.2659	12205.8167	2
2500	3	Female	399.2622	6457.7852	4
5000	4	Male	2128.4879	34548.1881	1
5000	4	Female	741.2313	11949.8933	24
Week 13					
Dose Level (mg/kg/day)	Group	Sex	C _{max} (ng/mL)	AUC ₍₀₋₂₄₎ (h*ng/mL)	t _{max} (h)
0	1	Male	75.8906	727.4643	8
0	1	Female	81.5502	1113.5239	8
1000	2	Male	196.2879	3119.3183	24
1000	2	Female	314.0428	4604.6880	24
2500	3	Male	994.1206	18004.6518	8
2500	3	Female	769.3162	12911.0451	4
5000	4	Male	1587.9538	22568.3707	4
5000	4	Female	664.2440	13785.5551	4

Mid and high doses were reduced to zero after 1st week. Dosing was resumed at 1500 and 2000 mg/kg/day, respectively, on study day 22.

SPP100B: Aliskiren hemifumarate

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3.2.3. Rising Dietary Dose Palatability/Toxicity Study in Wistar Rats

Key Study Findings: Dietary administration of aliskiren hemifumarate at increasing doses (concentration raised every 14 days) resulted in body weight gain reductions relative to control that were more severe for males (23 to 67%) than females (12 to 20%) at doses of 1500 or more mg aliskiren/kg/day. Females exhibited a slightly higher systemic exposure to aliskiren than did males. The mean daily food consumption was similar to control for most of the treatment period except during the last 2 weeks (at 2500 mg/kg/day) for males (28% less than control).

Study No.: 844833

Location of Report: EDR

Conducting Laboratory and Location: _____

Dates of Study: The animals were dosed from August 7 to September 30, 2002 and necropsied on October 1, 2002.

GLP Compliance: Yes

QA'd Report: yes (X) no ()

Drug, Lot #: Aliskiren hemifumarate, batch # TSDS102, — pure

Formulation: Aliskiren hemifumarate was admixed with the diet. Dietary test substance concentrations were adjusted according to mean food consumption and mean body weight of the animals. Concentration, homogeneity and stability of the dose formulations were determined at monthly intervals.

Species/Strain: Rats, Wistar (HanBrl:WIST (SPF))

#/Sex/Group: See Table 3.2.3.1.

Age: 6 weeks old at pretest

Weight: at pretest: Males: 112-152 gm, Females: 121-147 gm

Husbandry: Animals were housed in groups of three. Food and water was available *ad libitum*. Food but not water was withheld prior to blood sampling.

Dosing

Doses: Groups of 10 males and 10 females were given aliskiren hemifumarate admixed with the diet at increasing target doses of 1000, 1500, 2000 and 2500 mg/kg/day (dose levels are expressed as base) for 14 days/dose level for a total of 8 weeks. Additional (satellite) animals were treated with aliskiren hemifumarate in a similar way (9 rats per sex) for toxicokinetic analyses (see Table 3.2.3.1). The control group (n=10/sex) received untreated feed.

Observations and Measurements

Clinical Signs: All animals were observed twice daily for clinical signs and mortality.

Body Weight and Food Consumption: Recorded once during acclimatization and then twice weekly during the dosing period.

Hematology and Clinical Biochemistry: Blood was collected from all fasted (for 18 hr) main study animals from the retro-orbital plexus at the end of the dosing period.

Pathology: A complete necropsy was conducted on all animals in the toxicology group and on all animals that died during the study. The necropsy included internal and external examinations. Samples of the following tissues and organs were collected for possible further investigations: lungs (filled w/formalin at necropsy), head, nasal cavity, nasopharynx, gross lesions, stomach, and small intestine.

Toxicokinetics: Blood samples were collected from the retro-orbital plexus of the toxicokinetic animals under light isoflurane anesthesia during study weeks 2, 4 and 8 (1, 2, 4, 8, 16 and 24 hr, n= 3 males and 3 females/time point).

TABLE 3.2.3.1
STUDY DESIGN

	Sex	Control	1000, 1500, 2000 and 2500* mg/kg/day
		Group 1	Group 2
Toxicology	M	1-10	11-20
	F	30-39	40-49
Toxicokinetics	M	None	21-29
	F	None	50-58

* : Target dose levels

Results

Analysis of Formulations: The mean intake of the drug was in excess of the targeted dose levels for females (14 to 47% higher) throughout the 8 week treatment period and for males (16-17% higher) through the 6th week. During the 7th and 8th study weeks, mean drug intake for males was less than the targeted dose (11% lower) (Table 3.2.3.2).

TABLE 3.2.3.2
TEST SUBSTANCE INTAKE (MG/KG) WITH DIETARY ADMINISTRATION

Group	Treatment week	Target Dose (mg/kg)	Males		Females	
			Value	% of target dose	Value	% of target dose
1		0	---	---	---	---
2	1-2	1000	1170.92	117	1265.02	127
2	3-4	1500	1751.69	117	2205.37	147
2	5-6	2000	2329.36	116	2284.66	114
2	7-8	2500	2218.22	89	3056.63	122

Mortality: All animals survived until scheduled necropsy.

Clinical Signs: Soft feces were observed in most of the treated animals at study week 4 (when dose was 2000 mg/kg/day) and continued till necropsy. This was considered treatment-related.

Body Weight: Significant (p <0.01) reductions in body weight gain relative to control were observed for both males and females treated with aliskiren hemifumarate throughout the treatment period (Fig. 3.2.3.1). The reduction was dose-dependent and greater for males than females. Mean reductions in body weight gain relative to control at dose levels of 1000, 1500, 2000 and 2500 mg/kg/day were, respectively, 16, 33, 42 and 62% for males (Table 3.2.3.3) and 48, 38, 32 and 43% for females (Table 3.2.3.4). With the start of the highest dose, 2500 mg/kg/day, a gradual body weight loss was observed from study day 46 to day 55 for both males and females (8% and 1%, respectively, as a percentage of body weight prior to treatment at 2500 mg/kg/day).

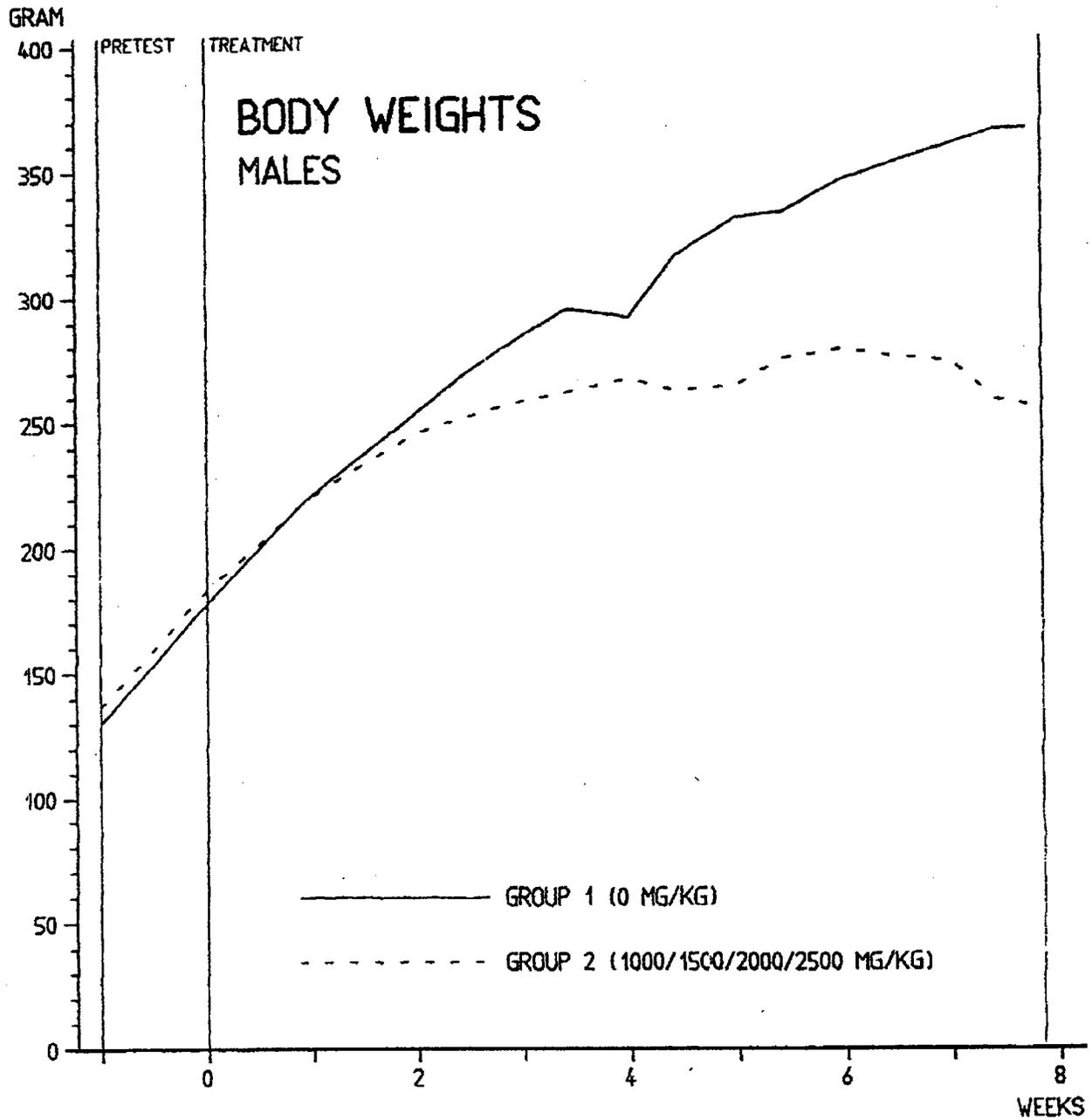


Fig. 3.2.3.1.: Group Mean Body Weights in Males

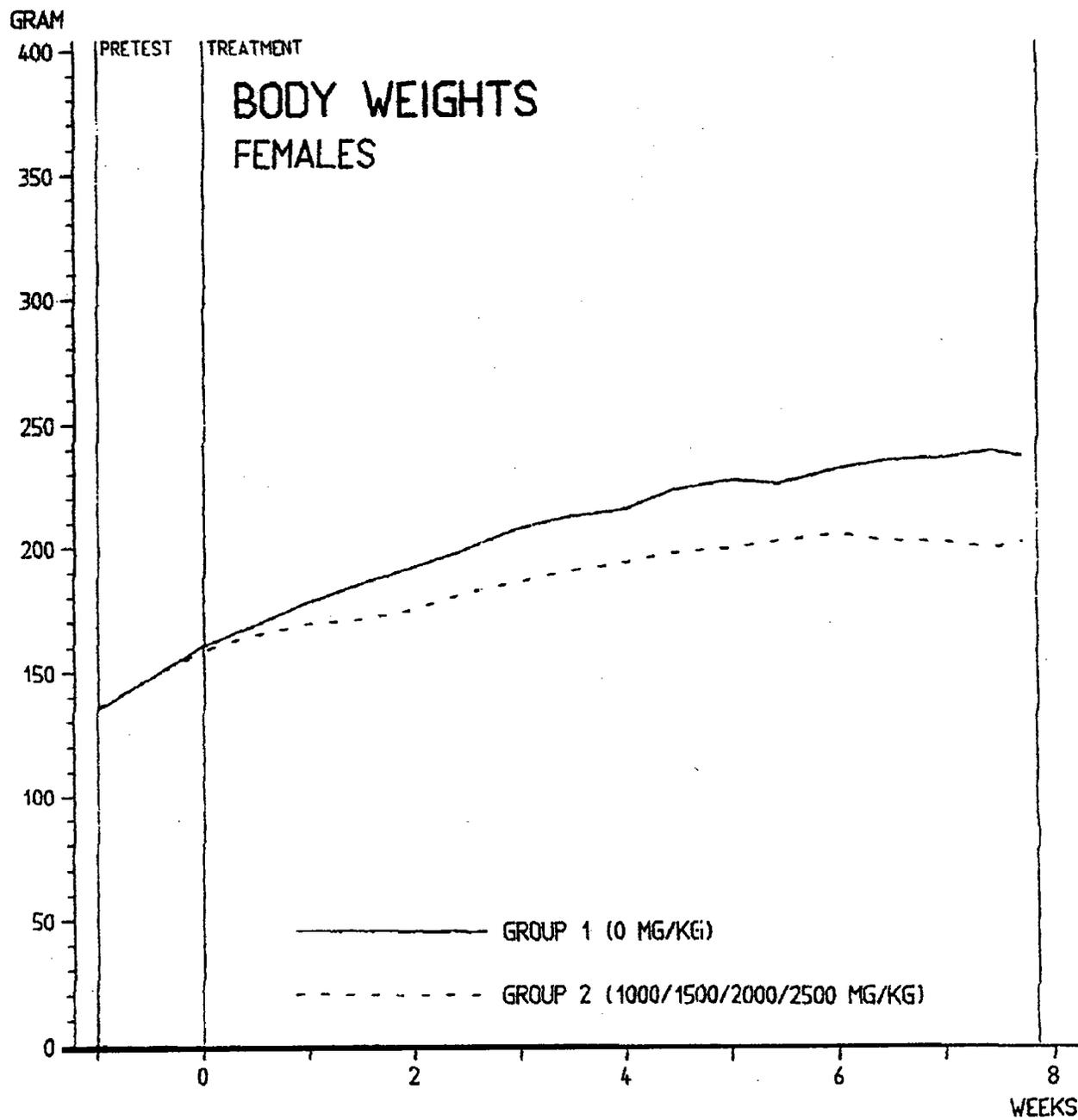


Fig. 3.2.3.2.: Group Mean Body Weights in Females

TABLE 3.2.3.3
GROUP MEAN BODY WEIGHTS AND BODY WEIGHT GAINS FOR MALES

TREATMENT	Group Mean Body Weight (Gram)			Group Mean Body Weight Gain (%)		
		GROUP 1 0 MG/KG	GROUP 2 1000/1500/2000/2500 MG/KG		GROUP 1 0 MG/KG	GROUP 2 1000/1500/2000/2500 MG/KG
DAY 1	MEAN	178	184		0.0	0.0
WEEK 1	ST. DEV.	12.6	10.4		0.0	0.0
	N	10	19		10	19
DAY 4	MEAN	198	199		10.8	8.5 **
WEEK 1	ST. DEV.	12.0	12.4		1.7	1.5
	N	10	19		10	19
DAY 8	MEAN	222	221		24.4	20.3 *
WEEK 2	ST. DEV.	11.8	16.1		3.6	3.9
	N	10	19		10	19
DAY 11	MEAN	235	232		31.8	25.9 **
WEEK 2	ST. DEV.	13.2	17.8		4.4	5.1
	N	10	19		10	19
DAY 15	MEAN	254	246		42.4	33.7 **
WEEK 3	ST. DEV.	16.0	20.4		6.1	6.8
	N	10	19		10	19
DAY 18	MEAN	268	252 *		50.6	36.8 **
WEEK 3	ST. DEV.	16.5	21.1		6.5	7.1
	N	10	19		10	19
DAY 22	MEAN	284	258 **		59.6	40.4 **
WEEK 4	ST. DEV.	18.7	24.2		8.9	9.1
	N	10	19		10	19
DAY 25	MEAN	295	262 **		65.7	42.3 **
WEEK 4	ST. DEV.	18.7	24.9		9.2	9.4
	N	10	19		10	19
DAY 29	MEAN	292	267 **		63.9	45.3 **
WEEK 5	ST. DEV.	16.8	24.1		11.5	9.7
	N	10	19		10	19
DAY 32	MEAN	316	262 **		77.5	42.5 **
WEEK 5	ST. DEV.	21.0	27.7		12.7	11.9
	N	10	19		10	19
DAY 36	MEAN	332	264 **		86.3	43.8 **
WEEK 6	ST. DEV.	22.4	28.4		13.5	12.5
	N	10	19		10	19
DAY 39	MEAN	334	275 **		87.4	49.5 **
WEEK 6	ST. DEV.	22.4	30.0		13.7	13.3
	N	10	19		10	19
DAY 43	MEAN	347	279 **		94.8	51.5 **
WEEK 7	ST. DEV.	22.3	31.1		14.3	13.5
	N	10	19		10	19
DAY 46	MEAN	353	276 **		98.3	50.0 **
WEEK 7	ST. DEV.	24.7	32.0		15.7	14.5
	N	10	19		10	19
DAY 50	MEAN	361	274 **		102.7	49.0 **
WEEK 8	ST. DEV.	24.9	32.1		15.3	14.8
	N	10	19		10	19
DAY 53	MEAN	366	259 **		105.8	40.6 **
WEEK 8	ST. DEV.	25.1	31.4		16.2	14.8
	N	10	19		10	19
DAY 55	MEAN	367	256 **		106.2	39.4 **
WEEK 8	ST. DEV.	25.4	31.2		15.1	14.3
	N	10	19		10	19

TABLE 3.2.3.4
GROUP MEAN BODY WEIGHTS AND BODY WEIGHT GAINS FOR FEMALES

TREATMENT		Group Mean Body Weight (Gram)			Group Mean Body Weight Gain (%)		
		GROUP 1 0 MG/KG	1000/1500/2000/2500	GROUP 2 MG/KG	GROUP 1 0 MG/KG	1000/1500/2000/2500	GROUP 2 MG/KG
DAY 1	MEAN	161		159	0.0		0.0
WEEK 1	ST.DEV.	8.9		8.4	0.0		0.0
	N	10		19	10		19
DAY 4	MEAN	168		165	4.6		3.5
WEEK 1	ST.DEV.	9.3		9.9	3.9		2.3
	N	10		19	10		19
DAY 8	MEAN	178		170	10.8		6.6 *
WEEK 2	ST.DEV.	10.3		11.6	4.1		3.8
	N	10		19	10		19
DAY 11	MEAN	184		171 **	14.6		7.3 **
WEEK 2	ST.DEV.	9.2		11.4	4.8		4.1
	N	10		19	10		19
DAY 15	MEAN	193		175 **	19.7		10.1 **
WEEK 3	ST.DEV.	9.5		10.6	5.6		4.0
	N	10		19	10		19
DAY 18	MEAN	198		181 **	23.3		13.7 **
WEEK 3	ST.DEV.	9.6		12.4	4.1		4.4
	N	10		19	10		19
DAY 22	MEAN	208		186 **	29.4		17.1 **
WEEK 4	ST.DEV.	10.3		13.1	5.6		4.6
	N	10		19	10		19
DAY 25	MEAN	213		191 **	32.2		19.7 **
WEEK 4	ST.DEV.	10.3		12.4	5.6		4.0
	N	10		19	10		19
DAY 29	MEAN	216		194 **	34.2		22.2 **
WEEK 5	ST.DEV.	11.7		12.6	3.6		4.9
	N	10		19	10		19
DAY 32	MEAN	224		198 **	39.0		24.5 **
WEEK 5	ST.DEV.	13.1		12.4	7.7		4.0
	N	10		19	10		19
DAY 36	MEAN	228		200 **	41.6		25.7 **
WEEK 6	ST.DEV.	12.9		11.9	7.2		3.5
	N	10		19	10		19
DAY 39	MEAN	226		203 **	40.7		27.4 **
WEEK 6	ST.DEV.	13.0		14.1	8.9		4.1
	N	10		19	10		19
DAY 43	MEAN	232		206 **	44.3		29.4 **
WEEK 7	ST.DEV.	14.4		13.7	9.1		4.0
	N	10		19	10		19
DAY 46	MEAN	236		203 **	46.4		27.6 **
WEEK 7	ST.DEV.	13.2		14.3	8.0		4.8
	N	10		19	10		19
DAY 50	MEAN	237		203 **	47.2		27.3 **
WEEK 8	ST.DEV.	13.1		15.6	8.1		4.9
	N	10		19	10		19
DAY 53	MEAN	240		200 **	49.0		25.8 **
WEEK 8	ST.DEV.	14.7		16.0	8.1		6.1
	N	10		19	10		19
DAY 55	MEAN	238		203 **	47.8		27.3 **
WEEK 8	ST.DEV.	16.4		16.7	9.2		6.1
	N	10		19	10		19

Food Consumption: Food intake was not statistically significantly different between control and treated groups except for males during study weeks 7 and 8 ($p < 0.01$). Relative food consumption for high dose (2500 mg/kg/day) males was 72% of control at the end of the 8th study week.

Hematology: Hematology investigations conducted at the end of the 8 week dosing period showed statistically significantly ($p < 0.05$) decreased red cell volume and hemoglobin concentration distribution widths, decreased reticulocyte count, decreased WBC counts and decreased basophils in male rats treated with aliskiren hemifumarate. Of these parameters, only WBC showed a significant difference from control ($p < 0.05$) in females treated with test article. Unlike males, females treated with aliskiren hemifumarate had a significant increase in WBC. In addition, treated females showed decreased ($p < 0.05$) hemoglobin and hematocrit (Table 3.2.3.5). The sponsor contends that, though the findings are test substance-related, they are minor in nature and indicate metabolic adaptation.

TABLE 3.2.3.5
GROUP MEAN VALUES FOR HEMATOLOGY

Parameter	Control group		Treated group	
	M	F	M	F
Red cell volume distribution width, relative	0.1188	0.1082	0.1102*	0.1108
Hemoglobin, mmol/l	10.07	9.94	10.32	9.45**
Hemoglobin Conc distribution width, mmol/l	1.638	1.235	1.417*	1.176
Hematocrit, relative	0.478	0.467	0.486	0.446**
Reticulocyte count, g/l	201.3	170.8	100.8**	181
WBC, g/l	10.611	5.895	8.627*	7.173*
Basophils, g/l	0.062	0.017	0.027**	0.017

*: $p < 0.05$; **: $p < 0.01$

Clinical Chemistry: There were many drug-related biochemical findings in both sexes (Table 3.2.3.6). Mean ALT, blood urea nitrogen, creatinine and creatinine kinase were significantly higher ($p < 0.05$, 1.4 to 2.1-fold) in treated males relative to control males. All but creatinine kinase were also significantly higher in treated females. In addition, statistically significant increases in bilirubin, AST, glutamate dehydrogenase and inorganic phosphorus in treated males and potassium in treated females were noted (glucose significantly decreased in treated males). Mean cholesterol, phospholipids and protein (due to significant decrease in globulin levels, A/G level increased) were significantly lower in treated males and females relative to control values (Table 3.2.3.6). According to the sponsor, these effects were the result of metabolic adaptation in the liver.

TABLE 3.2.3.6
GROUP MEAN VALUES FOR BLOOD CHEMISTRY

Parameter	Control group		Treated group	
	M	F	M	F
Glucose, mmol/l	5.37	4.844	3.846**	6.058**
Urea, mmol/l	6.514	6.517	9.401**	11.434**
Creatinine, μ mol/l	26.39	30.96	34.66**	34.43**
Bilirubin, total, μ mol/l	2.017	2.597	2.543*	2.207
Cholesterol	2.109	1.676	1.723**	1.285*
Phospholipids	1.866	1.973	1.445**	1.545**
AST, u/l	70.92	82.17	85.72*	82.42
ALT, u/l	40.17	30.90	59.50**	65.58**
Creatinine kinase, u/l	156.25	172.30	313.70*	202.89
Alkaline phosphatase, u/l	95.75	33.31	55.33**	44.75
Glutamate dehydrogenase, u/l	6.14	13.23	10.25*	12.58
K ⁺ , mmol/l	3.624	3.386	3.468	3.799**
Ca ²⁺ , mmol/l	2.844	2.815	2.772**	2.839
P, inorganic, mmol/l	1.955	1.54	2.262**	1.61
Protein, total g/l	70.139	73.155	63.449**	67.413**
Albumin, g/l	42.035	47.884	40.952	44.676*
Gobulin, g/l	28.104	25.271	22.497**	22.737**
A/G ratio	1.504	1.898	1.834**	1.97

*: p <0.05; **: p <0.01

Gross Pathology: There were no test substance-related macroscopic or microscopic findings observed at the scheduled necropsy.

Toxicokinetics: Plasma concentrations of aliskiren after repeated daily administration of test substance in the feed at escalating doses increased with the increasing dose levels (Table 3.2.3.7). At all doses, animals showed significant systemic exposure to aliskiren. Intake of aliskiren hemifumarate during weeks 7 and 8 was much lower for males than for females (intake 11% below target in males and 22% above target in females, Table 3.2.3.2); consequently, systemic exposure (AUC) to aliskiren in males at 2500 mg/kg/day was much lower than in the corresponding females. The AUC values for females at 1000, 1500 and 2500 mg/kg/day were 1.4, 1.2 and 1.4 times higher than the AUC values for the corresponding males (Table 3.2.3.8). Though females exhibited a slightly higher exposure to aliskiren than did males, reduction in body weight gain, or body weight loss, was more severe in males than in females.

TABLE 3.2.3.7
TOXICOKINETICS FOR ALISKIREN IN RATS AFTER DIETARY ADMINISTRATION FOR 8 WEEKS.
GROUP MEAN VALUES

Time (hours)	Mean Plasma Levels [ng/ml]				Mean Plasma Levels [ng/ml]				Mean Plasma Levels [ng/ml]			
	Group 2 - 2 weeks				Group 2 - 4 weeks				Group 2 - 8 weeks			
	M 1000 mg/kg		F 1000 mg/kg		M 1500 mg/kg		F 1500 mg/kg		M 2500 mg/kg		F 2500 mg/kg	
	[ng/ml]	[s.d.]	[ng/ml]	[s.d.]	[ng/ml]	[s.d.]	[ng/ml]	[s.d.]	[ng/ml]	[s.d.]	[ng/ml]	[s.d.]
1	261.5	12.6	497.3	351.1	391.6	76.5	519.2	180.4	810.2	317.5	472.8	85.2
2	277.1	114.2	355.0	89.8	281.2	74.0	487.7	158.9	541.6	67.6	541.0	201.2
4	299.3	135.7	344.8	100.1	388.8	101.1	502.0	108.7	490.7	82.5	627.3	139.9
8	196.1	46.3	345.7	139.3	318.5	79.8	347.6	155.9	629.2	159.1	437.7	51.7
16	235.8	63.0	284.1	26.5	417.3	122.6	460.1	160.1	521.9	183.9	1153.4	30.6
24	207.3	59.6	365.8	135.5	402.0	81.5	501.4	181.4	751.0	210.0	957.8	482.8
C _{average} [ng/ml]	246.2	40.4	362.1	40.6	366.6	53.9	469.6	63.0	624.1	131.0	698.3	290.9
Time interval [h]	1-24		1-24		1-24		1-24		1-24		1-24	
AUC ₀₋₂₄ [ng.h/ml]	5337		7465		8641		10269		13644		18615	

* : Time interval selected for C_{average}, M: Male; F: Female

TABLE 3.2.3.8
COMPARISON OF AUCs FOR ALISKIREN IN RATS AFTER DIETARY ADMINISTRATION OF ALISKIREN
HEMIFUMARATE AT RISING DOSES FOR 8 WEEKS. GROUP MEAN VALUES

Dose Dependency				
Groups	Dose	Factor	AUC _{0-t}	Factor
	mg/kg		[ng.h/ml]	
M 2w	1000	-	5337	-
M 4w	1500	1.5	8641	1.6
M 8w	2500	1.7	13644	1.6
F 2w	1000	-	7465	-
F 4w	1500	1.5	10269	1.4
F 8w	2500	1.7	18615	1.8
Sex Difference				
Groups	Dose	Factor	AUC _{0-t}	Factor
	mg/kg		[ng.h/ml]	
M 2w	1000	-	5337	-
F 2w	1000	1.0	7465	1.4
M 4w	1500	-	8641	-
F 4w	1500	1.0	10269	1.2
M 8w	2500	-	13644	-
F 8w	2500	1.0	18615	1.4

3.2.4. Ninety-Day Oral (gavage and feeding) Dose Range-Finding Study in Wistar Rats

Key Study Findings: Gavage administration of aliskiren hemifumarate resulted in ulceration and inflammatory exudates in the nasal cavities and nasopharynx and moderate erosion in the cecum and ulceration of the colon. Consequently, 6 males and 2 females at 500 mg/kg/day and 7 males and 8 females at 750 mg/kg/day were found dead or had to be sacrificed. A moderate decrease in mean body weight gain (16% less than control) was noted for males with gavage administration at 750 mg/kg/day. Body weights were less affected with dietary administration at 1000 mg/kg/day. Gavage administration at 500 mg/kg/day resulted in a higher AUC (1.1 to 1.4-fold) than dietary administration at 1000 mg/kg/day. (All of the above doses are expressed in terms of the aliskiren base.)

Study No.: 843804

Location of Report: EDR

Conducting Laboratory and Location: _____

Dates of Study: The animals were dosed from May 6 to August 4/5, 2002 and necropsied on August 5/6, 2002.

GLP Compliance: Yes

QA'd Report: yes (X) no ()

Drug, Lot #: Aliskiren hemifumarate, batch # S100B0201, S100B0202, S100B2001006, _____
pure

Formulation: Aliskiren hemifumarate was admixed with the diet. Concentrations, homogeneity and stability (after 3 hr and 7 days) of the dose formulations were determined in samples taken after the initiation of dosing. For gavage, aliskiren hemifumarate was suspended in 1% methylcellulose and was prepared weekly.

Species/Strain: Rats, Wistar (HanBrl:WIST (SPF))

#/Sex/Group: 10/sex/group for toxicology; 9/sex/group for toxicokinetics (Table 3.2.4.1).

Age: 6 weeks old at pretest

Weight: at pretest: Males: 128-158 gm, Females: 107-136 gm

Husbandry: Animals were housed in groups of three or five. Food and water was available *ad libitum*. Food but not water was withheld prior to blood and urine sampling.

Dosing

Doses: Groups 1, 2 and 3: 0, 750 or 1000 mg/kg/day (dose levels are given as base). Groups 4 and 5: 500 or 750 mg/kg/day (Table 3.2.4.1). The doses were selected on the basis of a 2 week oral study in which gavage administration of the hemifumarate at doses of 1000 or more mg aliskiren/kg/day resulted in deaths; GI tract, respiratory tract and kidneys were the target organs for toxicity. In the same study, dietary doses >1000 mg/kg/day caused body weight loss (11-36% according to the sponsor; data not provided).

Route, Mode and Duration of Administration: The first set of groups (2 and 3) received aliskiren admixed with the diet for 13 weeks. The control animals (groups 1) received pelleted control diet without the test drug. A second set of groups (4 and 5) received aliskiren orally by gavage (10 ml/kg), daily, for 13 weeks. Additional groups of aliskiren-treated rats (diet or gavage) were used for toxicokinetic study (Table 3.2.4.1).

TABLE 3.2.4.1
STUDY DESIGN

Allocation of animals	Sex	Drug admixed with diet			Oral by gavage	
		Control	750 mg/kg/day*	1000 mg/kg/day*	500 mg/kg/day	750 mg/kg/day
		Group 1	Group 2	Group 3	Group 4	Group 5
Toxicology	M	1-10	20-29	39-48	58-67	77-86
	F	96-105	115-124	134-143	153-162	172-181
Toxicokinetics	M	11-19	30-38	49-57	68-76	87-95
	F	106-114	125-133	144-152	163-171	182-190

* : Target dose levels

Observations and Measurements

Clinical Signs: All animals were observed twice daily for clinical signs and mortality.

Body Weight and Food Consumption: Recorded a week before treatment and then at weekly intervals for all animals.

Hematology and Clinical Biochemistry: Blood samples were collected from all animals in the toxicology group at the end of the dosing period. The animals were fasted overnight but allowed access to water *ad libitum* and blood was drawn from the retro-orbital plexus under light isoflurane anesthesia.

Urinalysis: Urine samples were collected overnight from the main study animals prior to blood sampling.

Pathology: A complete necropsy was conducted on all animals in the toxicology group and on all animals that died during the study. The necropsy included detailed internal and external examinations, weighing of selected organs and sampling of tissues for histopathological examination (Table 3.2.4.2). All tissues from all animals of the control group, the high dose diet group, the high dose gavage group and all animals that died or were sacrificed moribund, and all gross lesions from all animals were examined histopathologically. Histopathological examination of organs/tissues in the remaining animals was limited to kidneys, nasal cavities, trachea, lungs, stomach, and small and large intestines.

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TABLE 3.2.4.2
TISSUES/ORGANS SAMPLED FOR HISTOPATHOLOGICAL EXAMINATION

Adrenal glands*	Jejunum with Peyer's patches	Sciatic nerve
Aorta	Kidneys*	Seminal vesicles
Bone (sternum, femur including joint)	Larynx	Skeletal muscle
Bone marrow (femur)	Lacrimal gland, exorbital	Skin
Brain (cerebrum, cerebellum, brain stem) *	Liver*	Spinal cord - cervical, midthoracic, lumbar
Cecum	Lungs, filled w/formalin at necropsy	Spleen*
Colon	Lymph nodes - mesenteric, mandibular	Stomach
Duodenum	M	Testes (fixed in Bouin's solution)*
Epididymides (fixed in Bouin's solution)	Mammary gland area	Thymus
Esophagus	Nasal cavity	Thyroid (incl. parathyroid gland, if possible)*
Eyes with optic nerve (fixed in Davidson's solution)	Nasopharynx	Tongue
Harderian gland (fixed in Davidson's solution)	Ovaries*	Trachea (longitudinal section)
Heart*	Pancreas	Urinary bladder, filled w/formalin at necropsy
Ileum, with Peyer's patches	Pituitary gland*	Uterus*
	Prostate gland*	Vagina
	Rectum	Gross lesions
	Salivary glands - mandibular, sublingual	

*: Organ weighed

Toxicokinetics: Blood samples were collected from the retro-orbital plexus of the toxicokinetics animals under light isoflurane anesthesia on day 1 and during study week 13. Blood sampling time points were as follows.

FOR GROUPS 1-3:

Three animals per group were sampled according to the following scheme.

Blood sampling time points:

Time point	7.00 1h	08.00 2h	10.00 4h	14.00 8h	22.00 16h	6.00 24h
Males	11, 14, 17 30, 33, 36 49, 52, 55	12, 15, 18 31, 34, 37 50, 53, 56	13, 16, 19 32, 35, 38 51, 54, 57	11, 14, 17 30, 33, 36 49, 52, 55	12, 15, 18 31, 34, 37 50, 53, 56	13, 16, 19 32, 35, 38 51, 54, 57
Females	106, 109, 112 125, 128, 131 144, 147, 150	107, 110, 113 126, 129, 132 145, 148, 151	108, 111, 114 127, 130, 133 146, 149, 152	106, 109, 112 125, 128, 131 144, 147, 150	107, 110, 113 126, 129, 132 145, 148, 151	108, 111, 114 127, 130, 133 146, 149, 152

FOR GROUPS 4-5:

Each three animals per group were sampled according to the following scheme.

Blood sampling time points:

Time point blood sampling	Before dosing	1h	2h	4h	8h	24h
Males	68-70 87-89	71-73 90-92	74-76 93-95	68-70 87-89	71-73 90-92	74-76 93-95
Females	163-165 182-184	166-168 185-187	169-171 188-190	163-165 182-184	166-168 185-187	169-171 188-190

Results

Analysis of Formulations: The mean intake of aliskiren was, on average, slightly in excess of the targeted dose levels for each group throughout the dosing period (Table 3.2.4.3).

TABLE 3.2.4.3
TEST SUBSTANCE INTAKE WITH DIETARY ADMINISTRATION

Target dose, mg/kg/day	Males		Females	
	Achieved dose (mg/kg/day)	% of target dose	Achieved dose (mg/kg/day)	% of target dose
750	813.5	108.5	832.3	110.9
1000	1107.9	110.8	1101.6	110.1

Mortality: A total of 23 animals treated with aliskiren by gavage died or were sacrificed during the dosing period. At 500 mg/kg/day, 6 males (#61,63, 66, 68, 74, 76) and 2 females (#154, 158) were found dead during the treatment period. At 750 mg/kg/day, 7 males (#77,78, 81, 82, 88, 89, 93) and 8 females (#172, 173, 176, 177, 178, 179, 183, 189) were found dead or were sacrificed moribund during the 90 day treatment period (Table 3.2.4.4). Histopathology findings in these animals included ulceration and inflammatory exudates in the nasal cavities and nasopharynx, which were, according to the sponsor, due to aspiration of small amounts of the test article either during or after administration. All animals in the control and dietary administration groups survived until scheduled necropsy.

Clinical Signs: Among surviving rats in the 500 mg/kg/day (gavage) group, rales were observed in some and one female was found in poor health with hunched posture, labored respiration and ruffled fur. At 750 mg/kg/day (gavage), these findings, coupled with tachypnea and emaciation, were evident in a few surviving males. The clinical signs were considered by the sponsor to have resulted from aspiration of small amounts of test substance during the gavage administration. No test substance-related clinical signs were evident in the control animals or in animals treated by diet admixture.

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TABLE 3.2.4.4
ALISKIREN HEMIFUMARATE-TREATED RATS FAILING TO SURVIVE 90 DAYS OF
GAVAGE ADMINISTRATION

Dose, mg/kg/day	Animal #	Study day	Clinical signs during the study period
500	61 Toxicol, M	Died on day 52	No clinical signs noted
	63 Toxicol, M	Died on day 91	No clinical signs noted
	66 Toxicol, M	Died on day 12	No clinical signs noted
	68 T ¹ kinetics, M	Died on day 10	No clinical signs noted
	74 T ¹ kinetics, M	Died on day 89	Posture ventral recumbancy, labored respiration, in poor health
	76 T ¹ kinetics, M	Died on day 24	No clinical signs noted
	154 Toxicol, F	Died on day 35	Ventral recumbancy, labored respiration, in poor health
	158 Toxicol, F	Died on day 56	No clinical signs noted
750	77 Toxicol, M	Died on day 11	Labored respiration, ruffled fur, emaciated
	78 Toxicol, M	Died on day 13	No clinical signs noted
	81 Toxicol, M	Died on day 28	No clinical signs noted
	82 Toxicol, M	Died on day 11	No clinical signs noted
	88 T ¹ kinetics, M	Died on day 48	No clinical signs noted
	89 T ¹ kinetics, M	Died on day 55	Labored respiration
	93 T ¹ kinetics, M	Died on day 70	Ruffled fur
	172 Toxicol, F	Died on day 11	Labored respiration, ruffled fur, in poor health, emaciated
	173 Toxicol, F	Died on day 91	No clinical signs noted
	176 Toxicol, F	Died on day 12	Hunched posture, labored respiration, ruffled fur, in poor health, emaciated
	177 Toxicol, F	Died on day 85	No clinical signs noted
	178 Toxicol, F	Died on day 21	No clinical signs noted
	179 Toxicol, F	Died on day 74	No clinical signs noted
	183 T ¹ kinetics, F	Died on day 32	No clinical signs noted
189 T ¹ kinetics, F	Killed on day 37	Hunched posture, labored respiration, ruffled fur, in poor health, emaciated	

Body Weight: Mean body weight gain was statistically significantly decreased ($p < 0.05$) for high dose males receiving test substance by gavage relative to control from week 2 through week 13 of measurement (Fig. 3.2.4.1). The decrease was variable and was maximal (13% relative to control) on study day 91 (Table 3.2.4.5). Body weights were less affected with the dietary administration of the drug. Decrements in body weight gain ($p < 0.05$) were noted up to week 6 for high dose males (Fig. 3.2.4.1) and up to week 12 for high dose females (Fig. 3.2.4.2). Maximal effects (10% and 11% less body weight gain relative to controls) were recorded on study days 36 and 78, for males and females, respectively. However, reductions in body weight gain relative to control at the end of the study (day 91) for males and females at 1000 mg/kg/day (8.7% and 6%, respectively) were not statistically significant ($p > 0.05$) (Table 3.2.4.5).

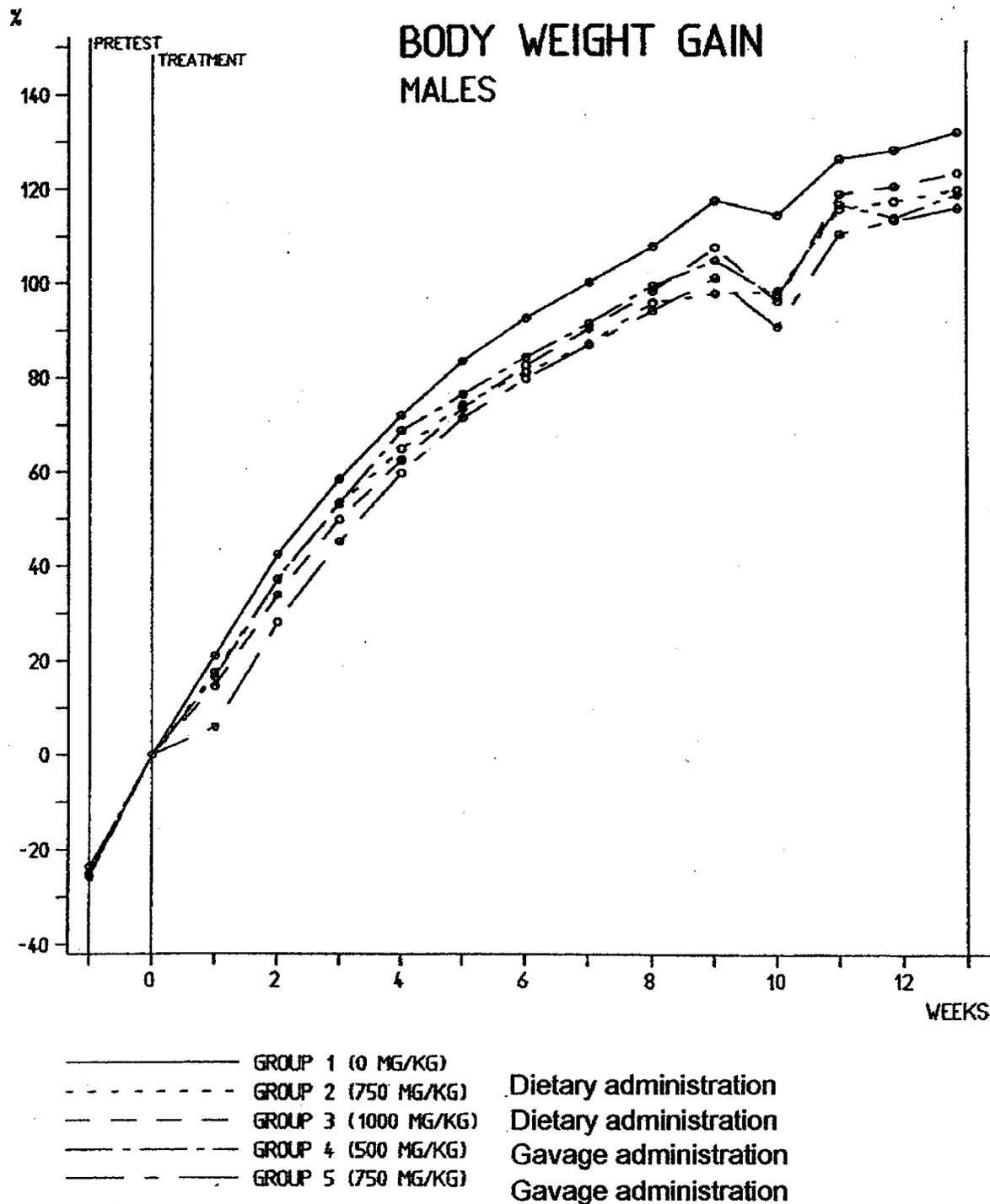


Fig .3.2.4.1.: Per cent Group Mean Body Weight Gain in Males

BODY WEIGHT GAIN FEMALES

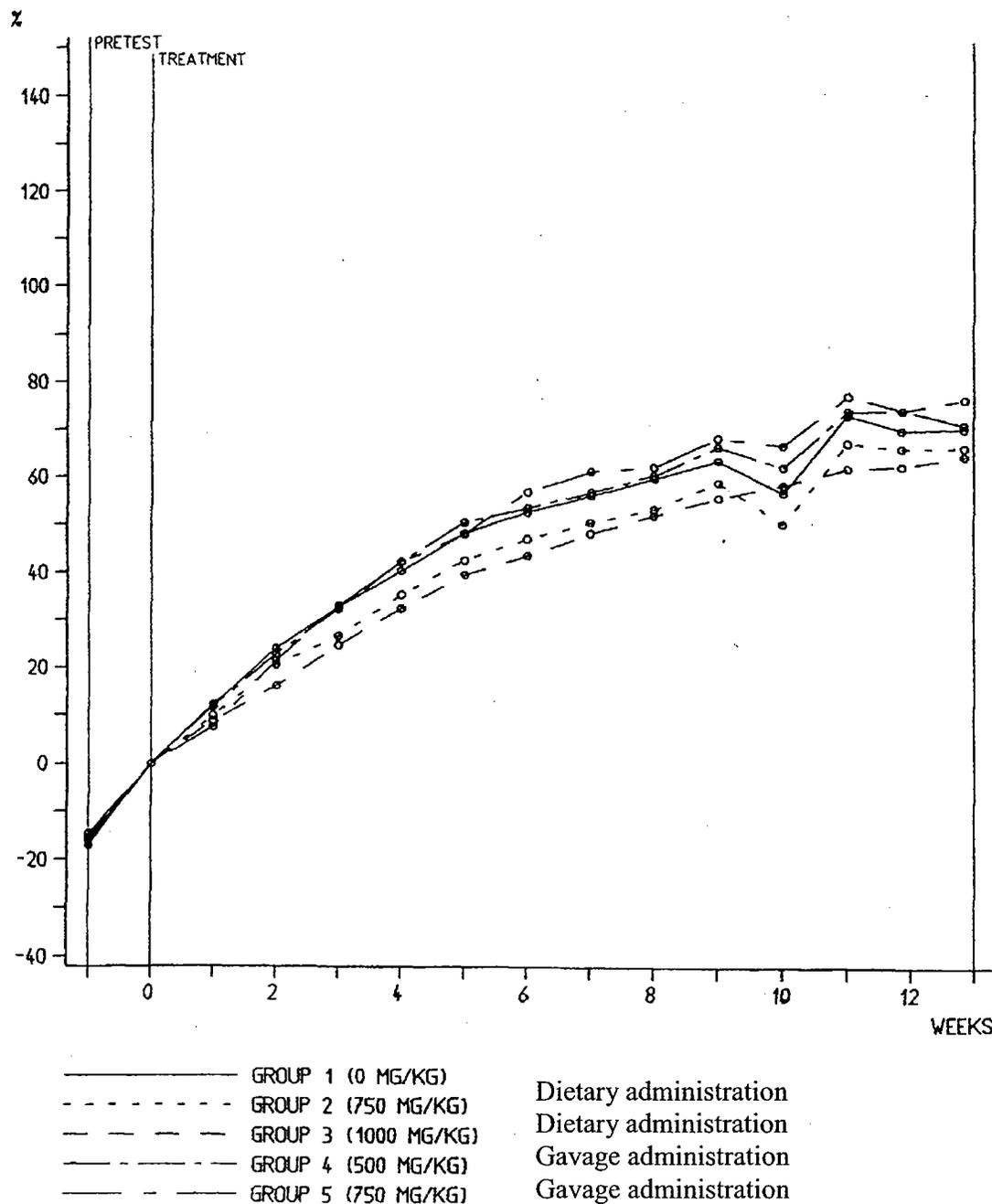


Fig. 3.2.4.2.: Per cent Group Mean Body Weight Gain in Females

TABLE 3.2.4.5
GROUP MEAN BODY WEIGHTS AND BODY WEIGHT GAINS¹
(COMBINED TOXICOLOGY AND TOXICOKINETICS GROUPS)

Study Week/ Day	M /F	Dose, mg/kg/day									
		Drug admixed with diet						Oral by gavage			
		Control, 0		750		1000		500		750	
		B.wt	%gain ¹	B.wt	% gain	B.wt	% gain	B.wt	% gain	B.wt	% gain
Prefest Wk 1	M	139 (19)		144 (19)		140 (19)		140 (19)		142 (19)	
	F	120 (19)		125 (19)		123 (19)		121 (19)		124 (19)	
Wk 1/ D 1	M	186 (19)	0.0	188 (19)	0.0	188 (19)	0.0	183 (19)	0.0	189 (19)	0.0
	F	145 (19)	0.0	148 (19)	0.0	146 (19)	0.0	144 (19)	0.0	146 (19)	0.0
Wk 2 D 8	M	225 (19)	21	221 (19)	17.4 85	215 (19)	14.5 69	214 (19)	16.5	200§ (19)	5.9§
	F	163 (19)	12.1	163 (19)	9.7 83	159 (19)	8.7 72	161 (19)	11.7	156 (19)	7.4
Wk 3/ D 15	M	265 (19)	42.3	258 (19)	37.0 89	252 (19)	33.8* 81	249 (17)	37.1	243* (16)	28.1§
	F	180 (19)	24.0	179 (19)	20.5 89	169* (19)	16.1§ 66	177 (19)	22.8	176 (17)	21.4
Wk 4/ D 22	M	295 (19)	58.4	288 (19)	53.0 92	282 (19)	49.9* 86	278 (17)	53.3	274* (15)	45.1§
	F	192 (19)	32.6	188 (19)	26.5§ 85	182* (19)	24.6§ 77	190 (19)	32.2	192 (16)	32.9
Wk 5/ D 29	M	321 (19)	72.1	310 (19)	64.9 90	306 (19)	62.5* 87	309 (16)	68.8	300 (14)	59.7§
	F	203 (19)	40.2	201 (19)	35.2 91	193 (19)	32.3§ 81	205 (19)	42.2	205 (16)	42.1
Wk 6/ D 36	M	342 (19)	83.5	328 (19)	74.3 88	327 (19)	73.6* 89	323 (16)	76.5	322 (14)	71.5*
	F	215 (19)	48.2	212 (19)	42.6 91	204 (19)	39.5§ 83	216 (18)	50.4	213 (15)	48.1
Wk 7/ D 43	M	359 (19)	92.7	341 (19)	81.2* 88	344 (19)	82.5 90	337 (16)	84.3	338 (14)	79.9*
	F	221 (19)	52.7	218 (19)	47.1 92	210* (19)	43.6§ 84	221 (18)	53.6	227 (14)	56.9
Wk 8/ D 50	M	372 (19)	100.3	353 (19)	87.3* 89	359 (19)	90.5 92	351 (16)	91.6	352 (14)	87.0
	F	226 (19)	56.1	223 (19)	50.5 93	216 (19)	48.3* 86	225 (18)	56.8	233 (14)	61.2
Wk 9/ D 57	M	388 (19)	108.0	369 (19)	96.0* 90	374 (19)	98.5 92	366 (15)	99.8	366 (13)	94.4*
	F	232 (19)	59.7	227 (19)	53.3 91	222 (19)	52.0* 87	229 (17)	60.5	235 (14)	62.2
Wk 10/ D 64	M	406 (19)	117.8	372* (19)	98.1§ 84	391 (19)	107.8 92	375* (15)	105.0	379 (13)	101.3
	F	237 (19)	63.6	236 (19)	58.9 96	227 (19)	55.7* 88	238 (17)	66.5	243 (14)	68.4

Study Week/ Day	M /F	Dose, mg/kg/day									
		Drug admixed with diet						Oral by gavage			
		Control, 0		750		1000		500		750	
		B.wt	%gain ¹	B.wt	% gain	B.wt	% gain	B.wt	% gain	B.wt	% gain
Wk 11/ D71	M	401 (19)	114.8	373 (19)	98.5 86	370 (19)	96.4 85	363 (15)	97.5	357 (12)	91.0
	F	227 (19)	56.8	223 (19)	50.4 92	231 (19)	58.4 104	232 (17)	62.2	241 (14)	66.8
Wk 12/ D 78	M	423 (19)	126.7	407 (19)	116.0 92	413 (19)	119.2 95	396 (15)	117.0	394 (12)	110.8
	F	251 (19)	73.2	248 (19)	67.3 94	236* (19)	62.0§ 85	249 (17)	74.1	256 (13)	77.3
Wk 12/ D 84	M	426 (19)	128.6	410 (19)	117.7 93	416 (19)	120.9 95	392* (15)	114.2	399 (12)	113.6
	F	246 (19)	70.0	246 (19)	66.3 97	237 (19)	62.5* 90	249 (17)	74.3	251 (13)	74.3
Wk 13/ D 91	M	433 (19)	132.5	415 (19)	120.3 92	421 (19)	123.8 94	405 (13)	119.3 90	404 (12)	116.3* 87
	F	247 (19)	70.4	247 (19)	66.4 97	240 (19)	64.6 92	245 (17)	71.3 99	252 (11)	76.7 104

Numbers in parentheses indicate number of animals that survived

¹: group mean body weight gain (%) relative to weight on study day 1 are shown in upper line (numbers copied from the sponsor's table), while the second line with bold face number denotes group mean body weight gain as % of control. The latter is calculated as: difference in body wt (as gm) between the study day and day 1 (initial weight) for the treated group X 100 / difference in body wt (as gm) for the control between the study day and day 1.

* and §: significantly different from the control group at 0.05 and 0.01, respectively, using Dunnett's T-test.

Food consumption: Food intake was statistically significantly decreased ($P < 0.05$) in females at all dose levels (gavage and dietary administration) during the first week of the study and in males receiving 750 mg aliskiren/kg/day by gavage during the first week of the study. The food consumption recovered in the 2nd week and posted gains in subsequent weeks for all dose groups.

Hematology: No test substance-related changes in hematology were recorded for any dose group.

Clinical Chemistry: Drug-related biochemical changes were restricted to dose-dependent elevations in serum enzymes in females receiving aliskiren by gavage. Mean AST and ALT were significantly higher relative to control for dose levels of 500 or more mg/kg/day (1.2 to 1.7 times control) and 750 mg/kg/day (1.44 times control), respectively. A slight but statistically significant and dose-dependent increase in inorganic phosphorus was noted for males at both dose levels of dietary administration. Urinalysis revealed a significant decrease ($p < 0.01$) in urine volume (3-fold less than control) resulting in increased relative density (1.073 vs. 1.035 for control) and increased osmolality (1.8-fold higher than control) in males receiving 750 mg aliskiren/kg/day orally by gavage. In addition, statistically significant increases in protein concentration (317%), bilirubin (8.5 $\mu\text{mol/l}$, absent in all other groups including the control) and erythrocytes (606%) relative to concurrent control were noted for this group.

Organ Weights: In males, statistically significant decreases ($p < 0.01$) in mean absolute (8 to 13%) and relative (19 to 23%) liver weight at all dose levels (gavage and feeding)

relative to the concurrent control group were noted. The decreases were dose dependent. Gross Pathology: No treatment-related macroscopic findings were observed at any dose level.

Histopathology: Histopathological changes noted in animals treated with aliskiren hemifumarate by gavage were confined to the respiratory tract and large intestine. Treatment (by gavage) at doses of 500 and 750 mg aliskiren/kg/day resulted in minimal to marked inflammatory exudate, minimal to marked ulceration, minimal to moderate squamous metaplasia, and slight mucoseropus exudate in the nasal cavities, nasopharynx and larynx (mostly in decedents). In intestine, a moderate erosion in the cecum and ulceration in the colon were observed in 3 of 10 females (2 decedents and one surviving animal) receiving 750 mg aliskiren/kg/day by gavage. There were no test substance-related microscopic findings observed at the scheduled necropsy in animals treated by dietary administration.

Toxicokinetics: With the exception of the high dose group, aliskiren was not detectable in plasma at most sampling times on day 1, after dietary administration. Thus, the calculated AUC for day 1 has limited value. After 13 weeks of dietary administration, systemic exposure (AUC_{0-24h}) to aliskiren increased with the dose in males but not in females, suggesting saturation in the process of absorption at the high dose level (1000 mg/kg/day) in females (Table 3.2.4.6).

After single or repeated oral gavage administration of aliskiren hemifumarate, systemic exposure (AUC_{0-24h}) to aliskiren in both males and females increased with the dose (Table 3.2.4.7). Systemic exposures (AUC_{0-24h}) after 13 weeks gavage administration at 500 mg/kg/day for males and females were, respectively, 5.3- and 2.4-fold higher than after single administration. The exposure differences at 750 mg/kg/day for males and females were, respectively, 2.0- and 3.2-fold, suggesting the attainment of steady state. It is difficult to predict from the limited pharmacokinetic data whether aliskiren has significant accumulating potential, though it has been suggested by the sponsor. Similar T_{max} (1-4 hr), elimination half-lives and mean residence times (MRT) were found, irrespective of dose and duration (Table 3.2.4.7). Any sex differences in exposure were masked by high standard deviations and failed to conform to a pattern.

After 13 weeks of dosing, gavage at 500 mg/kg/day resulted in exposures (AUC_{0-24h}) at least as high as attained by feeding at 750 mg/kg/day (2.0- and 1.1-fold higher for males and females, respectively) or 1000 mg/kg/day (1.4- and 1.1-fold higher for males and females, respectively) (Table 3.2.4.8). It may be noted that unlike with dietary administration, evidence for saturation of absorption was not observed with gavage administration at 750 mg/kg/day.

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TABLE 3.2.4.6
TOXICOKINETICS OF ALISKIREN ON DAY 1 (TOP) AND AFTER 13 WEEKS (BOTTOM) OF
DIETARY ADMINISTRATION

Time	Mean Plasma Levels [ng/ml]							
	Animal Groups 2 and 3							
	M 750 mg/kg (1x)		M 1000 mg/kg (1x)		F 750mg/kg (1x)		F 1000 mg/kg (1x)	
[hours]	[ng/ml]	[s.d.]	[ng/ml]	[s.d.]	[ng/ml]	[s.d.]	[ng/ml]	[s.d.]
1	62.1	32.5	77.3	71.3	0*	-	25.1	23.2
2	0*	-	0*	-	0*	-	9.4	16.3
4	0*	-	0*	-	0*	-	13.7	23.8
8	0*	-	0*	-	0*	-	0*	-
16	0*	-	20.1	-	0*	-	7.6	13.2
24	0*	-	63.4	-	0*	-	28.0	25.5
AUC _(0-24h)	[ng.h/ml]	31		453		0		241

*: Limit of detection was set to zero

Time	Mean Plasma Levels [ng/ml]								
	Animal Groups 2 and 3								
	M 750 mg/kg (13weeks)		M 1000 mg/kg (13weeks)		F 750mg/kg (13weeks)		F 1000 mg/kg (13weeks)		
[hours]	[ng/ml]	[s.d.]	[ng/ml]	[s.d.]	[ng/ml]	[s.d.]	[ng/ml]	[s.d.]	
1	151.2	53.7	166.9	35.3	619.4	286.0	322.1	131.1	
2	113.8	26.5	297.0	175.9	186.6	43.1	235.1	42.2	
4	125.3	25.3	181.8	9.9	252.8	81.2	161.4	23.6	
8	75.4	6.4	170.2	29.2	149.0	69.1	206.8	28.5	
16	109.4	30.3	155.5	24.3	215.4	151.3	182.1	105.2	
24	203.0	95.6	145.6	20.3	146.8	22.5	180.8	19.6	
Coverage	[ng/ml]	129.7	43.5	163.3	15.9	190.1	45.12	193.2	28.42
Time*interval	[h]	1-24		1-24		2-24		2-24	
AUC _(0-24h)	[ng.h/ml]	2762		3922		4553		4419	

*: Time interval selected for C_{average}

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TABLE 3.2.4.7
TOXICOKINETICS OF ALISKIREN ON DAY 1 (TOP) AND AFTER 13 WEEKS (BOTTOM) OF
GAVAGE ADMINISTRATION

Time	Mean Plasma Levels [ng/ml]							
	Animal Groups 4 and 5							
	M 500 mg/kg (1x)		M 750 mg/kg (1x)		F 500 mg/kg (1x)		F 750 mg/kg (1x)	
[hours]	[ng/ml]	[s.d.]	[ng/ml]	[s.d.]	[ng/ml]	[s.d.]	[ng/ml]	[s.d.]
0	27.5	24.6	10.1	17.6	53.8	28.1	8.1	14.0
1	81.7	40.6	644.4	802.9	199.7	160.3	305.5	89.3
2	97.1	50.5	206.4	108.5	431.7	381.0	320.0	185.3
4	101.8	45.5	97.6	37.1	132.6	79.9	210.0	133.8
8	41.5	51.9	199.4**	81.3	66.3	83.0	117.6	42.7
24	8.2	14.2	22.1*	-	22.1*	-	41.2	36.5
C _{max} [ng/ml]	101.8		644.4		431.7		320	
T _{max} [h]	4.0		1.0		2.0		2.0	
AUC _(0-24h) [ng.h/ml]	1027		3423		2112		2925	
AUC _(0-∞) [ng.h/ml]	1196		3615		2307		3401	
MRT _(area) [h]	8.0		8.0		8.3		11.2	
Elimination half-life [h]	5.8		6.0		6.1		8.0	
Selected time interval	4-24		1-24		2-24		2-24	
Corr. Coefficient	0.97		0.77**		0.82***		0.95	

* < LOD = 22.1 ng/ml, by this assumptions optimal evaluation and comparisons between AUC values of the different groups became possible.

** Less reliable values due to the value at 8 hours.

*** Due to the less reliable values.

Time	Mean Plasma Levels [ng/ml]							
	Animal Groups 4 and 5							
	M 500 mg/kg (13 weeks)		M 750 mg/kg (13 weeks)		F 500 mg/kg (13 weeks)		F 750 mg/kg (13 weeks)	
[hours]	[ng/ml]	[s.d.]	[ng/ml]	[s.d.]	[ng/ml]	[s.d.]	[ng/ml]	[s.d.]
0	0*	---	77.8**	---	78.0	41.4	83.2	50.2
1	826.3	254.7	914.8	255.7	905.8	126.3	1836.9	673.2
2	557.8***	---	477.8	23.5	676.4	253.2	986.0	27.3
4	431.8	160.2	496.3**	---	334.4	57.1	938.6	159.0
8	211.6	34.0	348.2	37.1	159.1	61.9	256.3	11.3
24	43.1***	---	37.7	53.3	51.6	5.9	66.4	22.6
C _{max} [ng/ml]	826.3		914.8		905.8		1836.9	
T _{max} [h]	1.0		1.0		1.0		1.0	
AUC _(0-24h) [ng.h/ml]	5419		6943		4966		9268	
AUC _(0-∞) [ng.h/ml]	5774		7237		5412		9761	
MRT _(area) [h]	7.7		7.3		8.2		6.6	
Elimination half-life [h]	5.7		5.4		6.0		5.2	
Selected time interval	1-24		1-24		1-24		1-24	
Corr. Coefficient	0.97		0.97		0.90		0.91	

* < LOD was set to zero.

** Only one value available.

*** Only one value due to individual outliers excluded from the mean

TABLE 3.2.4.8
COMPARISON OF AUCs FOR THE FEEDING AND THE GAVAGE GROUPS

Single Gavage (g) Versus Single Feeding (f)				
Groups	Dose	Factor	AUC_{0-24h}	Factor
	mg/kg		[ng.h/ml]	
M3 1x f	1000	-	453	-
M4 1x g	500	0.5	1027	2.3
M3 1x f	1000	-	453	-
M5 1x g	750	0.75	3423	7.6
F3 1x f	1000	-	241	-
F4 1x g	500	0.5	2112	8.7
F3 1x f	1000	-	241	-
F5 1x g	750	0.75	2925	12.1
Repeated Gavage (g) Versus Repeated Feeding (f)				
Groups	Dose	Factor	AUC_{0-24h}	Factor
	mg/kg		[ng.h/ml]	
M2 13w f	750	-	2762	-
M4 13w g	500	0.67	5419	2.0
M2 13w f	750	-	2762	-
M5 13w g	750	1.00	6943	2.5
M3 13w f	1000	-	3922	-
M4 13w g	500	0.5	5419	1.4
M3 13w f	1000	-	3922	-
M5 13w g	750	0.75	6943	1.8
F2 13w f	750	-	4553	-
F4 13w g	500	0.67	4966	1.1
F2 13w f	750	-	4553	-
F5 13w g	750	1.00	9268	2.0
F3 13w f	1000	-	4419	-
F4 13w g	500	0.5	4966	1.1
F3 13w f	1000	-	4419	-
F5 13w g	750	0.75	9268	2.1

M: male, F: female, g: gavage, f: feeding

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3.2.5 Twenty-Six Week Oral Gavage Study in Wistar Rats

Key Study Findings: A total of 6 animals, including two controls, were euthanized *in extremis* during the study. None of these deaths were attributed by the sponsor to an effect of the test substance. A significant decrement in group mean body weight gain relative to control was noted for animals receiving aliskiren at 150 (14% lower) or 250 (16% lower) mg/kg/day between study weeks 12 and 26. Histopathologic findings were confined to minor inflammatory and degenerative changes of the epithelium of the upper respiratory tract in a few animals from the 150 and 250 mg/kg/day groups. The NOAEL for this study was 50 mg/kg/day.

Study No: — 1940/18

Location of Report: EDR

Conducting Laboratory and Location: —

Dates of Study: Dosing was initiated on August 21, 2001 and terminal necropsies were begun on February 22, 2002.

GLP Compliance: Yes

QA'd Report: yes (X) no ()

Drug, Lot #: Aliskiren hemifumarate, batch #S100B-2001002

Formulation: Aliskiren hemifumarate was dissolved in purified water. The solution was divided into daily aliquots and was analyzed in weeks 1, 13 and 26.

Animals

Species/Strain: HsdBrlHan:WIST (equivalent to CrI:WI) rats

#/Sex/Group: 20/sex/group for toxicology; 9/sex/group for toxicokinetics

Age: 6 weeks at the time of dosing

Weight: males: 131.8 to 175 gm; females: 106 to 140 gm

Husbandry: Animals were housed in groups of five (same sex) in polypropylene/stainless steel cages. Food and water were given *ad libitum* throughout the study period except during the period of fasting (prior to sampling of blood for clinical pathology studies) when food, but not water was withheld.

Dosing

Doses: See Table 3.2.5.1. Dose levels were based on the results of a 13 week oral toxicity study in the same rat strain in which gavage administration of aliskiren hemifumarate at doses of 500 or more mg aliskiren/kg/day resulted in deaths. Deaths were also reported in two 2 week studies at doses of 600 or more mg aliskiren/kg/day.

Route, Mode and Duration of Administration: Orally by gavage (10 ml/kg), for all groups for 26 weeks.