STUDY DMPK R0400151– INTERACTION POTENTIAL OF PSC833 ON THE ABC TRANSPORTER LEVEL IN CACO-2 CELL MONOLAYERS

INVESTIGATOR AND SITE:

Not provided

REPORT # DMPK R0400151
EDR VOLUME 6
STUDY DATE: June 20, 2004 – July 6, 2004

OBJECTIVES:

To determine the potential effect of the efflux pump modulator PSC833 on the permeation potential of SPP100.

ABBREVIATIONS:

AP	apical (luminal side)
AP-BL / BL-AP	apical-to-basolateral / basolateral-to-apical
BL	basolateral (blood side)
P	permeability coefficient
J	flux

MATERIALS AND METHODS:

Test

Labeled preparation	[^{14}C]SPP100 — .: 611.8 g/mol, RSE 229-1, Batch No E-3277-147-39, 3.62 MBq/mg
Unlabeled preparation	CGP060536-NX-1: 551.77 g/mol, purity: > — (Novartis)

Reference

Preparation	PKF215-833-NX-1 (PSC833): 1214.65 g/mol, purity > — (Novartis)

Caco-2 cells were cultured on permeable polyethylene terephthalate membranes. Apical to basolateral transportation (upper chamber to lower chamber), basolateral to apical transportation and effect of transport inhibitor were determined by liquid scintillation counter.

RESULTS:

Aliskiren transportation from apical to basolateral was concentration dependent within the range of 1 to50 µM (Figure 1) while basolateral to apical transportation was already saturated at the lowest concentration used (1 µM) indicating that transportation of aliskiren was dependent on the transport direction. Basolateral to apical transportation was much higher than that of apical to basolateral transportation. This could be explained by the existence of efflux transporter on apical membrane. In fact, inhibitor of efflux protein (PSC833) effectively modulated aliskiren transportation (Figure 2). As PSC833 concentration increased, apical to basolateral transportation increased and reached a plateau at 0.4 µM with concomitant decrease in basolateral to apical transportation; which also reached a plateau at 0.4 µM. Km values (3 µM) calculated from Linweaver-Burk plot indicated that aliskiren had a moderate affinity for the efflux pump.

Figure 1Bi-directional SPP100 transport

Concentration-dependent transport of SPP100 across Caco-2 cell monolayers measured in the AP-to-BL and BL-to-AP direction, respectively.

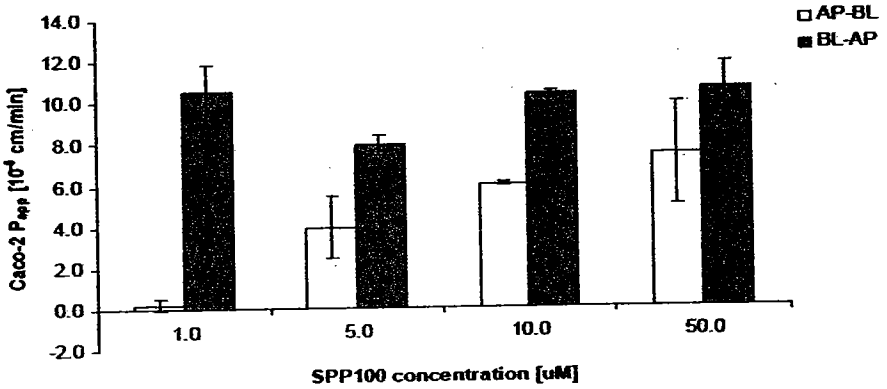
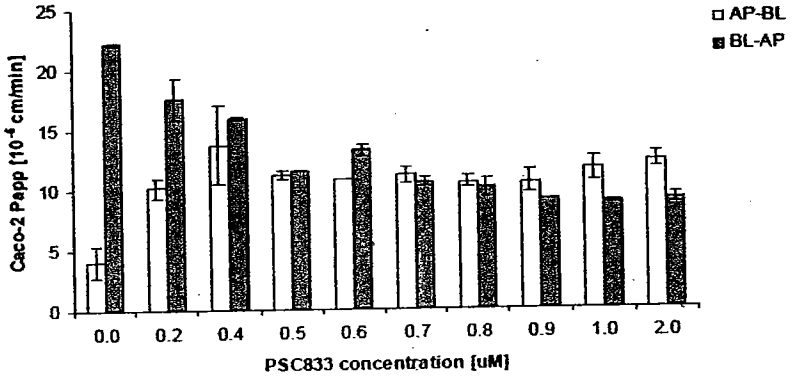


Figure 2SPP100 transport in the presence of PSC833

The role of potential efflux pump inhibitor PSC833 on the transport of SPP100 [1 μM] across Caco-2 cell monolayers measured in the AP-to-BL and BL-to-AP direction, respectively.



CONCLUSIONS:

Aliskiren was preferentially transported from the basolateral to apical direction. Inhibition of aliskiren transport by transporter inhibitor PCS 833 was observed at an IC_{50} value of about 1 μM. From calculated K_m (transporter affinity constant) and J_{max} (maximal transporter-mediated flux) values (3 μM and 30 x 10^{-5} nmol/min·cm^2, respectively), aliskiren has a moderate capacity to act as a substrate for the efflux pump(s).

In a previous study, SPP100 was identified to be a substrate for a prominent efflux system, possibly MRP2. Binding studies using apical-to-basolateral Caco-2 permeability data determined at different substrate concentrations revealed a K_m value of about 3 μM and a J_{max} value of about 29 x 10^{-5} nmol/min·cm^2 for SPP100. These data indicate that SPP100 is a moderate affinity, moderate capacity substrate for the efflux pump involved in its transport. Detailed analysis of the inhibition kinetics of PSC833, using apical-to-basolateral Caco-2 permeability data for the marker substrate SPP100 in the presence of varying inhibitor concentrations, revealed an IC50 value of about 0.1 μM. This finding is indicative of a high inhibitor potential for PSC833. Considering the inter-compound differences between the determined binding (K_m) and inhibition parameters (IC50), respectively, PSC833 is likely to have a concentration-dependant influence on SPP100 permeability by an inhibition interaction at the efflux transporter level.

REVIEWER'S COMMENT:
The reviewer concurs.

APPEARS THIS WAY
ON ORIGINAL

APPEARS THIS WAY
ON ORIGINAL

STUDY DM(EU) 18/1994 – METABOLIC STABILITY OF CGP 60 536 A *IN VITRO* WITH LIVER HOMOGENATE FRACTION OF MARMOSET AND MAN

INVESTIGATOR AND SITE:

Not provided

REPORT # DM(EU) 18/1994
EDR VOLUME 5
Release Date: October 20,1994

OBJECTIVES:
To investigate metabolic stability of aliskiren in postmitochondrial liver fraction (S12) from man.

MATERIALS AND METHODS:
Test Compound CGP 60 536A (Laboratory batch 4) by —

Aliskiren was incubated with human liver post mitochondrial fraction and analyzed by HPLC.

RESULTS:
Three metabolites (P3, P4 and P5) were found when aliskiren was incubated with post mitochondrial fraction of human liver. However, about 2/3 of aliskiren remained unchanged after 120 min of incubation. Molecular structure of metabolites were not determined in this study.

Figure 1 In vitro kinetics of aliskiren and potential metabolites
Time course of concentrations of aliskiren and two potential main and three minor metabolites using the postmitochondrial liver fraction (S12) from man is depicted below. The concentrations of the potential metabolites were estimated using the calibration factor of the parent compound aliskiren assuming a similar extinction coefficient (spectra were similar). Semiquantitative HPLC analysis on —
and UV detection at 230 nm.

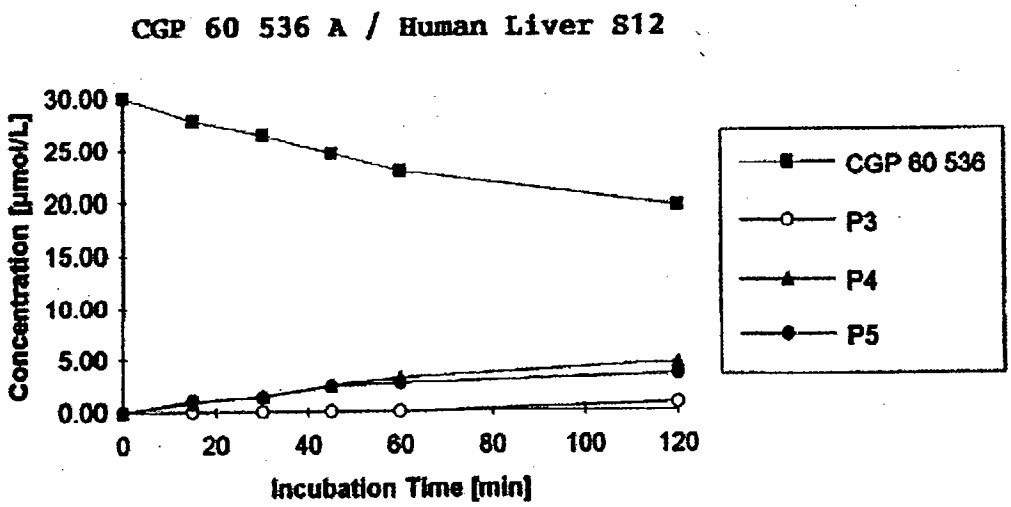


Figure 2 Chromatograms of incubations with human liver S12
Reversed-phase chromatogram (UV detection at 230 nm) of the supernatants of incubations of aliskiren (30 µmol/L initial concentration) with human liver postmitochondrial fraction:"0 min", 120 min incubation, and 120 min control incubation.

Table 1: Concentrations of CGP 60 536 and five potential metabolites in liver S12 incubations

Concentrations of CGP 60 536 five potential metabolites determined in the supernatants of incubations of CGP 60 536 A (30 µmol/L initial concentration) with the postmitochondrial liver fraction (S12) from marmoset and man. The concentrations of the potential metabolites were estimated using the calibration factor of the parent compound CGP 60 536 assuming a similar extinction coefficient (UV spectra were similar).
Semiquantitative HPLC analysis on ——— and UV detection at 230 nm.

Man

Sample		Concentration [µmol/L]					
	Incubation Time [min]	CGP 60 536	P1	P2	P3	P4	P5
Complete[1]	0						
Complete	15						
Complete	30						
Complete	45						
Complete	60						
Complete	120						
inact. S12[2]	120						
w/o S12[3]	120						

[1] Complete active incubation system
[2] Incubation using heat inactivated S12 (10 min at 100°C)
[3] Incubation without S12 as control for substance stability under incubation conditions

CONCLUSIONS:

Aliskiren was metabolized in postmitochondrial fraction of human liver with 2/3 remaining intact after 120 min of incubation. Thus, aliskiren is considered to be metabolically stable in human liver postmitochondrial fractions.

REVIEWER'S COMMENT:

Reviewer concurs with findings; however:

1. Detailed information on the test compound was not provided.
2. Study site not provided.

APPEARS THIS WAY
ON ORIGINAL

STUDY DMPK R0101129– IDENTIFICATION OF HUMAN CYTOCHROME P450 ENZYMES INVOLVED IN THE OXIDATIVE METABOLISM OF SPP100 (ALISKIREN)

INVESTIGATOR AND SITE: Not provided

REPORT # DMPK R0101129
EDR VOLUME 6
STUDY DATES: July – September 2004

OBJECTIVES:

1. To investigate which human cytochrome P450 isoenzymes are involved in the oxidative metabolism of SPP100.
2. To estimate the potential of a drug-drug interaction with co-medications.

MATERIALS AND METHODS:

Labeled Compound [^{14}C]SPP100 hemifumarate. (Batch number RSE 229-8E-13669-128-33 and E-30585-11-46) by Novartis Pharma AG, Switzerland

Commercially available human liver microsomes (HIV 1 and 2, HTLV 1 and 2 and Hepatitis B and C negative) and recombinant human CYP subtypes were used for the determination of metabolic oxidation of aliskiren.

RESULTS:

Four metabolites (M1, M3, M4 and M8) were discovered when aliskiren was incubated with post mitochondrial fraction of human liver. CYP3A4 is the major subtype contributing by more than 99 % of total metabolism. The apparent kinetic constant, Km is 43.8 ± 5.2 μM and for Vmax is 1807.6 ± 69.6 pool/min/mg. CYP 3A4 (99.6 %) and CYP 2D6 (0.4 %) were major contributors toward the metabolism of aliskiren when incubated with human liver microsomes. Almost the same metabolites as with the human liver microsome were obtained by incubation with human recombinant CYP3A4. The kinetic constants for both CYP3A4 (Km: 24.3 ± 2.2 μM; Vmax: 24618 ± 482 pmol/min·nmol and CYP2D6 (Km: 73.6 ± 7.0 μM; Vmax 5479 ± 258 pmol/min/nmol) were determined. Aliskiren metabolism by CYP3A was inhibited by known inhibitors, ketoconazole and troleandomycin (TAO). When human liver microsome was treated with CYP3A4 monoclonal antibody, metabolic oxidization of aliskiren was decreased by 90 %.

Table 1 Enzyme kinetic constants and estimated contribution of various CYPs to the metabolic clearance of SPP100 in human liver microsomes

Enzyme	K_m (μM)	V_{max} (pmol/(min.nmol CYP)	CL(Vm/Km) μl/(min. nmol CYP)	abundance[A] (pmol P450/mg)	Specific Conc. (% total CYP)	Rel. CL μl/(min.nmol total CYP)	Contribution[C] (% total Rel. CL)
CYP 1A1	nd	nd	nd				
CYP 1A2	nd	nd	nd	52	9.47%		
CYP 1B1	nd	nd	nd				
CYP 2A6	nd	nd	nd	36	6.56%		
CYP 2B6	nd	nd	nd	11	2.00%		
CYP 2C8	nd	nd	nd	24	4.37%		
CYP 2C9	nd	nd	nd	73	13.30%		
CYP 2C18	nd	nd	nd				
CYP 2C19	nd	nd	nd	14	2.55%		
CYP 2D6*1	73.6	5478.7	74.4	8	1.46%	1.08	0.38 %
CYP 2E1	31.9	46.2	1.4	61	11.11%	0.16	0.06 %
CYP 2J2	57.0	604.4	10.6				
CYP 3A4[B]	24.3	24617.7	1013.1	155	28.23%	286.02	99.6 %
CYP 3A5	28.7	1351.7	47.0				
CYP 3A7	32.6	112.7	3.5				
CYP 4A11	3.1	15.5	4.9				
CYP 4F2	11.3	89.1	7.9				
CYP 4F3A	nd	nd	nd				
CYP 4F3B	5.0	120.8	24.0				
CYP 4F12	nd	nd	nd				
Total				434	79.1%	287.27	100 %

Footnote to Table 1: Enzyme kinetic parameters Vmax and Km of CYP2D6, 3A4, were obtained from kinetic experiments (rates of parent drug disappearance). The values of other isoenzymes were estimated by solving the Michaelis-Menten equation V=Vmax·S/(Km+S) for the 2 concentrations of substrate and their rate numbers. A: the CYP abundance values were from (Rowland Yeo, et al 2004); B: for CYP3A4, the abundance value of CYP3A was used; C: the contribution of other CYPs (about 21%) could not be calculated due to the unknown abundance in HLM.

Figure 1 Inhibition of SPP100 metabolism in HLM by chemical inhibitors

Biotransformation of [^{14}C] SPP100 (1 and 20 mol/L) by human liver microsomes (0.3 mg/mL) was investigated in the presence and absence of different inhibitors. The supernatants of incubations after 15 minutes were analyzed by HPLC with radiodetection.

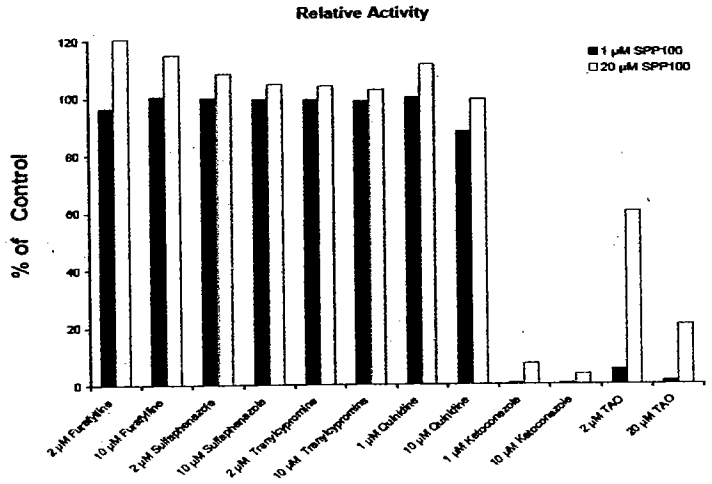
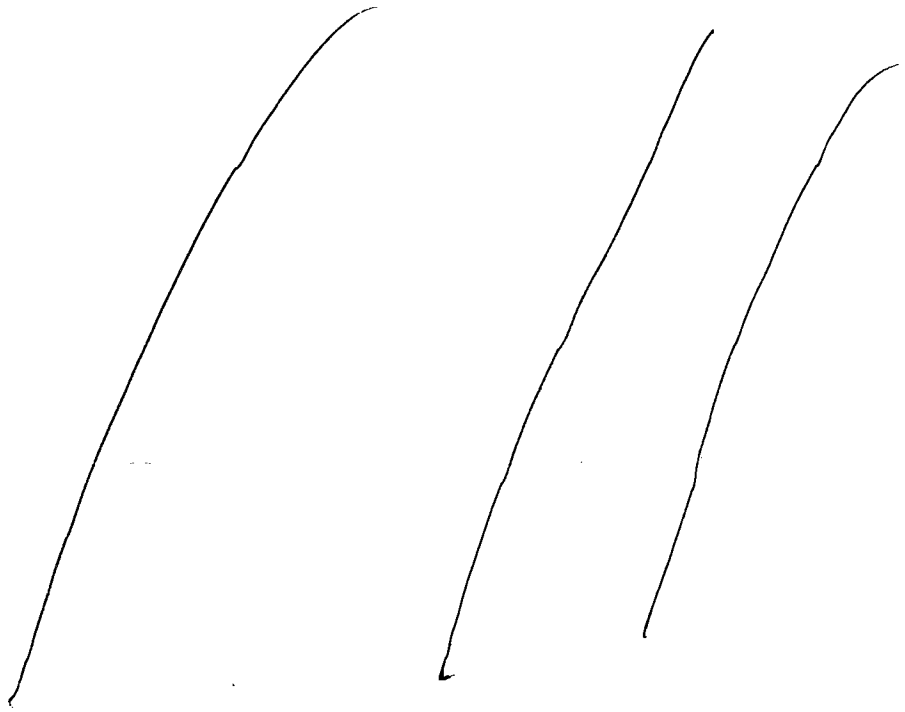


Figure 2 Chromatogram of [^{14}C]SPP100 incubated in human liver microsomes or CYP3A4 expressing cell lysate.

A Human Liver Microsome



CONCLUSIONS:

The subtypes of CYP contributing to aliskiren metabolism were determined as seen in Table 1. According to the results presented here, CYP3A4 is the major contributor to aliskiren metabolism. Chemical (ketoconazole) and biological (monoclonal antibody) inhibitors effectively inhibit aliskiren metabolism by CYP3A4.

REVIEWER'S COMMENT:

The reviewer concurs with study findings, however:

1. Study site was not provided.

APPEARS THIS WAY
ON ORIGINAL

APPEARS THIS WAY
ON ORIGINAL

STUDY DMPK 1997/452– IN VITRO BIOTRANSFORMATION OF CGP 60536B BY LIVER MICROSOMES OF RATS, MARMOSET AND MAN

INVESTIGATOR AND SITE: B. Wirz
Novartis Pharma AG
Preclinical Safety / Drug Metabolism and Pharmacokinetics
Biotransformation
4002 Basel, Switzerland

REPORT # DMPK 1997/452
EDR VOLUME 5
RELEASE DATE: April 15, 1998

OBJECTIVES:

To investigate the species differences of in vitro metabolism of aliskiren.

NOTE: Study results in man will only be reviewed.

MATERIALS AND METHODS:

Labeled Compound: [^{3}H]CGP60536 hemifumarate. (Batch number W 336.2B, 0.29 mg/mL aqueous solution, 12 MBq/mL, 41.6 MBq/mg, radiochemical purity: —, by Novartis Pharma

Human liver microsome from kidney donor liver (pooled from 8 individual, male and female) was used. Aliskiren was incubated with human liver microsomes containing NADPH generating system. Formed metabolites were analyzed by HPLC equipped with radio and UV detector. Metabolites were analyzed by LC/MS.

RESULTS:

After 120 min of incubation, 29, 3.7 and 5 % of aliskiren in remained unchanged from initial concentrations of 100, 10 and 1 µmol/L, respectively. Major metabolites found in the supernatant were P2 and P3. The structures of P2 and P3 were determined to be derivatives of aliskiren without metoxyproply side chain and demethylated derivative, respectively by LC/MS analysis.

Table 1 Pattern of metabolites incubated with human liver microsomes
Pattern of metabolites determined by HPLC with radiodetection incubated supernatants of incubations of 100, 10, and 1.0 µmol/L [^{3}H]CGP 60536B with human liver microsomes (single injections).

100 µmol/L initial [^{3}H]CGP 60536B concentration:

Incubation [min]	Proportion of radioactivity in chromatogram [%]					
	Region a 2 - 27.5 min	P2 tr: 28 min	P3 tr: 29 min	Region b 29.5-40.5min	CGP 60536 tr: 41 min	Region c 41.5-60 min
0						
15						
30						
60						
120						
120 (Control 1)						
120 (Control 2)						

10 µmol/L initial [^{3}H]CGP 60536B concentration:

0						
15						
30						
60						
120						
120 (Control 1)						
120 (Control 2)						

1.0 µmol/L initial [^{3}H]CGP 60536B concentration:

0
15
30
60
120
120 (Control 1)
120 (Control 2)

tr, retention time;
nd, not detected;
Control 1, 120 min control incubations with heat inactivated (10 min at 100°C) liver microsomes;
Control 2, 120 min control incubations with liver microsomes in the absence of an NADPH regenerating system.

Figure 1Chromatogram pattern of aliskiren after 60 min incubation with human liver microsomes

CONCLUSIONS:

Aliskiren metabolism in human liver microsome was determined. Aliskiren was metabolized to its derivative without the metoxyproply side chain and demethylated further to other compounds. Although the precise molecular structures were not completely determined, metabolite structures were partially estimated by LC-MS analysis. More than 95 % of aliskiren in supernatant fraction was converted to metabolites after 120 min of incubation in concentrations below 10 µM. Since the sponsor used purified human liver microsomes, the metabolite proportions were higher in comparison to study #18-1994. Even if the protein concentration in the reaction mixture were the same, the exact amounts of enzymes were not equal.

REVIEWER COMMENTS:

Reviewer concurs with study results, however:

1. Since HPLC condition for LC-MS analysis and radioactive counting were different, the chromatographic pattern of LC-MS ought to be presented. Although the sponsor wrote that both chromatograms were comparable, we could not judge without seeing both.
2. Total recovery of radioactivity should have been presented. Up to 7.4 % of the radioactivity was found in precipitate fraction. However, the results in Table 1 represent the proportion of radioactivity in the chromatogram and not the proportion of total radioactivity.

STUDY PCS(EU) R0101128– INHIBITION OF SPECIFIC P450 ENZYME ACTIVITIES BY SPP100 IN HUMAN LIVER MICROSOMES

INVESTIGATORS AND SITE:

Not provided

REPORT # PCS(EU) R0101128
VOLUME in EDR, Section 5
RELEASE DATE: September 15, 2003

OBJECTIVES:
To determine the inhibition capacity of SPP100 on specific CYP450 enzymes in human liver microsomes.

MATERIALS AND METHODS:
SPP100 (Batch No. NE-5810-Batch-02-01, Lot NE-5810-B-11-10 cryst 13#1, purity , by

Pooled human liver microsomes (HIV1, 2, HTLV1, 2 and Hepatitis B, C negative) were used. Inhibition capacity of aliskiren on CYP subtypes was investigated using a specific substrate for each subtype.

RESULTS:
Significant inhibition on 2C9, 2C19 and 2D6 were observed. However, the in vitro concentration range required to achieve inhibition (200μM) is beyond clinically achievable blood concentrations since Aliskiren's C_{max} when administered at a dose of 150 mg to healthy volunteer is observed to be about 0.03 μM.

Table 1 Inhibitory effect of aliskiren on specific substrate

Subtype	Maximal inhibition
1A2	84.8%
2C8	95.5%
2C9	67.9%
2C19	57.5%
2D6	67.1%
2E1	112.8%
3A4/5	116.6%*

*** Maximum concentration used for 3A4/5 subtype was 10 μM.**

CONCLUSIONS:
Although aliskiren has demonstrated to have inhibitory effect on CYP2C9, 2C19, and 2D6, the concentration required for inhibition was not clinically achievable. Thus the inhibitory effects of aliskiren on CYP enzymes is not of clinical relevance.

REVIEWER'S COMMENT:
1. This study is acceptable except for the results with CYP3A4/5. The maximum concentration of aliskiren used in CYP3A4/5 was 10 μM whereas 200 μM was used in other subtypes. Higher concentrations were re-examined for the inhibition of CYP3A4/5 in study DMPK R0101128-01.
2. Study site not provided.
3. Information on study drug not provided.

STUDY DMPK R0101128-01– *IN VITRO* ASSESSMENT OF CYTOCHROME P450 3A4 INHIBITION BY SPP100

INVESTIGATOR AND SITE: H. Schiller
Novartis Pharma AG
Drug Metabolism and Pharmacokinetics
ADME Section
4002 Basel, Switzerland

REPORT # DMPK R0101128-01
VOLUMES in EDR, Section 5
RELEASE DATE: February 11, 2005

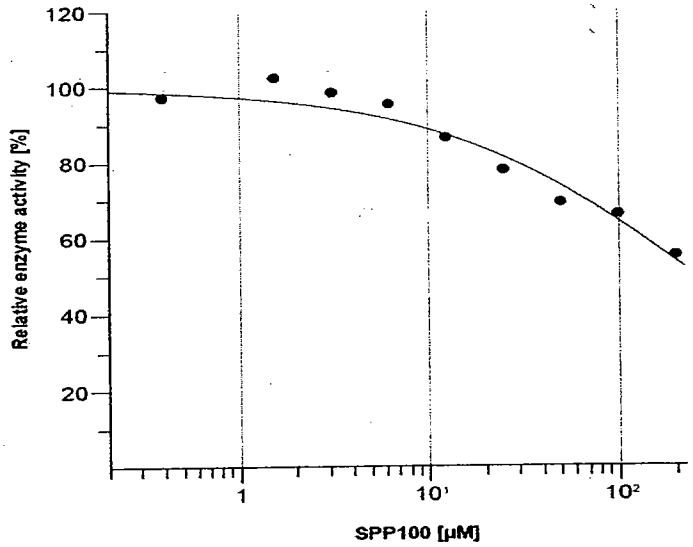
OBJECTIVES:
To determine the inhibition capacity of SPP100 on CYP3A4.

MATERIALS AND METHODS:
SPP100 (Batch No. NE-5810-Batch-02-01, Lot NE-5810-B-11-10 cryst 13#1, purity ___ by

Pooled human liver microsomes were used. Inhibition capacity of aliskiren on CYP 3A4/5 was investigated using two different substrates specific for CYP3A4/5.

RESULTS:
Significant inhibition on CYP3A4 was observed for both substrates. However, the concentration range required to achieve inhibition (IC_{50} - testosterone 6β-hydroxylation 71 μM, midazolam 1'-hydroxylation 251 μM) were higher than clinically achievable since the Cmax of aliskiren when administered at a dose of 150 mg to healthy volunteers is about 0.03 μM.

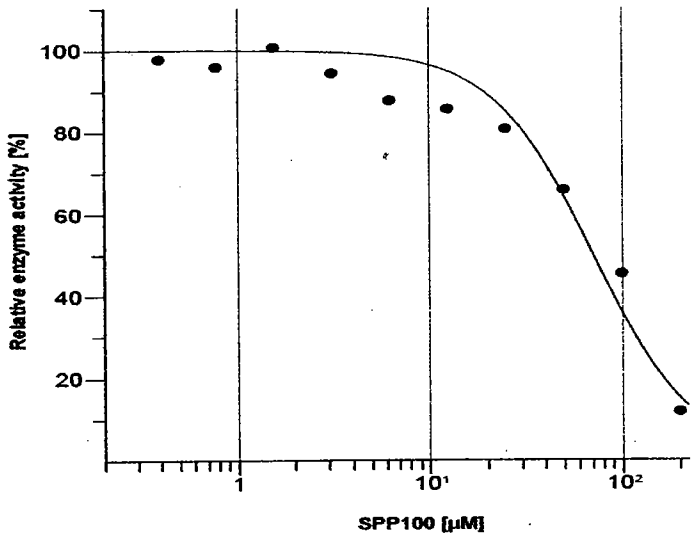
Figure 1 Effect of SPP100 on CYP3A4-mediated midazolam 1'-hydroxylation in pooled human liver microsomes
Results represent the mean of two experiments.



IC_{50} = 251 ± 49 μM

Figure 2 Effect of SPP100 on CYP3A4-mediated testosterone 6ß hydroxylation in pooled human liver microsomes

Results represent the mean of two experiments.



$IC_{50} = 71 \pm 7$ µM

CONCLUSIONS:

This study was conducted to re-examine the inhibitory capacity of aliskiren on CYP3A4 since the concentration of aliskiren was not high enough in the previously conducted study R0101128 where a concentration of 10 µM was used.

Although inhibitory effect on CYP3A4/5 was observed, the concentration range required for inhibition is greater than the observed aliskiren C_{max} (0.03 µM) administered at a dose of 150 mg to healthy subjects. As a result, the inhibitory effect is considered to be clinically negligible.

REVIEWER'S COMMENT:

The reviewer concurs on study results, however:
Detailed information on study drug not provided.

STUDY DMPK R0400734– INTERACTION OF SPP100 WITH ORGANIC CATION TRANSPORTER SUBTYPES OF RAT (rOCT1) AND HUMAN (hOCT1 AND hOCT3)

INVESTIGATOR AND SITE: Not provided

REPORT # DMPK R0400734
VOLUMES in EDR, Section 5
RELEASE DATE: November 23, 2005

OBJECTIVES:
To determine the possible involvement of transporter Organic Cation Transporter (OCT) on aliskiren transportation.

MATERIALS AND METHODS:

Labeled preparation	0.57 µM [^{14}C]SPP100 hemifumarate: Batch RSE 229-10, Batch No.: E-15086-47-39, 1.988 MBq/µmol, supplied by the Isotope Laboratory of Novartis Pharma
Unlabeled Preparation	0.1 pM – mM SPP100 free base, HWB no.11109627, Novartis Pharma AG

NOTE: Only human study results will be assessed.

Human transporter hOCT1 or hOCT3 were used. The effects of aliskiren on 1-methyl-4-phenyl-pyridium (MPP) transport by OCT proteins were determined.

RESULTS:
Aliskiren inhibited MPP incorporation through hOCT1; however, affinity of aliskiren to this transporter was considered low based on the calculated Ki value of approximately
100 µM (see Figure 1). Yet MPP uptake was not affected by the addition of aliskiren (Figure 2). Overexpression of OCT1 had no effect on aliskiren uptake while MPP uptake was significantly low in wild type cells (Figure 3 and 4).

Figure 1 Inhibition of hOCT1 mediated [^{3}H]MPP uptake by different concentrations of SPP100

Uptake of 0.1 µM [^{3}H]MPP into CHO cells that were stably transfected with human OCT1 (hOCT1) was measured. Measurements were performed at 37°C using an incubation period of 1 s. The incubation buffer was PBS containing 0.1 µM [^{3}H]MPP or 0.1 µM [^{3}H]MPP plus the indicated concentrations of SPP100 (SPP). Mean values ± SEM were calculated from the indicated number of individual measurements that were obtained from 1 (3-4 measurements), 2 (7-8 measurements) or 3 (10-11 measurements) independent experiments. The uptake rates are presented as percentage of the mean value of the measurements performed in the absence of SPP100. The number of measurements is indicated in brackets. In A, mean values that are significantly different from the uptake in the absence of SPP100 are indicated (***P<0.001). In B the Hill equation was fitted to the data and the IC_{50} value of 93 ± 17 µM was calculated.

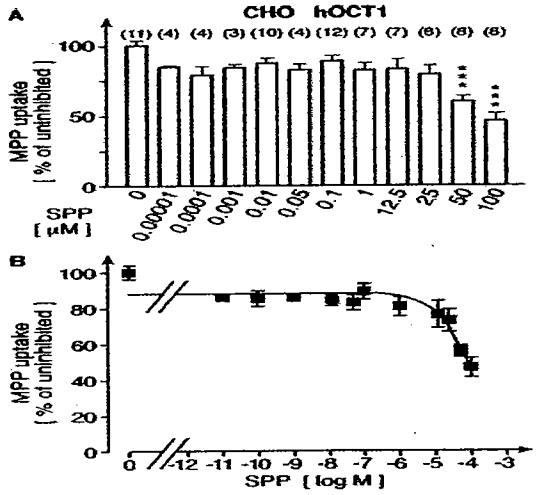


Figure 2 **Inhibition of hOCT3 mediated [^{3}H]MPP uptake by different concentrations of SPP100**

Uptake of 0.1 µM [^{3}H]MPP in CHO cells stably transfected with human OCT3 (hOCT3) was measured. The measurements were performed and are presented as in Figure 1. Mean values + SEM of 10-13 measurements from 3 independent experiments are shown. The number of measurements is indicated in brackets.

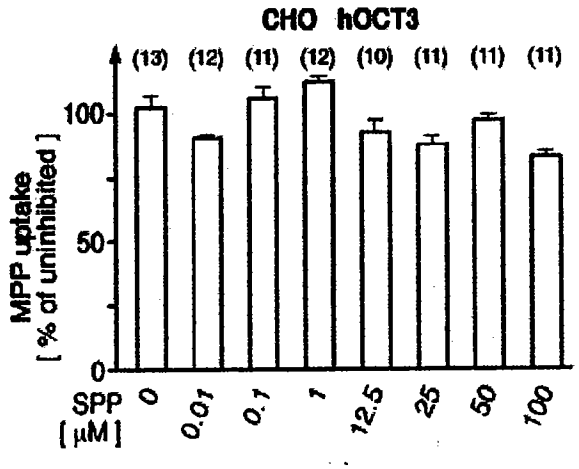


Figure 3 **Uptake of MPP and SPP100 in CHO cells that were stably transfected with hOCT1**

Uptake of 0.1 µM [^{3}H]MPP (MPP) or 0.57 µM [^{14}C]SPP100 into hOCT1 expressing CHO cells was measured in the absence or presence of unlabelled substrates as indicated. Mean values ± SEM of 8 individual measurements from 2 independent experiments are indicated. (**P<0.01, **P<0.001).

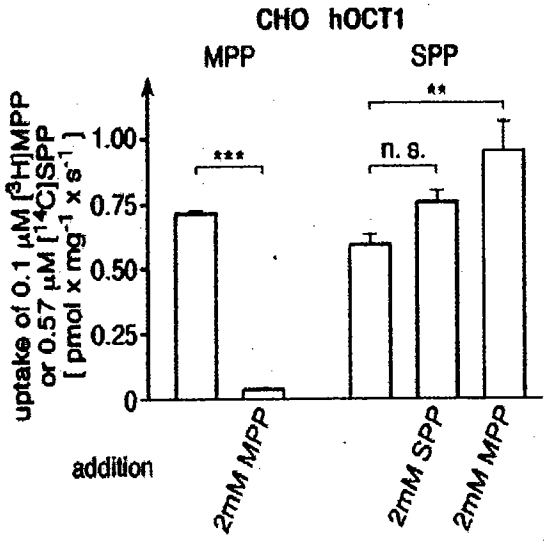
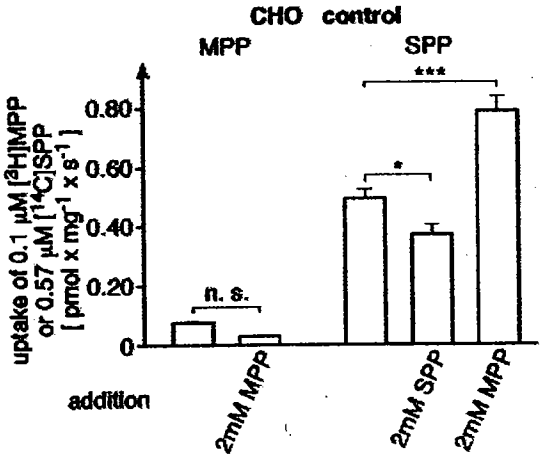


Figure 4 Uptake of MPP and SPP100 into non transfected CHO cells

Uptake of 0.1 µM [^{3}H]MPP (MPP) or 0.57 µM [^{14}C]SPP100 (SPP) into non transfected CHO cells was measured in the absence or presence of unlabelled substrates as indicated. Mean values ± SEM of 8 individual measurements from 2 independent experiments are indicated. (P<0.01, **P<0.001).**



CONCLUSIONS:

The affinity of aliskiren to hOCT1 was low and almost no affinity to hOTC3. Thus, transport of aliskiren was not likely to occur through this transporter.

REVIEWER COMMENT:

The incubation time for the uptake was only 1 sec which is sufficient for binding but not enough time for uptake. As a result, it is not possible to evaluate this study thoroughly.

APPEARS THIS WAY
ON ORIGINAL

INVESTIGATORS AND SITE:

Not provided

REPORT # DMPK R0200734
VOLUMES in EDR, Section 5
STUDY DATES: March 8 – March 15, 2002

OBJECTIVES:

1. To determine the predictive absorption, particularly the involvement of a non-passive diffusion system in the transport of SPP100 across the intestinal barrier.
2. To identify the transporter for SPP100 identified to be a substrate for a prominent efflux system.

MATERIALS AND METHODS:

Labeled preparation	[^{14}C]SPP100 hemifumarate, Code CFQ12601, 3.873 MBq/mg, (HPLC)according to product specification dated 27-Jul-2001.
Unlabeled preparation	NE-5810-Batch-02-01, > — (HPLC) according to substance sheet dated 21-Mar-01

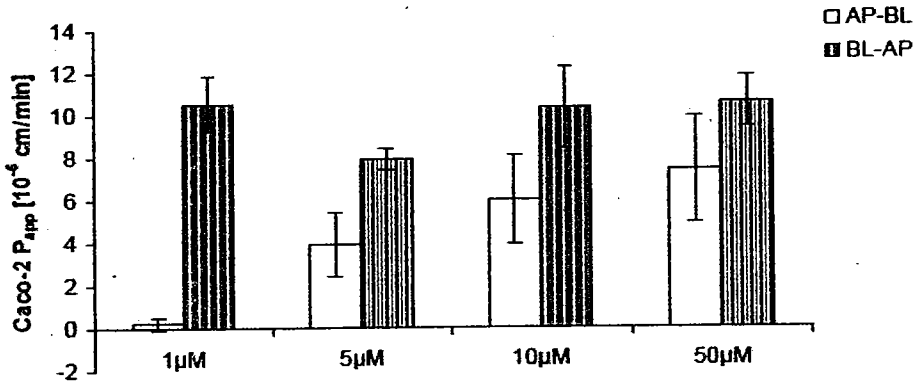
Caco-2 cells were cultured on permeable polyethylene terephthalate membranes. Apical to basolateral transportation (upper chamber to lower chamber) or basolateral to apical transportation and effect of transport inhibitor were determined by liquid scintillation counter or LC/MS/MS method.

RESULTS:

Aliskiren transportation from apical to basolateral was concentration dependent within the range of 1 to50 μM while basolateral to apical transportation was saturated at a concentration of 1 μM. Transport capability of aliskiren was dependent on direction. Basolateral to apical transport was greater than that of apical to basolateral transport. Potential transporter inhibitor cyclosporine A (CsA) increased apical to basolateral aliskiren transportation. However, Verapamil, another transporter inhibitor had no effect on aliskiren transportation from the apical to basolateral direction.

Figure 1 **Bi-directional SPP100 transport**

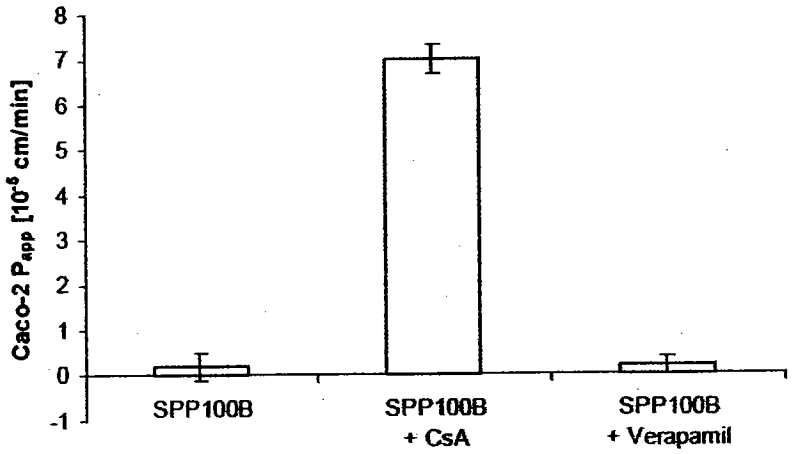
Transport of SPP100 [1, 5, 10 and 50 μM] across Caco-2 cell monolayers measured in the AP-to-BL and BL-to-AP direction



Addition of CsA increased aliskiren transportation from apical to basolateral side (Figure 2).

Figure 2Inhibition of transport

The role of the efflux pump inhibitors CsA and Verapamil on the transport of SPP100 [1 μM] across Caco-2 cell monolayers measured in the AP-to-BL direction



CONCLUSIONS:

Transport of aliskiren occurred both apical to basolateral and basolateral to apical direction. However, transport from apical to basolateral side was much less than that of basolateral to the apical side. This could be explained by the existence of a potential efflux pump on the apical membrane. The results support the conclusions that the efflux system involved in aliskiren transport is MDR1.

REVIEWER'S COMMENT:

1. Verapamil is known as an MDR1 inhibitor, yet there was no explanation as to why this compound had no effect on aliskiren transportation.
2. It seems that the Standard Deviation bars for the 10 μM results in Figure 1 were not the same as depicted in Table 8-1; which is presented on page 10 the original document (0.1 for AP-BL and 0.2 for BL-AP).

STUDY DMPK R0500381– DETERMINATION OF THE HEPATIC UPTAKE CLEARANCE OF SPP100 USING PRIMARY HUMAN AND RAT HEPATOCYTES AND IDENTIFICATION OF THE INVOLVED TRANSPORTERS USING CRNA INJECTED XENOPUS LAEVIS OOCYTES

INVESTIGATOR AND SITE: —

Not provided

REPORT # DMPK R0500381
VOLUMES in EDR, Section 6
RELEASE DATE: October 10, 2005

OBJECTIVES:

1. Determination of the potential and mechanism of hepatic active uptake of SPP100 in rats and human.
2. Identification of the transporter system(s) involved in hepatic carrier-mediated drug uptake of SPP100 and determination of the corresponding kinetic parameters (K_m and V_{max}).

MATERIALS AND METHODS:

Unlabeled test Compound	NVP-CGP060536-AE-1 (hemifumarate salt): Novartis, 609.8 g/mol, purity: > —
Labeled test compound	[^{14}C]SPP100 — 611.8 g/mol, RSE 229-1, Batch No E-3277-147-39, 2.904 MBq/mg

NOTE: Only results from findings in man will be reviewed.

Commercially available human primary cultured hepatocytes were used for the determination of uptake of aliskiren. *Xenopus laevis* oocyte overexpressing human OATP1, 2 and 8 were also used.

RESULTS:

Uptake of aliskiren into human hepatocyte was carrier mediated. However, transport inhibitor PCS833 at 10 μM was not effective in preventing incorporation of aliskiren (Figure 2). Possible carrier for this transportation was OATP1. Since increased uptake of aliskiren into OATP1 overexpressing *Xenopus laevis* oocytes was observed but no increases were observed for cells overexpressing OATP2 or 8.

Figure 1 Concentration-dependency of SPP100 uptake by isolated human hepatocytes for 2 minutes

Each point represents mean ± SD. ■, the nonspecific uptake at 4 °C; ●, the apparent uptake at 37 °C; ○, the calculated carrier-mediated uptake at 37 °C (table 8-5).

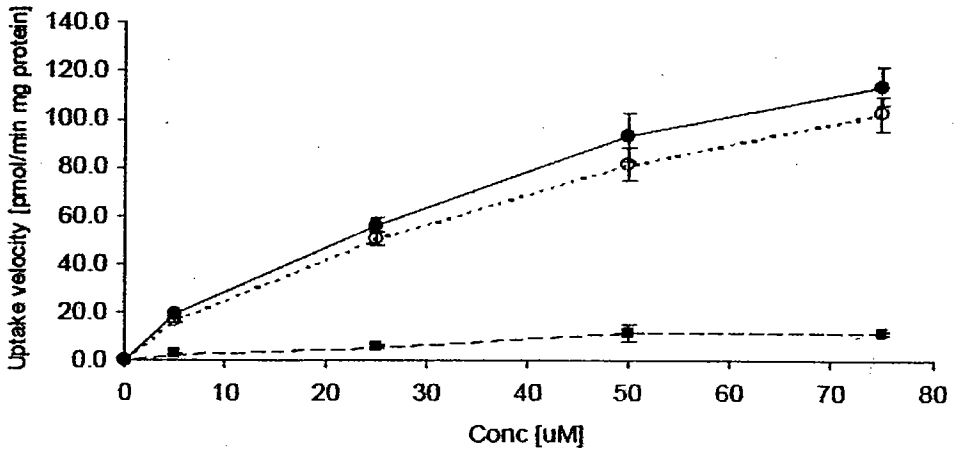


Figure 2 **Inhibition of SPP100 uptake into human hepatocytes by PSC833**
Concentration-dependency of SPP100 uptake in the presence and absence of transporter inhibitor PSC833 (10 µM). ●, the apparent uptake at 37 °C in the absence of inhibitor; ■, the apparent uptake at 37 °C in the presence of inhibitor (table 8-5).

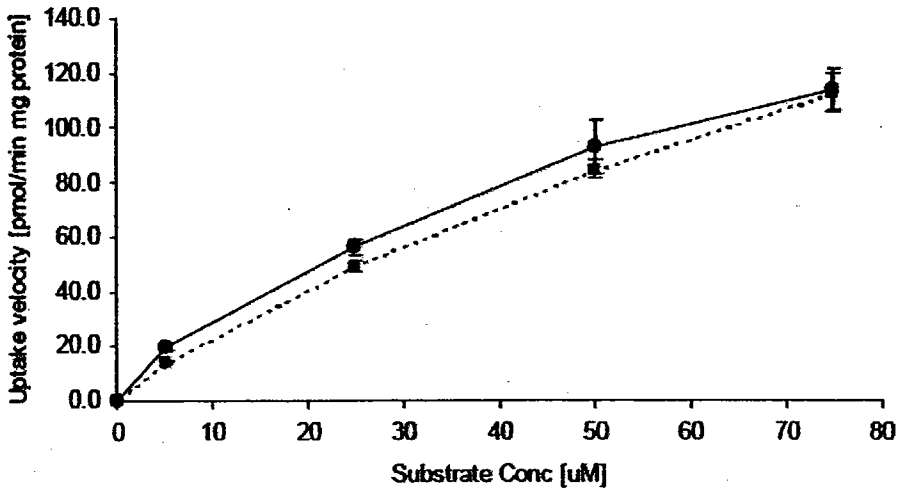


Table 1 **Uptake of different SPP100 concentrations into Xenopus laevis oocytes overexpressing transporters from the human OATP-family**

Conc. [µM]	Uptake into control oocytes [pmol/oocyte/min]	Uptake into OATP8 transportocytes [pmol/oocyte/min]	Uptake into OATP1 transportocytes [pmol/oocyte/min]	Uptake into OATP2 transportocytes [pmol/oocyte/min]
5	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.03 ± 0.01
25	0.01 ± 0.00	0.01 ± 0.01	0.05 ± 0.01	0.03 ± 0.01
50	0.01 ± 0.00	0.03 ± 0.02	0.08 ± 0.04	0.06 ± 0.02
75	0.03 ± 0.01	0.04 ± 0.01	0.10 ± 0.02	0.05 ± 0.02
100	0.04 ± 0.01	0.06 ± 0.02	0.14 ± 0.09	0.06 ± 0.03
200	0.10 ± 0.01	0.10 ± 0.02	0.19 ± 0.02	0.06 ± 0.01

CONCLUSIONS:

This study was conducted for the clarification of the possible hepatic uptake mechanism in human. Human OATP1 is a candidate for hepatic uptake of aliskiren. However, it expressed mainly in brain and hepatic expression was relatively low. Thus, the precise mechanism of hepatic uptake of aliskiren is still controversial.

REVIEWER'S COMMENT:

The reviewer concurs with study findings however:

The labeled test compound in this study was the — salt whereas hemifumarate was used in other Studies (DMPK R0500660, DMPK R0101129, DMPK 1997/452, DMPK R0400734, and DMPK R0200734). The sponsor should clarify this point. Aliskiren's absorption does not seem to be limited or inhibited by PgP or by CYP3A4.

STUDY DMPK (CH) R0100669– IN VITRO BLOOD DISTRIBUTION AND PLASMA PROTEIN BINDING OF ^{14}C-LABELED SPP100 IN MOUSE, RAT, MARMOSET, RABBIT AND HUMAN

INVESTIGATOR AND SITE:

Not provided

REPORT # DMPK (CH) R0100669
VOLUMES in EDR, Section 6
RELEASE DATE: October 23, 2001

Objective(s)	**Investigate the *in vitro* blood/plasma distribution and plasma protein binding of SPP100 in mouse, rat, marmoset, rabbit and human. Protein binding will also be investigated in human serum.**
Test compound	**[^{14}C]SPP100 = [gem dimethyl-14C], Batch Code: CFQ12601** **Specific radioactivity : 3.87 MBq/mg** **Purity : —** **(see full details in Section 3.1)**
Concentrations	**10, 50, 250, 500, 5'000, 10'000 ng/mL (free base)** **(investigations in human matrix covered only the 10-500 ng/mL range)**
Species/strain/sex	mice CD-1(ICR)BR, males albino rats WI(Glx/BRL/Han)BR, males marmoset Callithrix jacchus, males and females rabbit NZW/Kbl BR (New Zealand White rabbit), males human healthy human Caucasian volunteers, males
Matrix	Blood, plasma and serum Anticoagulant: lithium heparin for animals, sodium citrate for man For animals: pools with N ≥ 3, for human: individual analysis, N = 3 (blood and plasma investigations) and N=2 (serum investigations)
Matrix	Blood, plasma and serum Anticoagulant: lithium heparin for animals, sodium citrate for man For animals: pools with N ≥ 3, for human: individual analysis, N = 3 (blood and plasma investigations) and N=2 (serum investigations)
Methods	• *In vitro* blood distribution Incubation at 37°C for 1 h, fraction in plasma (F_P) obtained after cell separation by centrifugation (10 min, 1500 *g*, 37°C). Temperature-dependency investigated at 4°C *versus* 37°C • *In vitro* plasma/serum protein binding Incubation at 37°C for 1 h, free or unbound fraction (F_u) investigation by the ultrafiltration method (10 min, 2000 *g*, 37°C). • Detection technique: ^{14}C-Liquid scintillation counting

Results:

- **Irrespective of the species, at 37°C [^{14}C]SPP100 distributed well into the blood cells although the largest fraction was found in plasma (>50%). The fraction in plasma (F_P) amounted to:**

Concentration range	Species	F_p [%]
10-500 ng/mL	Human 1	69.6 ± 0.9
	Human 2	74.7 ± 0.8
	Human 3	70.1 ± 2.3

Blood distribution was species dependent (F_p: rat < rabbit < mouse ≈ human < marmoset) and concentration independent except in mouse and marmoset: in those species F_p decreased with increasing concentrations, but only at concentrations >500 ng/mL.

Blood distribution achieved at 37°C is stable at 4°C (tested in human).

In vitro blood distribution kinetic of [^{14}C]SPP100 in human

Human blood spiked at 250 ng/mL [^{14}C]SPP100 was incubated at 37°C for 0.5, 1 and 2 hours (triplicates).

Human (H = 41%)						
Incubation time [h]	Actual concentration Blood [ng/mL]	Actual concentration Plasma [ng/mL]	F_P Mean ± [%]		SD	C_b/C_p
0			95.0	±	0.8	0.62
0.5			73.3	±	1.5	0.80
1			69.6	±	1.0	0.85
2			69.3	±	0.9	0.85

In vitro blood distribution of [^{14}C]SPP100 in fresh heparinized human blood with sodium-citrate as anticoagulant. Incubation was for 1 hour at 37°C. Human blood was from three individuals.

Human 1 (H = 41%)						
SPP100 Nominal Concentration [ng/mL]	Actual concentration Blood [ng/mL]	Actual concentration Plasma [ng/mL]	F_P Mean ± [%]		SD	C_b/C_p
10			70.8	±	3.3	0.84
50			68.8	±	1.1	0.86
250			69.3	±	1.2	0.85
500			69.5	±	0.5	0.85
	Human 1 $F_{p\ 10\ to\ 500\ ng/mL}$		69.6	±	0.9	

Human 2 (H = 37%)						
SPP100 Nominal Concentration [ng/mL]	Actual concentration Blood [ng/mL]	Actual concentration Plasma [ng/mL]	F_P Mean ± [%]		SD	C_b/C_p
10			75.3	±	1.3	0.84
50			75.2	±	2.2	0.84
250			74.5	±	1.0	0.85
500			73.6	±	1.1	0.86
	Human 2 $F_{p\ 10\ to\ 500\ ng/mL}$		74.7	±	0.8	

Human 3 (H = 40%)						
SPP100 Nominal Concentration [ng/mL]	Actual concentration Blood [ng/mL]	Actual concentration Plasma [ng/mL]	F_P Mean ± [%]		SD	C_b/C_p
10			73.4	±	3.2	0.82
50			68.1	±	1.8	0.88
250			69.5	±	0.7	0.86
500			69.2	±	0.9	0.87
	Human 3 $F_{p\ 10\ to\ 500\ ng/mL}$		70.1	±	2.3	

H: hematocrit

***In vitro* blood distribution of [^{14}C]SPP100 at 4°C versus 37°C in human**

Human blood spiked at 10 and 500 ng/mL [^{14}C]SPP100 was (A) incubated at 4°C and 37°C for 1 hour (triplicates) and (B) preincubated for 1 h at 37°C and additionally incubated at 4°C for 0.25, 0.5 and 1 h.

A.

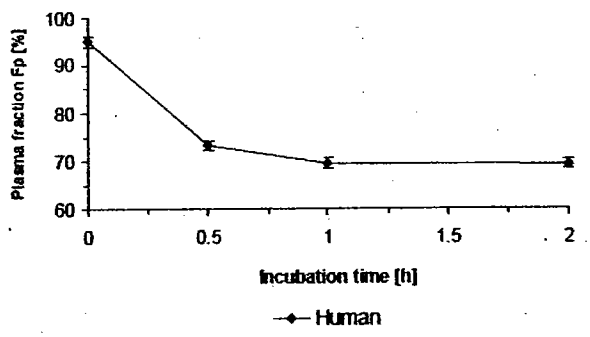
Human (H = 41%)						
SPP100 Nominal Concentration [ng/mL]	Incubation temperature [°C]	Actual concentration Blood [ng/mL]	Actual concentration Plasma [ng/mL]	F_P Mean ± SD [%]		C_b/C_p
10	4			98.6	± 2.8	0.60
	37			72.6	± 4.4	0.81
500	4			96.5	± 2.6	0.61
	37			68.8	± 0.8	0.85

B.

Human (H = 40%)						
SPP100 Nominal Concentration [ng/mL]	Incubation temperature [°C]	Incubation time	Actual concentration Blood [ng/mL]	Actual concentration Plasma [ng/mL]	F_P Mean ± SD [%]	C_b/C_p
10	37	1			69.8 ± 5.0	0.86
	4	0.25			67.7 ± 1.1	0.89
		0.5			65.5 ± 2.8	0.92
		1			64.2 ± 5.2	0.93
500	37	1			70.2 ± 1.3	0.85
	4	0.25			70.0 ± 1.4	0.86
		0.5			69.6 ± 1.1	0.86
		1			70.7 ± 0.8	0.85

***In vitro* blood distribution kinetic of [^{14}C]SP100 in human**

Human blood spiked at 250 ng/mL [^{14}C]SPP100 was incubated at 37°C for 0.5, 1 and 2 hours (triplicates).



- Irrespective of the species, [^{14}C]SPP100 was moderately bound to plasma proteins. The plasma free fraction or unbound fraction (F_u) was large (>> 5%) and amounted to:

Concentration range	Species	F_u [%]
10-500 ng/mL	Human 1	49.8 ± 1.7
	Human 2	47.3 ± 1.3
	Human 3	51.4 ± 2.4

Protein binding was species dependent (F_u: marmoset << mouse < rat ≈ rabbit < human) and concentration independent except in mouse (only slight concentration dependency) and even more concentration dependent in marmoset (factor 2 between F_u at the lowest and highest concentrations investigated). In those latter species F_u increased with increasing concentrations, but only at concentrations >500 ng/mL suggesting saturation of the plasma protein binding sites. This is in line with the observed increased cell uptake of [^{14}C]SPP100 at the highest concentrations investigated (5'000 and 10'000 ng/mL) in mice and marmosets.

In human, the fraction unbound in serum was slightly lower than that in plasma indicating anticoagulant effect on the protein binding of SPP100.

Overall, SPP100 with respect to blood/plasma distribution and extent of plasma protein binding behaves remarkably different in the marmoset when compared to all species investigated including human.

Conclusion:

SPP100 distributes in plasma more readily than blood at a ratio of about 0.81 to 0.86 (C_b/C_p). Protein binding is concentration independent and is about 50% in humans.

Reviewer Comments:

Reviewer concurs.

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Appendix III:
Individual Review of Clinical Pharmacokinetic Studies

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STUDY SPP100A 2205 – A RANDOMIZED, OPEN-LABEL, FOUR-TREATMENT, FOUR PERIOD, CROSSOVER STUDY TO EVALUATE THE DOSE PROPORTIONALITY OF ALISKIREN (75, 150, 300, 600 MG) CAPSULE IN HEALTHY SUBJECTS.

STUDY INVESTIGATOR AND SITE:

REPORT # 2205
VOLUME in EDR, Section 6
STUDY DATES: February 23 – March 22, 2004

Objectives:

Primary objective

- **To evaluate the dose proportionality of aliskiren 75, 150, 300 and 600 mg in healthy volunteers**

Secondary objective(s)

- **To monitor the safety and tolerability of oral dose administrations of aliskiren 75, 150, 300 and 600 mg in healthy volunteers**

FORMULATION:

Aliskiren	75 mg capsule, Novartis (Batch No: X238 0903; Re-test Date: June 2005)

Design: This was an open-label, single-dose, four- period, four-treatment, randomized crossover design with a 7-day washout between each period. A total of 32 healthy subjects were enrolled and 30 completed all study procedures and treatments.

Subjects had a minimum body weight of 50 kg and a BMI between 17-25 kg/m^2.

Subjects were screened during a 21-Day period and, if eligible, proceeded to a baseline visit prior to each treatment (four baseline evaluations in total). There was one end of study evaluation prior to discharge from the study site.

Subjects were randomized into 4 dosing sequence groups with 8 subjects per group. The subjects were admitted to the study center at least 12 hours prior to the initial dosing in each period for baseline evaluations, and were confined to the clinic at least 48h postdose in each period. Following an inter-dose interval of at least 7 days, each subject returned to the study site and was crossed over to receive the alternate treatment. All subjects received each of the following treatments once during the study according to a randomization schedule:

- Treatment A: Aliskiren 75 mg capsule, single dose
- Treatment B: Aliskiren 150 mg capsule, single dose (two, 75 mg capsules)
- Treatment C: Aliskiren 300 mg capsule, single dose (four, 75 mg capsules)
- Treatment D: Aliskiren 600 mg capsule, single dose (eight, 75 mg capsules)

Except for medication required to treat adverse events, no medication other than study drug was allowed from the initial day of screening until all of the final study evaluations had been completed. Administration of paracetamol was acceptable but had to be documented in the CRF. Decisions regarding replacements of subjects requiring concomitant medication had to be discussed with the sponsor on a case by case basis. In this study, no subjects were replaced for concomitant medications requirements.

Study medication was administered by the study center personnel with 200 mL of water between 0730 and 0900, after an overnight fast of at least 10-hours. All subjects were dosed within a maximum of a 1-hour interval. Subjects were instructed not to chew the medication, but to swallow it whole. The investigator or his/her designate checked each subject's mouth to ensure that the medication was swallowed.

The subjects were confined to the study center for at least 12 hours before administration of study drug until 48 hours thereafter.

Presence of a physician on site was required for at least the first 4 hours after dosing on all days of dosing.

During recruitment, informed consent review, and baseline period, the subjects had to observe the following restrictions:

- No strenuous physical exercise (e.g., weight training, aerobics, football) for 7 days before dosing until after the study completion evaluation.
- No alcohol for 72 hours before dosing until after the study completion evaluation.

All subjects had to fast for at least prior to the (first) dose of study medication and continue to fast for at least 2 hours thereafter. Lunch and dinner were served at ~1300 and 1730, respectively, and a large snack was served at 2100.

During waking hours, subjects were required to have a fluid intake every 4 hours in addition to fluid taken with the medication.

Intake of xanthine (e.g., caffeine) containing food or beverages had to be discontinued 48 hours before dosing. Consumption of these foods and beverages (i.e., coffee, tea, soda, chocolate) was not permitted at any time while the subjects were domiciled.

Plasma samples for determination of aliskiren were obtained for 96 h period after the dose in each treatment segment.

For all treatment periods, subjects fasted for a minimum of 10 h predose to 4 h postdose. Subjects were considered to have completed the study when all safety and pharmacokinetic evaluations had been completed.

ANALYTICAL METHODS:

Assay performance was adequate and validated. The LLOQ was 1 ng/mL.

PK SAMPLE COLLECTION, CALCULATIONS, AND STATISTICAL ANALYSIS:

At each time of SPP100 determination in plasma, 5 mL venous blood were to be drawn from a forearm vein into lithium heparin tubes at the following times: at pre-dose and 1, 2, 3, 4, 8, 12, 24, 36, 48, 72 and 96 h post dose.

Pharmacokinetic parameters

Pharmacokinetic parameters were determined using non-compartmental method(s) using WinNonlin Pro (Version 4.0).

AUC_{0-t}	The area under the concentration-time curve from time zero to t [amount x time x volume^{-1}]
$AUC_{0-\infty}$	The area under the plasma concentration-time curve from time zero to infinity [amount x time x volume^{-1}]
C_{max}	The maximum (peak) plasma, blood, serum, or other body fluid drug concentration after single dose administration [amount x volume^{-1}]
$t_{½}$	The elimination half-time associated with the terminal slope (λ_z) of a semilogarithmic concentration-time curve [time]. Use qualifier for other half-lives.
t_{max}	The time to reach peak or maximum concentration following drug administration [time]

Statistical Analysis

For assessing the dose proportionality of Aliskiren, the pharmacokinetic parameters $AUC_{(0\text{-}inf)}$, $AUC_{(0\text{-}t)}$, and C_{max} were analysed.

The following mixed effects model was applied to the logarithmic scale of the pharmacokinetic parameter:

$$\ln(PK_{ijk\iota}) = (\mu+S_\iota+\eta_{1i}) + \pi_j + \beta^* \ln(Dose_k) + \varepsilon_{ijk},$$

for the pharmacokinetic parameter on the *k*th dose (k= 1,..., 4) in the *j*th period (j= 1,...,4) for the *i*th subject (i= 1,....,32) associated with sequence ι (ι = 1,...,4) . The model contains random between-subject variability $\eta_{1i} \sim N(0,\xi^2)$ in the intercept parameter μ and random error $\varepsilon_{ijk} \sim N(0, \sigma^2)$.

The model above is a log-transformed version of a power model since of the form:

$$PK_{ijk} = \alpha * X_k^{\beta},$$

where α depends on sequence, period, random effect of subjects nested within sequence and error. The power β above is the parameter used to assess dose proportionality. In fact, dose proportionality occurs if and only if $\beta = 1$. A criteria based on the precision of the slope estimate as measured by the 90% confidence interval for it, is formulated as follows. Dose-proportionality across the entire dose range can be concluded, if the 90% confidence interval (β_L, β_U) for the slope β is completely contained within a pre-specified critical region (b_L, b_U). Otherwise, a smaller dose range where dose proportionality holds can be identified.

The slope β and the relative 90% confidence interval for β were estimated. Given the dose range ratio considered (highest dose/lowest dose = R_d), the critical region within which the 90% confidence interval for the slope needs to be contained in order to conclude dose proportionality can be derived as ($1+\ln 0.8/\ln R_d$, $1+\ln 1.25/\ln R_d$), where 0.8 and 1.25 are the standard bioequivalence limits. In this case, $R_d = 8$, the critical region for the 90% confidence interval for the slope in order to conclude dose-proportionality for a dose range of 8 was: (0.89, 1.11).

proportionality can be derived as ($1+\ln 0.8/\ln R_d$, $1+\ln 1.25/\ln R_d$), where 0.8 and 1.25 are the standard bioequivalence limits. In this case, $R_d = 8$, the critical region for the 90% confidence interval for the slope in order to conclude dose-proportionality for a dose range of 8 was: (0.89, 1.11).

RESULTS:

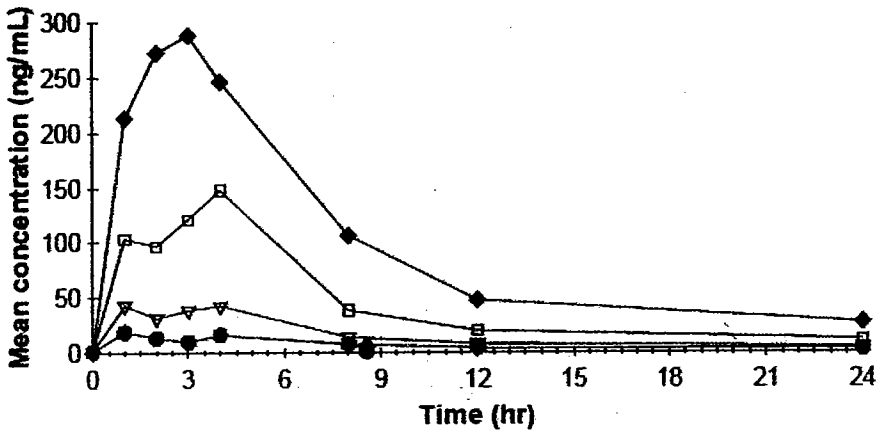
Plasma concentrations increased with increasing doses in a more than dose-proportional manner as illustrated below:

Mean (SD) pharmacokinetic parameters of single doses of Aliskiren to 30 subjects

Dose	T_{max}* (hr)	C_{max} (ng/ml)	AUC_{0-t} (ng·h/mL)	$AUC_{0-\infty}$ (ng·h/mL)	$T_{1/2}$ (hr)	CL/F (L/h)
75	1	26 (31)	266 (235)	356 (217)‡	54 (16)†	294 (178)†
150	2.5	72 (62)	530 (360)	627 (401)‡	41 (11)‡	321 (165)‡
300	3	202 (119)	1480 (806)	1620 (895)	37 (8)	254 (166)
600	2	420 (325)	3240 (1950)	3520 (2130)	34 (7)	222 (115)

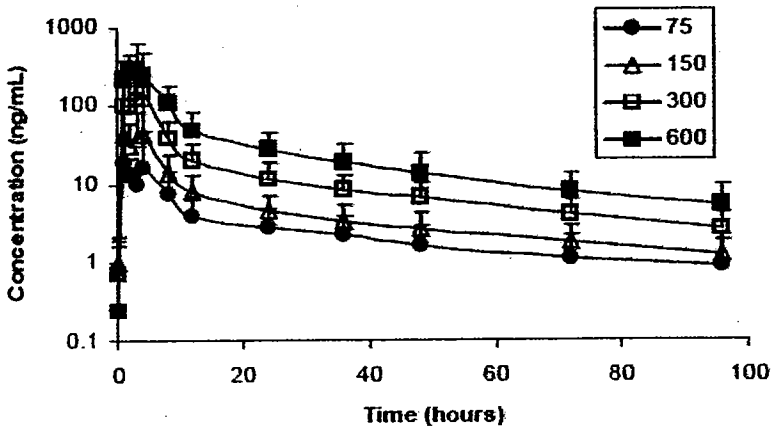
*Median, †n=23, ‡n=29

Linear mean plasma Aliskiren concentration profiles (n=30)



75 mg (•), 150 mg (▽), 300 mg (□) and 600 mg (■)

Log-transformed mean plasma Aliskiren concentration profiles (n=30)



The model predicted pharmacokinetic parameters for AUC and Cmax would increase 2.3 and 2.6 fold, respectively indicating a deviation from true dose-proportionality across the 8-fold range of 75 to 600 mg. Inter-subject variability was high for Cmax (59-117%) and AUC (55-64%).

Expected response in PK parameters when doubling the dose of aliskiren (SPP100) in healthy subjects (n=30)

PK parameter	Expected response when doubling the dose
C_{max} (ng/ml)	2.6 fold
AUC_{0-t} (ng·h/mL)	2.4 fold
$AUC_{0-\infty}$ (ng·h/mL)	2.25 fold

There were 7 subjects in the 75 mg dose group and 6 subjects in the 150 mg dose group who had residual concentrations 5-13% of Cmax. Therefore, the data was analyzed by including all 30 subjects as well as excluding only the subjects presenting carry-over above 5 % in that period. The overall results and conclusions did not alter significantly when the data was analyzed using either method. Therefore, the complete set of data (including all 30 subjects) was used for further statistical analysis.

SAFETY:

There were no serious adverse events.

All 32 subjects enrolled received at least 1 dose of study therapy and are included in the analysis of clinical adverse experiences.

	Subjects N=32
ENTERED:	32
Male (age range)	22 (18-45)
Female (age range)	10 (18-38)
COMPLETED STUDY:	30
DISCONTINUED STUDY:	2
Clinical adverse event	1
Deviation from protocol	1

CONCLUSIONS:

Aliskiren is more than dose-proportional when the dose is doubled. High-inter-subject variability was observed in Cmax and AUC. Some residual in Cmax was observed; but did not have an impact when adjusted for in the linear model used.

REVIEWER'S COMMENT:

1. The reviewer concurs and

STUDY (SPP100CRD16) SPP100A 0024 – ALISKIREN – A PHASE I, DOUBLE-BLIND, PLACEBO-CONTROLLED, ASCENDNG MULTIPLE-DOSE, SAFETY, TOLERABILITY AND PHARMACOKINETIC STUDY IN HEALTHY MALE SUBJECTS.

STUDY INVESTIGATOR AND SITE:

REPORT # (CRD16) 0024
VOLUME in EDR, Section 8
STUDY DATES: July 31 – October 22, 2001

Objectives:

Primary Objective
To determine the safety and tolerability of ascending multiple oral doses of Aliskiren in healthy male subjects.

Secondary Objective
To determine the multiple oral dose pharmacokinetics and pharmacodynamics (as measured by the renin-angiotensin system [RAS] parameters) of Aliskiren in healthy male subjects.

FORMULATION:

Aliskiren — powder (Batch No: SPP100B2001002; Re-test Date: June 2002) diluted in Bitrex® solution (1.5 mg/L, Batch # 26556), by Speedel

Placebo — Bitrex® solution (1.5 mg/L, Batch # 26556, Re-test Date: October 2003) by Macfarlan Smith Ltd., Edinburgh, UK

This was a double-blind, placebo-controlled, ascending multiple oral dose, sequential group study.

It was initially planned to study three dose levels of Aliskiren of 900, 1200 and 1800 mg. At the lowest dose level, however, the displacement volume of Aliskiren during dose preparation was not accounted for (detailed in Section 3.8) and the dose level studied was 850 mg, rather than 900 mg. The 1200 and 1800 mg dose levels were studied as planned.

Three separate groups of eight subjects (Groups A, B and C) were studied in ascending order of dose. There was an interval of at least 6 days between groups, to allow a satisfactory review of the safety and pharmacokinetic data from the lower dose levels prior to progression to the next higher dose levels.

Group	Planned treatment	Actual treatment	Subject numbers
A	900 mg Aliskiren/placebo	850 mg Aliskiren/placebo	1 to 8
B	1200 mg Aliskiren/placebo	1200 mg Aliskiren/placebo	9 to 16
C	1800 mg Aliskiren/placebo	1800 mg Aliskiren/placebo	17 to 24

The treatment randomisation was produced by the using a computer-generated pseudo-randomisation procedure. On acceptance into the study, subjects in each group were sequentially assigned to receive either Aliskiren or placebo.

In each study group, six subjects received Aliskiren and two subjects received placebo. All doses of Aliskiren and placebo were administered in the fasted state. It was planned that each subject would receive nine doses of Aliskiren or placebo during the study: a single dose administration on the morning of Day 1 and once daily dosing on the mornings of Days 4 to 11, inclusive.

Each dose (850, 1200 or 1800 mg Aliskiren, or placebo) was administered orally as a 100 mL solution. The dose container was then rinsed twice with 30 mL of water and the subject drank the rinsing solutions. All treatments were given in the fasted state. Following dosing, each subject was offered a mint to suck, to remove any lingering bitter taste.
Subjects were instructed to adhere to the following restrictions and requirements during the study:

Xanthines and Grapefruit
Consumption of caffeine-containing food and beverages was not allowed for 48 h prior to the first dosing occasion until discharge from the CRU on Day 14.

Grapefruit and grapefruit juice were not be allowed for 7days prior to the first dosing occasion until discharge from the CRU on Day 14.

Alcohol
Subjects were to adhere to the following alcohol restrictions during the study:

- No alcohol permitted for 48 h prior to the screening visit
- No alcohol permitted from 48 h before the start of dosing until discharge on Day 14
- Up to 2 units of alcohol per day were allowed from discharge on Day 14 to 48 h prior to the post-study visit
- No alcohol was permitted during the 48 h period prior to the post-study visit

Diet:

Time	Food and fluid details
Evening prior to first dose	Evening meal
From 22:00 on day prior to dosing	Fast from food and fluids except water
From 1 h before dosing	No fluids allowed including water (Days 1 and 11 only)
At dosing	Dose solution administered
From 2 h post-dose	Free access to water (Days 1 and 11 only)
Approximately 4.5 h post-dose*	Lunch
Approximately 7.5 h post-dose	Afternoon snack
Approximately 11 h post-dose	Evening meal
After 24 h post-dose	Breakfast

Screening was performed in the 3-week period prior to dosing. Each subject participated in one treatment period only and remained resident in the CRU from approximately 16:00 on Day–1 (the day before the first dose) until Day 14 (72 h post-dose on Day 11). Dosing occurred on Day 1 and Days 4 to 11, for each subject.

ANALYTICAL METHODS:

The plasma and urine samples were analysed for Aliskiren using a solid phase radioimmunoassay (RIA) method.

The lower limit of quantification (LLOQ) of Aliskiren in plasma was 0.1358 ng/mL. The inter-assay precision of quality control samples analysed throughout the study was 12.1% at 0.2715 ng/mL, 10.1% at 1.1313 ng/mL, 11.7% at 2.2625 ng/mL, 14.6% at 135.75 ng/mL, 10.2% at 362 ng/mL and 10.6% at 905 ng/mL. Inter-assay accuracy varied between 92.3 and 105.3%.

In urine, the LLOQ of Aliskiren was 113.125 ng/mL. The inter-assay precision of quality control samples analysed throughout the study was 9.4% at 226.25 ng/mL, 8.2% at 452.5 ng/mL, 14.3% at 905 ng/mL, 8.4% at 4525 ng/mL and 17.5% at 13575 ng/mL. Inter-assay accuracy varied between 98.9 and 114.3%.

PK SAMPLE COLLECTION, CALCULATIONS, AND STATISTICAL ANALYSIS:

Pharmacokinetics:			
Blood sampling for Aliskiren			Days 1 and 11: Pre-dose, 0.5, 1, 3, 6, 10, 24, 30, 36, 48 and 72 h post-dose Days 5 and 7: Pre-dose
Urine collection for Aliskiren			Days 1 and 11: Pre-dose (spot sample) and 0 to 6, 6 to 12 and 12 to 24 h post-dose
Pharmacodynamics:			
Blood sampling for active renin and plasma renin activity			Day 1: Pre-dose Day 11: Pre-dose, 0.5, 1, 3, 6, 10 and 24 h post-dose

Pharmacokinetic parameters

Pharmacokinetic parameters were determined from the plasma and urine concentrations of Aliskiren using non-compartmental procedures. The pharmacokinetic parameters determined are presented in the following table:

Parameter	Definition
$AUC(0\text{-}t_z)$	Area under the plasma drug concentration-time curve from time zero up to the last quantifiable concentration on Day 1
$AUC(0\text{-}\tau)$	Area under the plasma drug concentration-time curve over a dosing interval on Days 1 and 11
$AUC(0\text{-}\infty)$	Area under the plasma drug concentration-time curve from zero time to infinity on Day 1
%AUCex	Percentage of $AUC(0\text{-}\infty)$ extrapolated from the time of the last quantifiable plasma drug concentration on Day 1
C_{max}	Maximum measured plasma drug concentration on Days 1 and 11
t_{max}	Time of the first occurrence of C_{max} on Days 1 and 11
t_z	Time of occurrence of the last plasma drug concentration above the lower limit of quantification
C_{min}	Plasma concentration at the end of the dosing interval on Days 1 and 11
λ_z	Terminal elimination rate constant on Days 1 and 11
$t_{½}$	Terminal elimination half-life on Days 1 and 11
RA_1	Accumulation ratio based on $AUC(0\text{-}\tau)$
RA_2	Accumulation ratio based on C_{max}
RL	Linearity ratio
MRT	Mean residence time
Ae	Amount of unchanged drug excreted in the urine
fe	Fraction of administered dose excreted in the urine as unchanged drug

$\tau = 24$ h

In addition, dose and body weight normalised (norm) values for AUC(0-t_z), AUC(0-∞) and C_{max}.

For the calculation of the pharmacokinetic parameters and dose normalisation, the dose level of Aliskiren (administered as the salt form) was converted to free base equivalents, using the correction factor of 75/82.88.

Statistical Analysis

Data for all subjects who entered the study are presented in the data listings and are included in all summary tables and figures. For Subject 19 (1800 mg Aliskiren), who received his last dose on Day 7, plasma concentrations of Aliskiren are listed and included in the summaries and pharmacokinetic parameters on Day 1 for this subject are included in the statistical analyses.

The following pharmacokinetic parameters of Aliskiren were subjected to statistical analysis:

AUC(0-t_z)
AUC(0-τ)
AUC(0-∞)
C_{max}
C_{min}
t_{max}
$t_{½}$
MRT
Ae(0-τ)

As the variance for all pharmacokinetic parameters (except t_{max}) increases as the mean increases, these parameters were log-transformed (base e)[3] prior to analysis.

AUC(0-τ), C_{max}, C_{min}, $t_{½}$, MRT and Ae(0-τ) values derived following the first dose on Day 1 were compared to the values obtained on Day 11 using a mixed ANOVA model[4] of the form:

RESPONSE	=	TREATMENT
	+	SUBJECT
	+	DAY
	+	TREATMENT x DAY
	+	RANDOM ERROR

In the above model, the term 'Subject' was fitted as a random effect and the terms 'Treatment', 'Day' and the 'Treatment by Day Interaction' were fitted as fixed effects.

A comparison of AUC(0-τ) on Day 11 and AUC(0-∞) on Day 1 was also performed for each dose level using the above model.

For each dose level of Aliskiren, mean differences between Day 11 and Day 1 were calculated. The residual variance from the model was used to calculate 95% confidence intervals (CI) for the differences between Day 11 and Day 1. These values were

backtransformed to give point estimates and 95% CI for the ratios of Day 11 relative to Day 1.

Residual plots were produced to assess the distribution of the data and the adequacy of the model. There was no evidence within the plots to suggest significant departures from normality, and therefore, an additional analysis was not required.

Pharmacokinetic parameters were analysed by analysis of variance (ANOVA) or nonparametric methods. Geometric least squares means or medians, ratios or median differences, and 95% confidence intervals (CI) were calculated for the comparisons between Days 11 and 1. Slopes, 95% CI and P-values are presented for the assessment of dose proportionality.

Pharmacodynamic parameters were analysed by ANOVA or nonparametric methods. Least squares means or medians, differences or median differences, and 95% CI were calculated for the comparisons between each dose level of Aliskiren and placebo.

RESULTS:

Twenty-four subjects were recruited and entered the study. Twenty-three subjects completed the study in accordance with the protocol, protocol amendments and the treatment randomisation. Subject 19 (1800 mg Aliskiren) was withdrawn from the study on Day 8 (received his last dose on Day 7), due to drug-related adverse events.

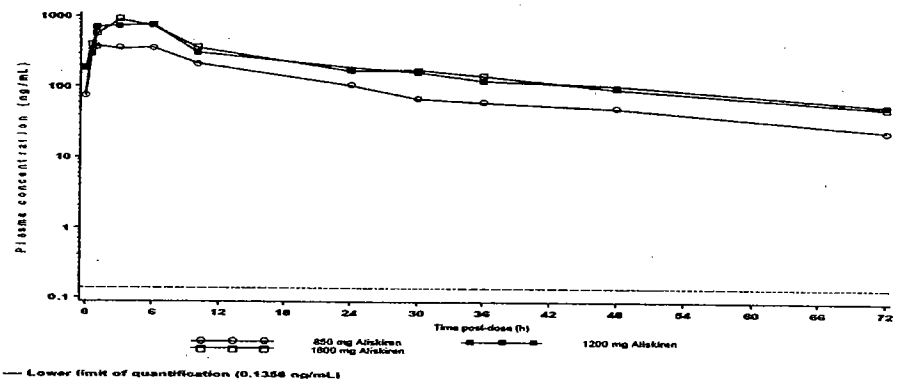
Pharmacokinetic Results:

	Dose of Aliskiren					
	850 mg		1200 mg		1800 mg	
Parameter	Day 1 (N=6)	Day 11 (N=6)	Day 1 (N=6)	Day 11 (N=6)	Day 1 (N=6)	Day 11 (N=5#)
$AUC(0-t_z)$ (ng.h/mL)	2141 (25.2)	NC	4397 (43.3)	NC	5561 (78.9)	NC
$AUC(0-\tau)$ (ng.h/mL)	1580 (26.2)	5609 (50.9)	3368 (47.0)	9576 (66.7)	4245 (77.4)	10625 (28.5)
$AUC(0-\infty)$ (ng.h/mL)	2523 (30.6)	NC	4765 (42.7)	NC	6037 (79.8)	NC
C_{max} (ng/mL)	207 (57.3)	652 (35.9)	357 (68.2)	931 (67.6)	543 (41.7)	1053 (41.0)
t_{max} † (h)	3.00 (1.00-6.00)	3.50 (0.500-6.00)	3.00 (0.500-6.00)	3.00 (1.00-6.00)	0.750 (0.500-10.0)	3.07 (1.00-6.00)
C_{min} (ng/mL)	19.2 (32.6)	106 (60.1)	42.2 (39.9)	188 (78.7)	53.0 (86.9)	170 (25.9)
$AUC(0-t_z)$ (norm)	211 (16.9)	NC	300 (35.9)	NC	238 (94.3)	NC
$AUC(0-\tau)$ (norm)	156 (18.8)	553 (42.4)	230 (39.2)	653 (57.2)	181 (94.0)	441 (36.9)
$AUC(0-\infty)$ (norm)	249 (24.2)	NC	325 (35.1)	NC	258 (94.6)	NC
C_{max} (norm)	20.4 (55.1)	64.3 (34.0)	24.3 (58.4)	63.4 (58.9)	23.2 (57.7)	43.7 (43.1)
C_{min} (norm)	1.89 (27.7)	10.4 (53.9)	2.88 (34.6)	12.6 (71.0)	2.27 (107)	7.07 (35.2)
$t_{\frac{1}{2}}$ (h)	32.3 (64.2)	30.0 (68.4)	24.1 (15.1)	31.2 (27.2)	23.9 (31.8)	27.1 (28.6)
MRT (h)	31.6 (54.6)	26.1 (34.2)	23.0 (12.3)	28.6 (13.6)	22.7 (28.9)	25.6 (9.19)
RA_1	1.60 (14.8)[a]	3.55 (50.2)	1.41 (5.62)[a]	2.84 (39.2)	1.44 (10.2)[a]	2.84 (45.2)
RA_2		3.16 (93.4)		2.61 (14.7)		2.02 (55.0)
RL		2.22 (49.1)		2.01 (39.0)		1.97 (48.3)

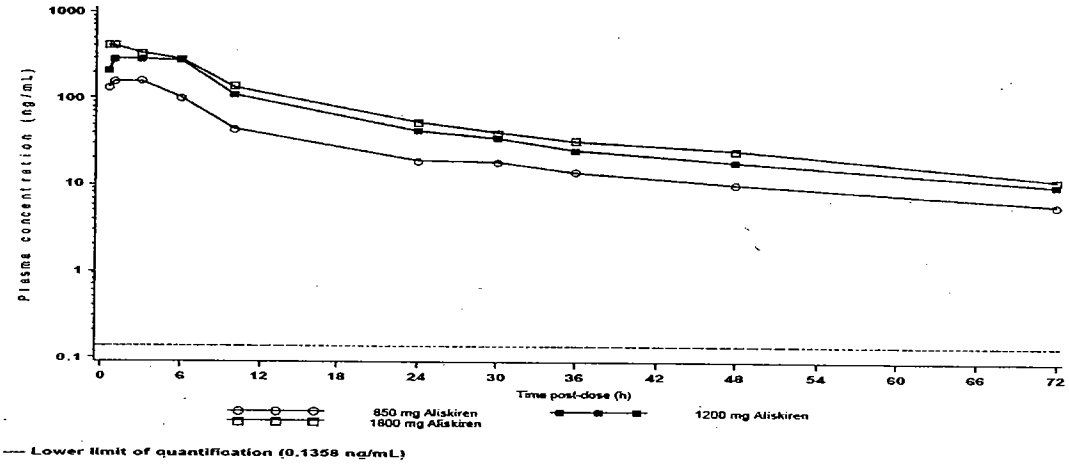
Source: Section 10.3 (Table 3)
Geometric mean (CV%) data are presented
† Median (min-max)
N= Number of subjects studied
# Subject 19 withdrawn from study (received his last dose on Day 7)
(norm) = Normalised for dose and body weight (mg/kg)
NC = Not calculated
$\tau = 24$ h
[a] = predicted value

Half-life values ranged from 17.0 to 86.3 hours across all dose levels. The highest intra-subject variability was observed with a C_{max} of 40%.

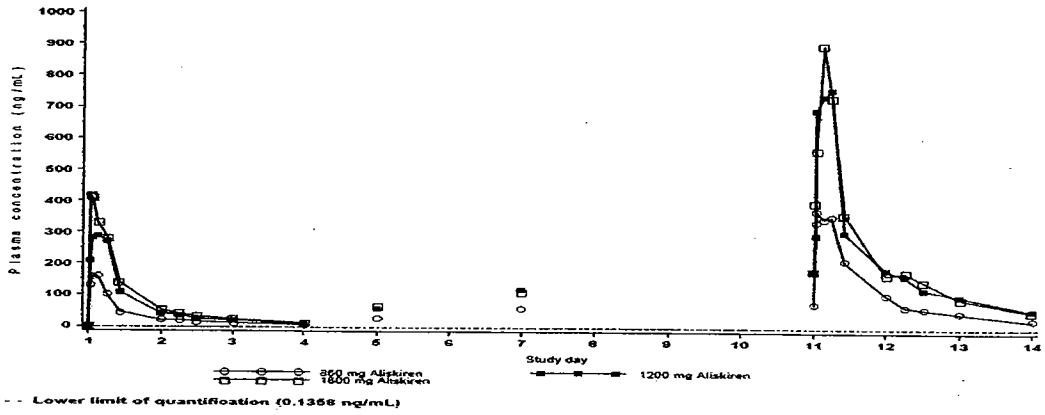
Geometric Mean Plasma Concentrations of Aliskiren on Day 1 (semi-logarithmic scale)



Geometric Mean Plasma Concentrations of Aliskiren on Day 11 (semi-logarithmic scale)



Geometric Mean Plasma Concentrations of Aliskiren Following Multiple Oral Doses (linear scale) (Days 1 to 14)



The total amount of unchanged drug eliminated in the urine was low at all dose levels on Day 1 (0.3 to 0.4%) to Day 11 (0.9 to 1.3% and rapid with the greatest amount eliminated over the first collection period of 0 to 6 hours post-dose.

A summary of the urinary excretion of Aliskiren is presented in the following table:

	Dose of Aliskiren					
	850 mg		1200 mg		1800 mg	
Parameter	Day 1 (N=6)	Day 11 (N=6)	Day 1 (N=6)	Day 11 (N=6)	Day 1 (N=6)	Day 11 (N=5#)
Ae(0-τ) (μg)	2300 (21.5)	6688 (44.3)	4576 (44.0)	14037 (52.0)	5199 (46.9)	19376 (44.9)
fe(0-τ) (%)	0.299 (21.5)	0.870 (44.3)	0.421 (44.0)	1.29 (52.0)	0.319 (46.9)	1.19 (44.9)

Geometric mean (CV%) data are presented
N = Number of subjects studied
# = Subject 19 withdrawn from study
τ = 24 h

Pharmacodynamic results:
A summary of the pharmacodynamic parameters of active renin on Day 11 is presented in the following table:

		Dose of Aliskiren		
Parameter	Placebo (N=6)	850 mg (N=6)	1200 mg (N=6)	1800 mg (N=5#)
AUC_{AR}(0-24 h) (pg.h/mL)	133 (31.2)	20743 (70.3)	24857 (84.3)	47495 (66.1)
AR_{max} (pg/mL)	9.30 (48.0)	1862 (76.1)	1883 (68.1)	3512 (66.4)
tAR_{max}† (h)	6.00 (6.00-24.0)	6.00 (6.00-6.00)	6.00 (6.00-6.00)	6.00 (6.00-6.00)

Arithmetic mean (CV%) data are presented
† Median (min-max)
N= Number of subjects studied
# = Subject 19 withdrawn from study

Statistical analysis of the pharmacodynamic parameters of active renin confirmed the AR_{max} values to be significantly higher for all doses of Aliskiren, compared to placebo. For AUC_{AR}(0-24 h), statistical analysis confirmed the values were significantly greater than placebo at the 1200 and 1800 mg dose levels, however, the increase in AUC_{AR}(0-24 h) following the 850 mg dose, did not achieve statistical significance.

A summary of the pharmacodynamic parameters of PRA on Day 11 is presented in the following table:

		Dose of Aliskiren		
	Placebo (N=6)	850 mg (N=6)	1200 mg (N=6)	1800 mg (N=5#)
AUC_{PRA}(0-24 h) (ng/mL)	15.7 (42.1)	8.29 (41.1)	6.53 (70.0)	4.66 (23.4)
PRA_{min} (ng/mL/h)	0.303 (54.8)	0.0650 (55.7)	0.0800 (186)	0.108 (43.1)
$tPRA_{min}$† (h)	0.500 (0.500-3.00)	0.500 (0-3.00)	3.00 (0-10.0)	1.00 (0.500-1.00)

Arithmetic mean (CV%) data are presented
† Median (min-max)
N= Number of subjects studied
# = Subject 19 withdrawn from study
* Spurious values, possibly due to stress-related samples

For the placebo treatment, PRA was higher at all time-points post-dose compared to the three doses of Aliskiren, with maximal activity being observed at 6 h post-dose. The AUC for PRA over the 24-h period post-dose generally decreased in a dose-related manner. The greatest decreases in PRA occurred from 0.5 to 3 h post-dose and were similar across all dose levels of Aliskiren. The inter-subject variability in AUC (0-24 h), as assessed from the CV%, was high at the 1200 mg dose level (up to 70%), compared to being low to moderate at the 850 and 1800 mg doses levels (18 to 42%).

Statistical analysis confirmed the visual assessments of the PRA data, with AUC_{PRA}(0-24 h) and the PRA_{min} being significantly lower for all doses of Aliskiren compared to placebo.

SAFETY:

No serious or severe adverse events were experienced during the study.

Aliskiren was well tolerated when administered as multiple oral doses at 850 mg. The tolerability, however, decreased at the 1200 and 1800 mg dose levels, due to the onset of diarrhoea and an increase in the number and severity of adverse events of gastrointestinal (GI) origin. At the 1200 mg dose level, Aliskiren was moderately well tolerated with three subjects experiencing diarrhoea. At the 1800 mg dose level, however, Aliskiren was poorly tolerated with four subjects experiencing diarrhoea and one subject (Subject 19) being withdrawn after 4 days of multiple dosing due to diarrhoea and abdominal pain. Further frequently reported, GI-related adverse events included abdominal pain, nausea and loose stools.

Note: Subject 19 had a comparatively high systemic exposure to aliskiren on Day 1.

Other drug-related adverse events occurring in more than one subject included headache and dizziness; both were reported following all treatments, including placebo.

At the highest dose level of 1800 mg, the overall increase in the frequency, duration and severity of the GI-related adverse events were clear, dose-limiting factors. Additionally, with increasing dose of Aliskiren, the adverse events occurred earlier in the dosing period, such that at the 1800 mg Aliskiren dose, approximately 34% of the drug-related adverse events had an onset after the first day of multiple dosing (Day 4).

CONCLUSIONS:

- Multiple oral doses of Aliskiren, administered once daily for 8 days, were safe and well tolerated at the 850 mg dose level, moderately well tolerated at the 1200 mg dose level and poorly tolerated at the 1800 mg dose level. The increase in adverse events at the 1200 and 1800 mg dose levels were of gastrointestinal origin, most notably diarrhoea, abdominal pain, nausea and loose stools.
- Aliskiren was rapidly absorbed with maximum plasma concentrations occurring at a median t_{max} of 0.75 to 3.5 h post-dose for all doses. Following C_{max}, plasma concentrations declined in a biphasic manner.
- The mean apparent elimination half-life of Aliskiren was estimated to be between 24 and 32 h, although this was longer in some subjects.
- Steady-state plasma concentrations of Aliskiren were achieved by Day 11, 7 days after the start of multiple dosing. There was a 2.0 to 3.6-fold accumulation of Aliskiren in plasma following multiple dosing at all dose levels, which was slightly greater than predicted.

- **Exposure to Aliskiren appeared to be dose-proportional over the dose range 850 to 1800 mg Aliskiren on Days 1 and 11. The inter and intra-subject variability in systemic exposure over all dose levels was high (53% for pooled inter-subject variability and 31 to 40% for pooled intra-subject variability).**
- **Urinary excretion of Aliskiren was very low (up to 1.3% of the dose).**
- **Aliskiren caused a marked increase in active renin and inhibition of PRA, these effects being maximal at approximately 6 h and 0.5 to 3 h post-dose on Day 11, respectively. The increase in active renin and decrease in PRA appeared to be dose-related. The effects of Aliskiren on both these parameters were long-lasting (greater than 24 h post-dose).**

REVIEWER'S COMMENT:

1. The drug clearly accumulates with repeated dosing. Some subjects individual pharmacokinetic parameters increased by 10 fold in C_{max} (subject# 8 at 850 mg with a 6 fold increase in corresponding T_{max} and over a 7 fold increase in $AUC_{0-\tau}$ on day 11), and an 11 fold in C_{min} (subject# 9 at 1200 mg) even after weight normalization.
2. Half-life for subject#1 increased from 16.8 to 86.3 hours (850 mg group). However, the sampling time was over less than 2 half-lives for this subject as well as most other subjects.
3. When assessing individual plasma trough concentrations from day 5, 7 and 11, none of the subjects had the same trough concentrations on day 5, 7 or 11. Assessment of steady-state between days 7 and 11 is not possible since trough concentrations were missing for days 8 through 10. In addition, one can not assume that 3 to 5 half-lives would put subjects in a steady-state condition with data from this study since sampling was not taken out beyond the 72 hour time-point. Additional drug accumulation may take place beyond a 7-day dosing interval.

APPEARS THIS WAY
ON ORIGINAL

STUDY SPP100 02 MD – TOLERABILITY, PHARMACOKINETICS AND PHARMACODYNAMIC EFFECTS OF SPP100 IN HEALTHY VOLUNTEERS UPON REPEATED ONCE-A-DAY ADMINISTRATION OF 40, 80, 160, OR 640 MG AS AN ORAL SOLUTION FOR 8 DAYS.

STUDY INVESTIGATOR AND SITE:

REPORT # 02 MD
EDR VOLUME 8
STUDY DATES: February 15 – May 16, 1999

Objectives:

- To determine the safety and tolerability of multiple oral doses of 40, 80, 160 and 640 mg of SPP 100 administered once a day as an oral solution for 8 days.
- To determine the pharmacodynamic effects (effects on RAS and on blood pressure) of SPP 100 following multiple oral doses of 40, 80, 160 and 640 mg SPP 100 administered once a day as an oral solution for 8 days.
- To determine the pharmacokinetics of SPP 100 at steady state with multiple oral doses of 40, 80, 160 and 640 mg SPP 100 administered once a day as an oral solution for 8 days.

Formulation:

Aliskiren by Speedel

- SPP 100: Placebo - bottles containing an appropriate amount of lactose to match the respective dose level of SPP 100
  Batch No. SPP 100 – PIB – 010
- SPP 100: bottles containing 40 mg of dry active ingredient
  (36.2 mg free base, powder-in-bottle, P.I.B.)
  Batch No. SPP 100 – PIB – 020
- SPP 100: bottles containing 80 mg of dry active ingredient
  (72.4 mg free base, powder-in-bottle, P.I.B.)
  Batch No. SPP 100 – PIB – 030
- SPP 100: bottles containing 160 mg of dry active ingredient
  (144.8 mg free base, powder-in-bottle, P.I.B.)
  Batch No. SPP 100 – PIB – 040
- SPP 100: bottles containing 640 mg of dry active ingredient
  (579.1 mg free base, powder-in-bottle, P.I.B.)
  Batch No. SPP 100 – PIB – 050

The solvent solution was freshly prepared on each study day by dissolving 1.5 mg of Bitrex in 1 L of water. Adding approximately 50 ml of the Bitrex solution dissolved the content of each SPP 100 or placebo bottle. Then, the necessary volume of Bitrex solution was added to obtain a final volume of 100 ml.

Enalapril	Reniten® 5 mg tablets (Charge # HH07590, Exp. Date: 12-2000) supplier information not given
Placebo	Lactose (Charge # SPP100-PIB-010) by Speedel

Study Design:

The study employed a placebo- and active-controlled, randomised, parallel-group, crossover design. The study was double blinded with respect to allocation of placebo or active drug in each dose group, but open and sequential with respect to dose escalation for safety reasons and the application of enalapril. All doses of Aliskiren are expressed as the hemifumarate salt SPP 100B.

A total of 18 male subjects in two cohorts of nine volunteers were included into the study. In each group of nine volunteers, 6 subjects received Aliskiren and placebo; 3 subjects received Aliskiren and enalapril. The volunteers were allocated to one of two treatment groups as shown in table below (Subjects 1-9: 40 and 80 mg Aliskiren; subjects 10-18: 160 and 640 mg Aliskiren). The two dose levels of Aliskiren were tested in a sequential and ascending way (low before high). Safety and tolerability were evaluated before proceeding to the next higher dose level.

Salt intake, which can affect renin production, was controlled throughout the study for three days prior to and during the eight days of drug administration in each period.

Day	-3	-2	-1	1	2	3	4	5	6	7	8	9	10
Diet (100 mmol Na^+)	X	X	X	X	X	X	X	X	X	X	X	X	X
Aliskiren				X	X	X	X	X	X	X	X	X	X
PK Sampling				X							X	X	X
PD Sampling				X							X		

Due to the sensitivity of the RAS to posture and meal intake, subjects were fasting at the time of drug intake on Days 1 and 8 and received their first meal after the 6-hour blood sample. On Days 2 to 7 all subjects received the daily morning dose of study drug before breakfast. All subjects took all meals at the hospital cafeteria three days prior to Day 1 of each session and throughout the session (Days 1 to 10). All meals were prepared by the hospital dietician to meet the 100 mmol salt diet requirement.

The placebo or enalapril active control treatments were randomised across the three administration sessions, the Aliskiren dose was always given as the lower dose before the higher dose as follows. The randomisation code is shown in the appendices. Subjects were randomised sequentially at screening.

Treatment Group 1A	Aliskiren 40 mg and 80 mg, Placebo
Treatment Group 1B	Aliskiren 40 mg and 80 mg, 20 mg Enalapril
Treatment Group 2A	Aliskiren 160 mg and 640 mg, Placebo
Treatment Group 2B	Aliskiren 160 mg and 640 mg, 20 mg Enalapril

Enalapril was chosen as a proven inhibitor of ACE at a dosage (20 mg) known to clearly inhibit ACE and affect plasma concentrations of ANG I and ANG II.

Male Caucasian subjects were from 18-45 years of age and in good health as determined by past medical history, physical examination, electrocardiogram, laboratory tests and urinalysis.

Criteria for evaluation:

Efficacy: Plasma concentrations of Active Renin (AR), Plasma Renin Activity (PRA), Angiotensin I and II concentrations (ANG I, ANG II), plasma concentrations of Aliskiren

ANALYTICAL METHODS:

The quantitative determination of Aliskiren in plasma was performed by RIA, with a limit of quantification (LOQ) of approximately 0.05 ng/ml (Lit 4).

PK SAMPLE COLLECTION, CALCULATIONS, AND STATISTICAL ANALYSIS:

Blood samples were drawn at the following times. Subjects were fasted at the time of drug ingestion on Days 1 and 8; remaining fasted until after the 6-hour blood sample.

Days 1 to 2: pre-dose on Day 1 (=blank), 0.5, 1, 3, 6, 10, and 24 h post-dose.

Days 3, and 5: pre-dose (=trough)

Days 8 to 10: pre-dose on day8 (=trough), 0.5, 1, 3, 6, 10, 24, 32, and 48 h post-dose (=end-of-study).

Pharmacokinetic parameters

The following pharmacokinetic parameters were determined using model independent methods:

C_{max}:	Highest observed Aliskiren plasma concentration (ng/ml).
t_{max}:	Sampling time (h) corresponding to C_{max}.
$AUC_{(0-24)}$:	Area under the plasma concentration-time curve (ng/ml*h), calculated using the trapezoidal rule over the sampling period 0-24 h.
$t_{½}$:	Apparent terminal elimination half-life (h) determined from the slope of the regression straight line that described the terminal log-linear segment of the plasma concentration-time curve.
$A_{e(0-24)}$	Total amount of Aliskiren excreted in urine, calculated over the sampling time period 0-24 h expressed as μg/24 hours and percentage of dose.

Pharmacodynamic assessments

Plasma renin activity and ANG II were measured by the method of Nussberger (Lit. 1, 2). Aldosterone in urine and plasma were measured by microassay (Lit. 5, 6).

RESULTS:

Pharmacokinetics:

The rise in plasma concentrations was dose dependent. Maximal plasma concentrations were reached between 1 and 6 hours. The pharmacokinetic parameters are estimates because only 7 to 9 blood samples were taken. Aliskiren was detectable in plasma up to 48 hours following the final dose on Day 8.

Pharmacokinetic Parameters

Dose (mg)	C_{max} (ng/ml)	t_{max} (h)	AUC (ng/ml*h)
		Single Dose (Day 1)	
40	5.1 ± 2.1	1.6 ± 1.8	28.5 ± 13.5
80	11.0 ± 3.6	2.8 ± 2.5	96.4 ± 31.6
160	21.5 ± 9.6	2.7 ± 2.1	214.8 ± 73.5
640	146.2 ± 61.6	2.1 ± 1.1	1193.4 ± 588.6
		Multiple Dose (Day 8)	
40	6.2 ± 2.9	2.1 ± 3.1	46.7 ± 29.2
80	12.7 ± 4.0	1.8 ± 2.4	136.9 ± 54.0
160	39.1 ± 24.6	4.1 ± 2.4	362.7 ± 146.5
640	335.1 ± 196.2	3.2 ± 1.8	2972.5 ± 1263.2

Aliskiren was measured in urine over 24 hours of Day 1 and 8; single vs. multiple dosing, respectively. At the highest dose of 640 mg of Aliskiren, only 0.3% ± 0.2% and 1.1% ± 0.6% of the dose administered were excreted into urine as unchanged compound.

Pharmacodynamics:

Aliskiren inhibited plasma renin activity in a dose-dependent manner; both the extent and duration of inhibition being clearly dose dependent. PRA was significantly inhibited for up to 24 hours following the 80-mg administration of Aliskiren. Maximal inhibition of PRA was reached within one hour of administration. More importantly, the inhibition of PRA was not attenuated after 8 days of dosing. Similar results were observed in the other dosage groups.

Dose[1] (mg)	PRA (ng/ml/h)	Active Renin (pg/ml)	ANG II (fmol/ml)
		AUC (0-24 h) - Single Dose (Day 1) Mean ± SD	
0	27.0 ± 11.9	505.7 ± 570.1	119.5 ± 52.0
Enalapril	119.4 ± 101.8	2167.2 ± 1398.4	29.8 ± 26.7
40	23.1 ± 10.5	486.1 ± 168.3	76.7 ± 37.3
80	14.7 ± 4.3	919.0 ± 329.6	48.6 ± 17.2
160	11.6 ± 10.2	1896.2 ± 1325.5	63.9 ± 63.5
640	6.5 ± 5.0	4183.1 ± 2490.5	29.8 ± 26.5
Dose[1] (mg)	PRA (pg/ml/h)	Active Renin (pg/ml)	ANG II (fmol/ml)
		AUC (0-24 h) - Multiple Dose (Day 8)	
0	25.3 ± 9.6	353.1 ± 262.4	101.7 ± 54.8
Enalapril	245.4 ± 112.4	4843.4 ± 2549.5	47.3 ± 19.9
40	18.4 ± 5.9	628.5 ± 244.8	72.8 ± 23.8
80	13.7 ± 5.4	1308.3 ± 448.3	61.8 ± 36.3
160	10.5 ± 6.1	3089.1 ± 2359.2	80.6 ± 62.2
640	7.2 ± 3.7	11870.0 ± 2991.3	49.3 ± 17.6

There was an inverse relationship between PRA and plasma concentrations of Aliskiren.

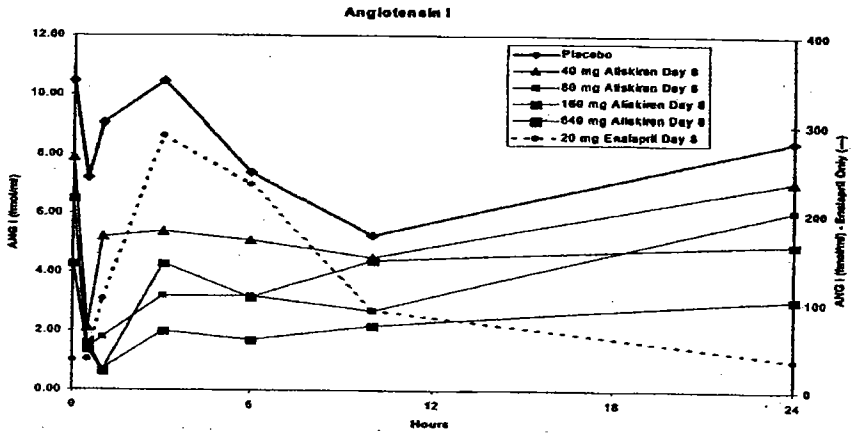
The baseline activity of PRA on Day 8 (prior to the last dose) was slightly decreased over that measured on Day 1 (before the first dose). The baseline PRA was lower on Day 8 of treatment compared to pre-dose levels on Day 1 for all dosages.

The time course of inhibition of PRA correlated well with that of the plasma concentrations of Aliskiren. As the doses of Aliskiren increased, not only did the maximal inhibition increase, but also the duration of inhibition, approaching 24 hours at the higher doses. Total inhibition of PRA was not significantly different between the 160 and 640 mg doses as measured by the total AUC of activity. All doses of Aliskiren suppressed PRA in the first 6 hours following dosing. Doses of 80 mg and above inhibited PRA for up to 24 hours after dosing on Day 1.

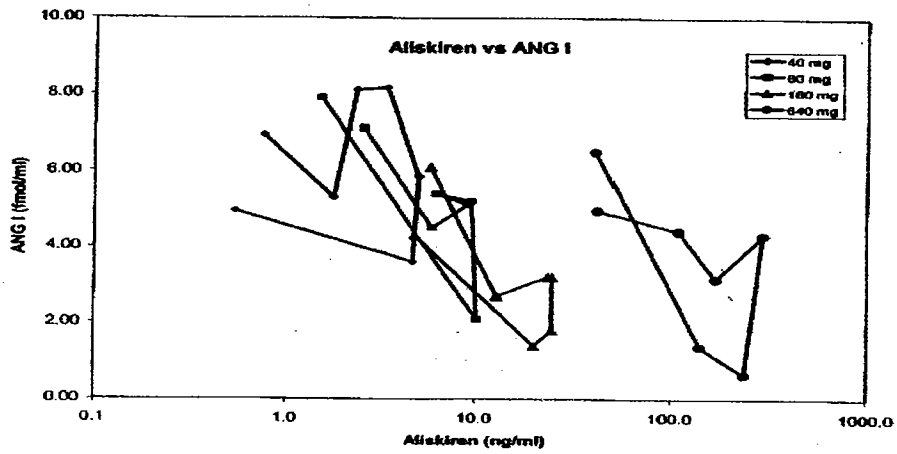
On Day 8, Aliskiren at doses of 160 and 640 mg also decreased the baseline activity of PRA as evidenced by the decrease in PRA at time 0 (trough activity).

Angiotensin I in plasma (Day 8) at all doses

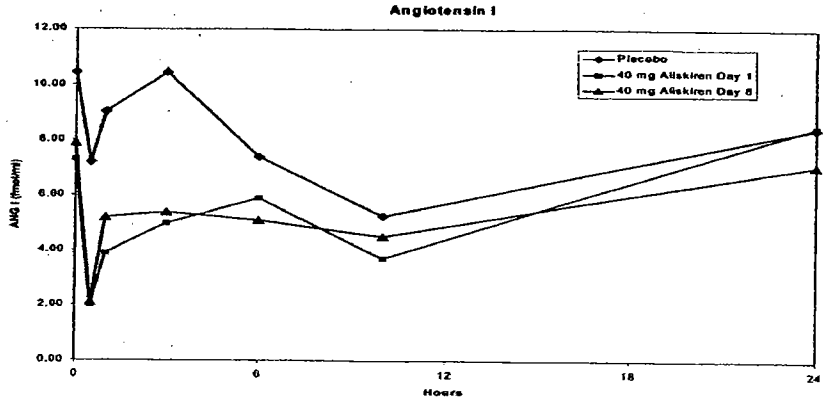
Please note that the curve for enalapril uses the right y-axis due to the high concentrations measured.



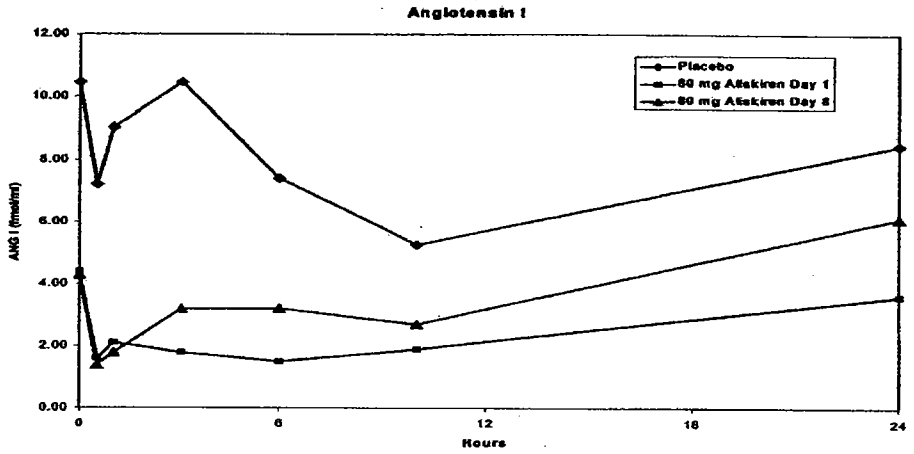
Aliskiren vs ANG I – All Doses



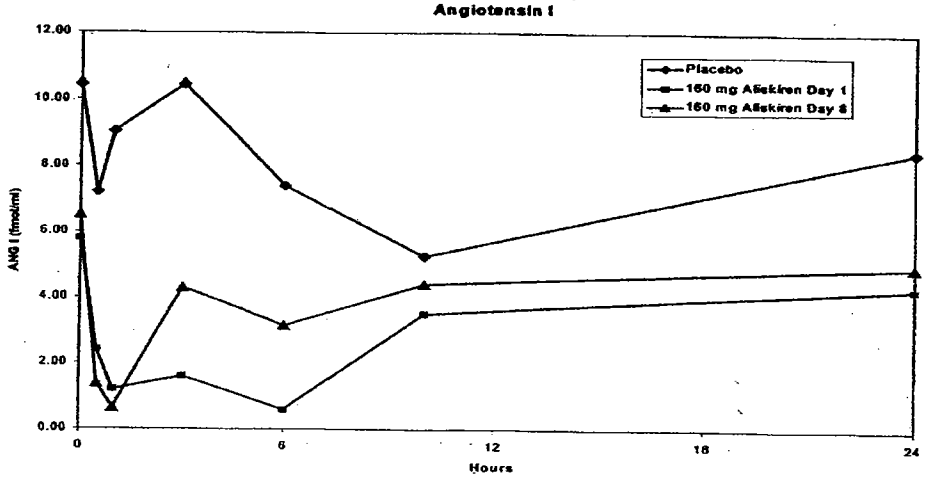
Angiotensin I in plasma (day 1 and 8) 40 mg of Aliskiren



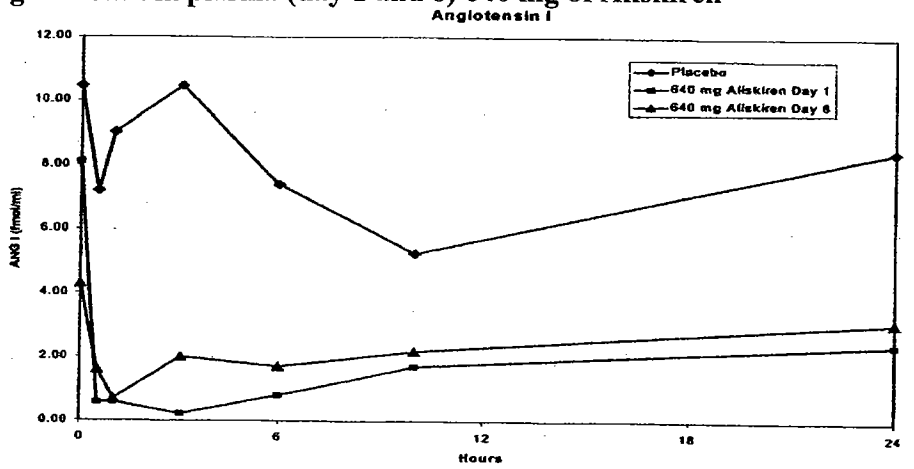
Angiotensin I in plasma (day 1 and 8) 80 mg of Aliskiren



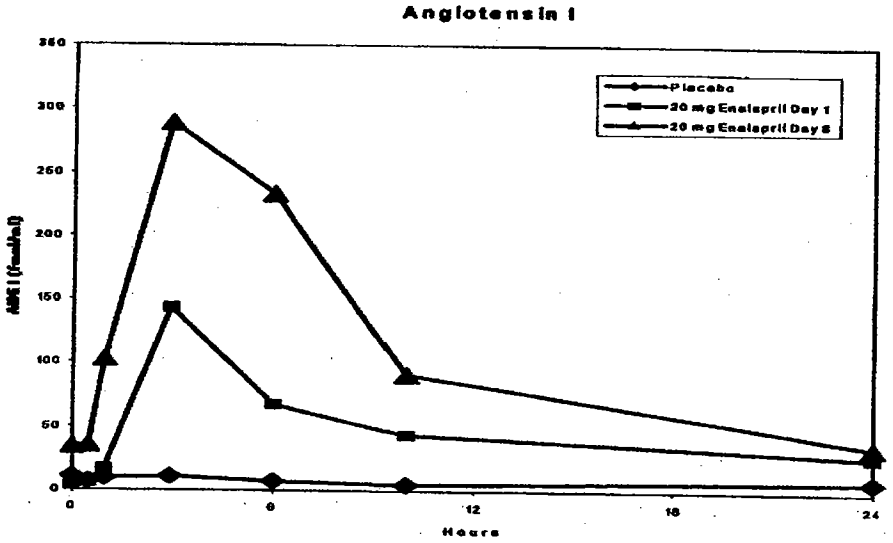
Angiotensin I in plasma (day 1 and 8) 160 mg of Aliskiren



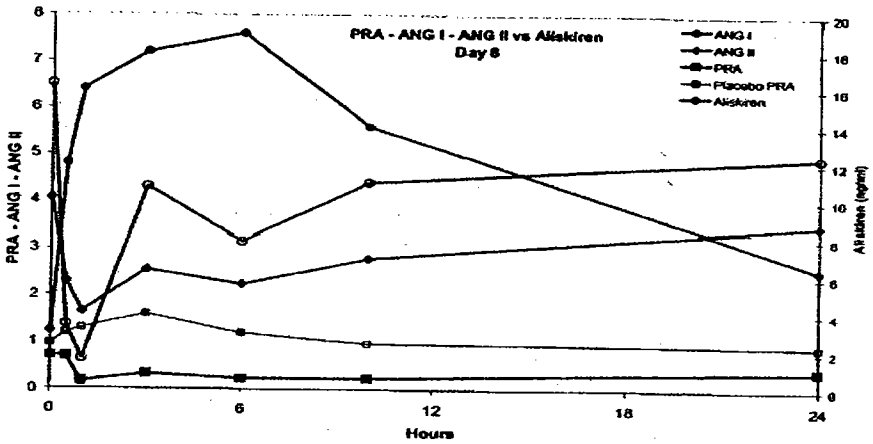
Angiotensin I in plasma (day 1 and 8) 640 mg of Aliskiren



Angiotensin I in plasma (day 1 and 8) 20 mg of Enalapril



Comparison of PRA, ANG I and II versus Plasma Aliskiren Following 160 mg of Aliskiren on Day 8



Units ANGI (fmol/ml)
ANGII (fmol/ml)
PRA (ng/ml/h)

Supine and Sitting Blood Pressure and Heart Rate Following Placebo, Enalapril 20 mg, and Aliskiren 40, 80, 160 and 640 mg on Day 1

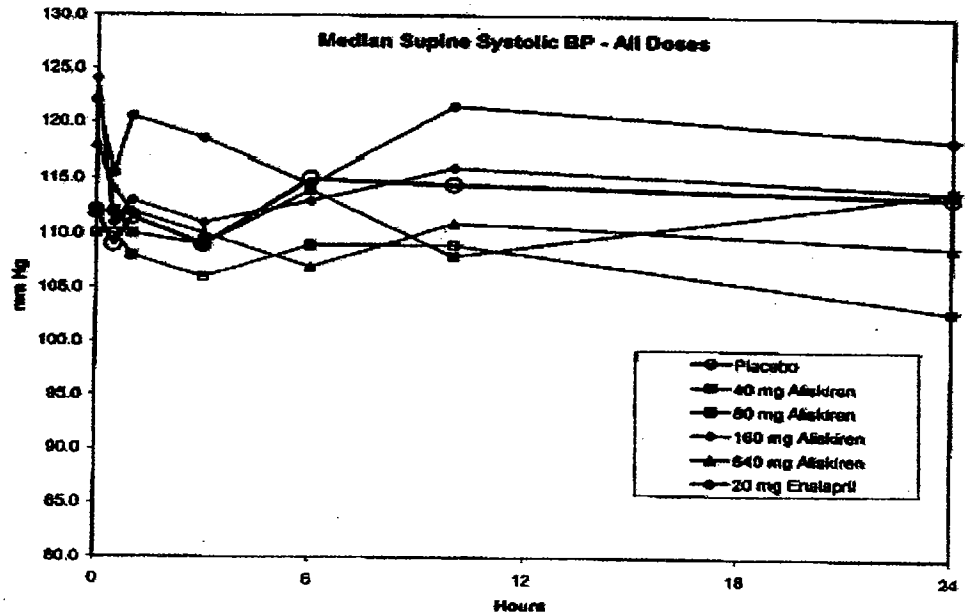
Dose Time	Supine Systolic BP (mm Hg)	Supine Diastolic BP (mm Hg)	Sitting Systolic BP (mm Hg)	Sitting Diastolic BP (mm Hg)	Supine HR (bpm)	Sitting HR (bpm)
Placebo						
0 hours	113.2 ± 12.6	64.5 ± 8.4	123.8 ± 8.7	77.4 ± 5.2	70.3 ± 19.0	71.5 ± 16.9
3 hours	114.9 ± 9.2	64.8 ± 7.3	116.1 ± 8.7	73.0 ± 7.1	54.6 ± 8.1	77.4 ± 13.6
Enalapril						
20 mg 0 hours	111.0 ± 5.5	63.3 ± 3.2	117.8 ± 3.2	73.3 ± 7.9	61.5 ± 13.7	66.5 ± 5.2
20 mg 3 hours	100.7 ± 8.3	56.5 ± 3.5	103.0 ± 8.3	67.5 ± 5.9	54.8 ± 8.7	75.2 ± 18.1
Aliskiren						
40 mg 0 hours	115.1 ± 8.7	67.1 ± 6.3	121.4 ± 9.7	75.9 ± 4.4	57.8 ± 7.6	76.6 ± 13.9
40 mg 3 hours	109.0 ± 3.9	60.3 ± 6.1	106.0 ± 7.4	66.7 ± 8.2	64.9 ± 17.6	77.6 ± 15.9
80 mg 0 hours	111.1 ± 4.0	66.1 ± 6.0	116.4 ± 5.9	70.4 ± 5.2	54.9 ± 9.9	71.2 ± 19.5
80 mg 3 hours	106.1 ± 6.9	58.1 ± 6.9	106.0 ± 9.6	66.0 ± 7.9	53.9 ± 9.2	77.6 ± 22.5
160 mg 0 hours	121.7 ± 10.5	70.7 ± 4.7	116.1 ± 16.8	71.8 ± 10.0	75.2 ± 12.6	66.4 ± 16.8
160 mg 3 hours	114.9 ± 10.3	65.0 ± 5.1	106.4 ± 16.1	78.3 ± 17.3	66.8 ± 22.6	75.2 ± 12.6
640 mg 0 hours	115.8 ± 16.2	68.4 ± 12.1	118.4 ± 6.9	73.4 ± 3.4	70.2 ± 15.2	60.0 ± 11.1
640 mg 3 hours	111.6 ± 7.8	63.6 ± 4.3	112.2 ± 11.6	72.1 ± 6.3	56.9 ± 8	81.0 ± 14.2

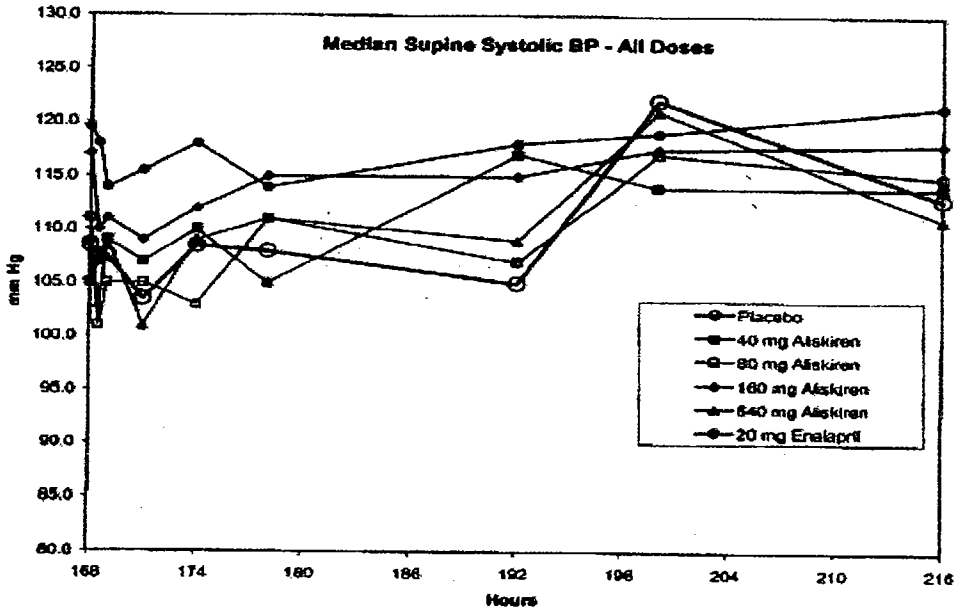
Supine and Sitting Blood Pressure and Heart Rate Following Placebo, Enalapril 20 mg, and Aliskiren 40, 80, 160 and 640 mg on Day 8

Dose/Time	Supine Systolic BP (mm Hg)	Supine Diastolic BP (mm Hg)	Sitting Systolic BP (mm Hg)	Sitting Diastolic BP (mm Hg)	Supine HR (bpm)	Sitting HR (bpm)
Placebo						
0 hours	116.4 ± 8.7	71.4 ± 7.9	116.8 ± 7.8	71.4 ± 7.8	67.4 ± 15.1	69.0 ± 14.3
3 hours	110.8 ± 8.5	65.0 ± 5.1	115.5 ± 12.7	73.5 ± 6.8	54.3 ± 7.7	75.7 ± 14.1
Enalapril						
20 mg 0 hours	107.0 ± 9.1	63.7 ± 3.4	109.5 ± 11.3	73.3 ± 4.7	62.5 ± 20.6	67.2 ± 6.3
20 mg 3 hours	101.3 ± 6.4	57.5 ± 5.1	105.0 ± 9.5	65.3 ± 5.6	53.3 ± 7.9	80.3 ± 16.2
Aliskiren						
40 mg 0 hours	111.0 ± 9.3	64.3 ± 9.0	110.5 ± 7.9	73.6 ± 4.7	63.1 ± 17.6	70.1 ± 15.6
40 mg 3 hours	106.2 ± 7.1	61.8 ± 5.0	104.8 ± 4.6	67.6 ± 4.7	55.1 ± 9.7	76.7 ± 12.2
80 mg 0 hours	114.0 ± 7.2	67.9 ± 7.6	113.1 ± 6.1	70.3 ± 7.0	62.4 ± 21.1	69.7 ± 14.5
80 mg 3 hours	105.3 ± 10.2	56.8 ± 9.2	99.8 ± 6.4	64.4 ± 6.5	53.8 ± 10.9	77.9 ± 13.4
160 mg 0 hours	117.3 ± 10.5	75.8 ± 6.7	116.8 ± 5.4	71.7 ± 5.4	74.3 ± 15.0	70.2 ± 13.4
160 mg 3 hours	108.4 ± 9.4	63.6 ± 6.1	113.1 ± 11.4	72.4 ± 6.7	57.6 ± 10.8	80.6 ± 13.2
640 mg 0 hours	106.9 ± 9.6	64.1 ± 6.0	114.1 ± 9.6	69.5 ± 6.4	71.8 ± 14.8	61.6 ± 7.3
640 mg 3 hours	102.7 ± 7.2	60.4 ± 4.8	108.2 ± 7.4	70.1 ± 7.3	57.1 8.7	80.8 ± 10.4

Median Supine Systolic Blood Pressure – All Doses

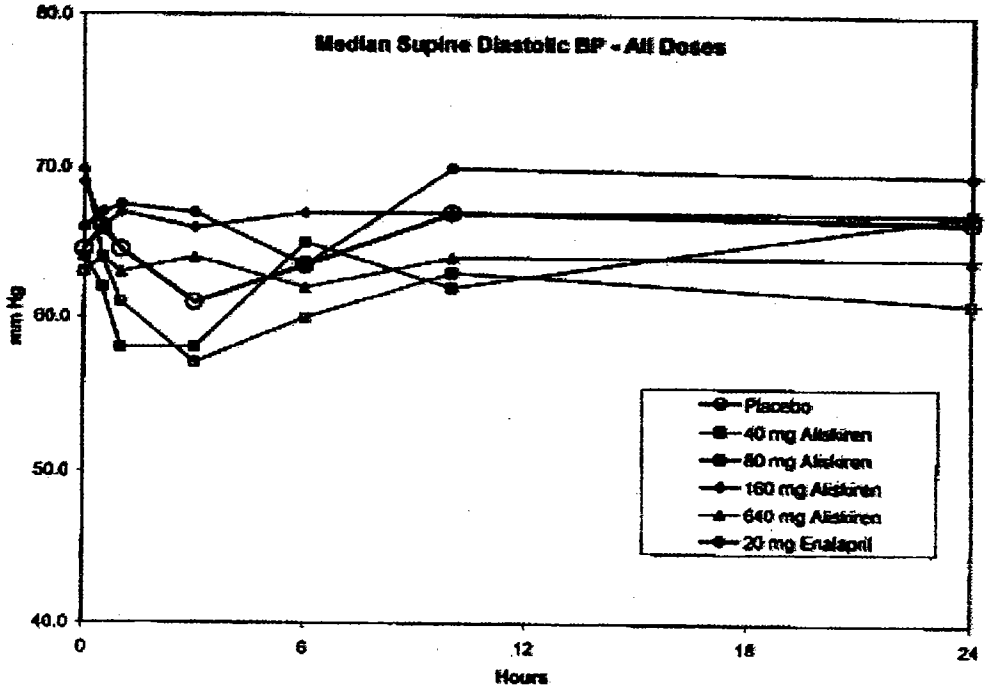
Day 1 top, Day 8 bottom

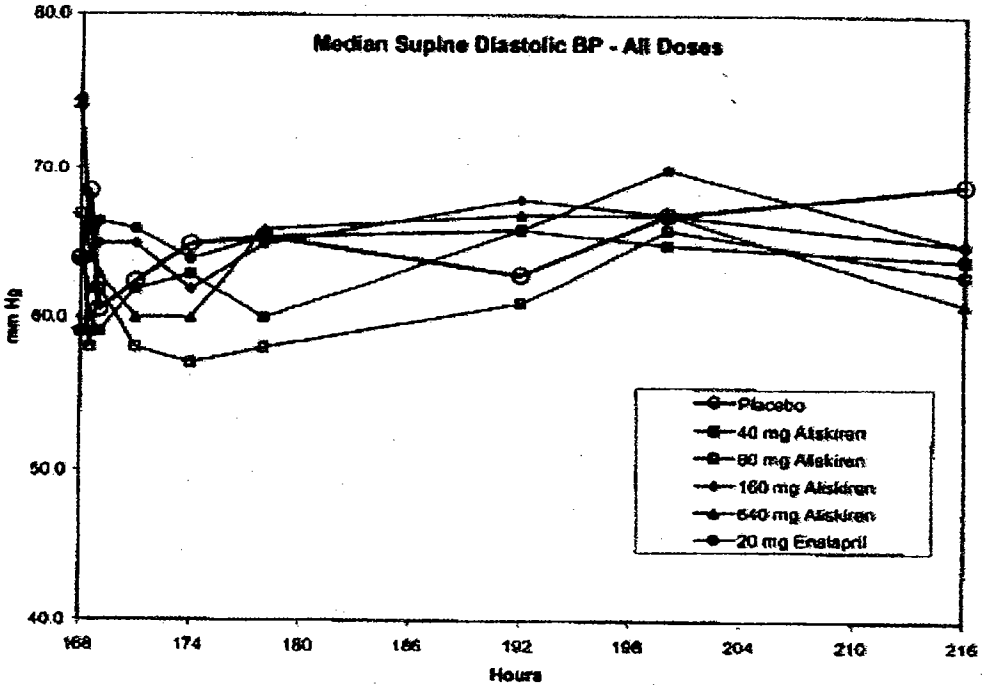




Median Supine Diastolic Blood Pressure – All Doses

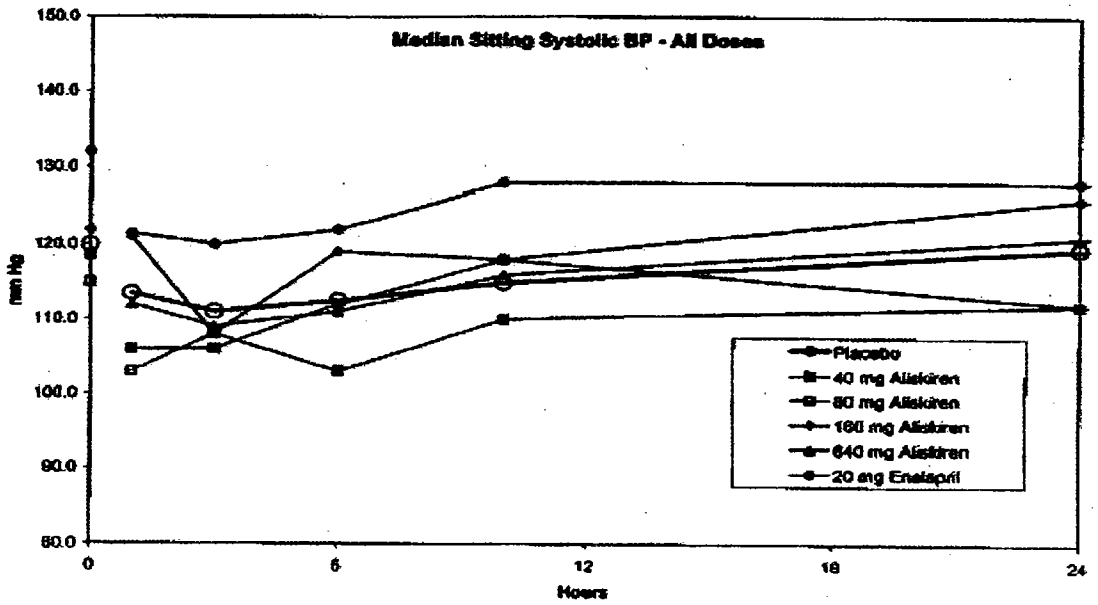
Day 1 top, Day 8 bottom

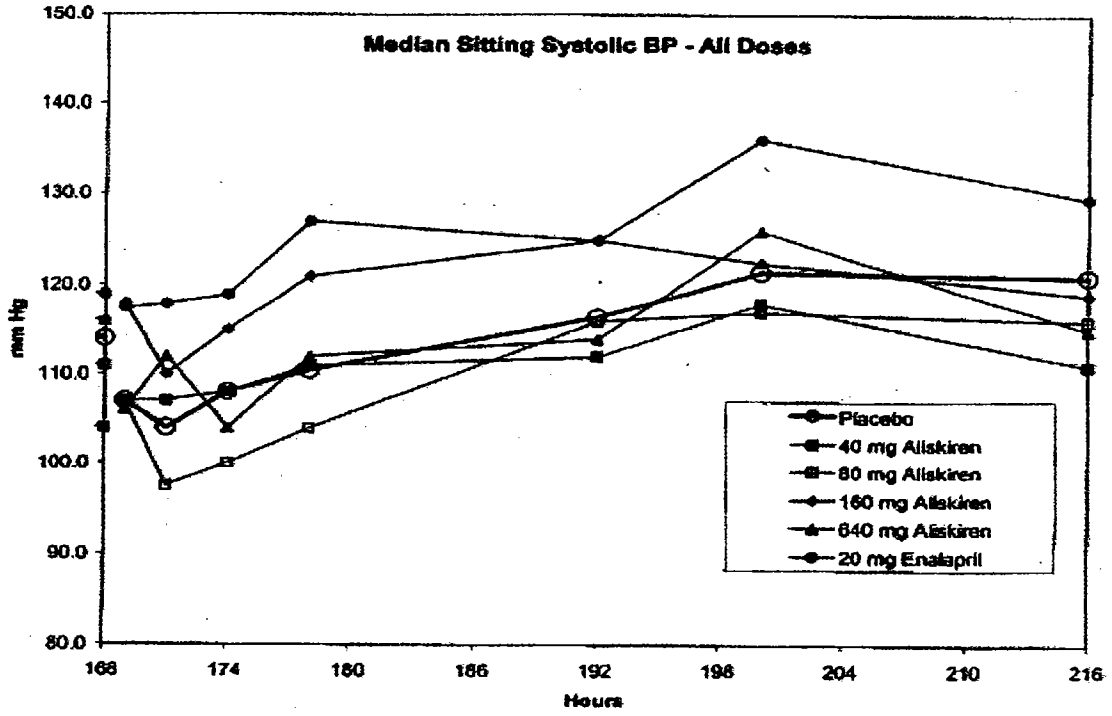




Median Sitting Systolic Blood Pressure – All Doses

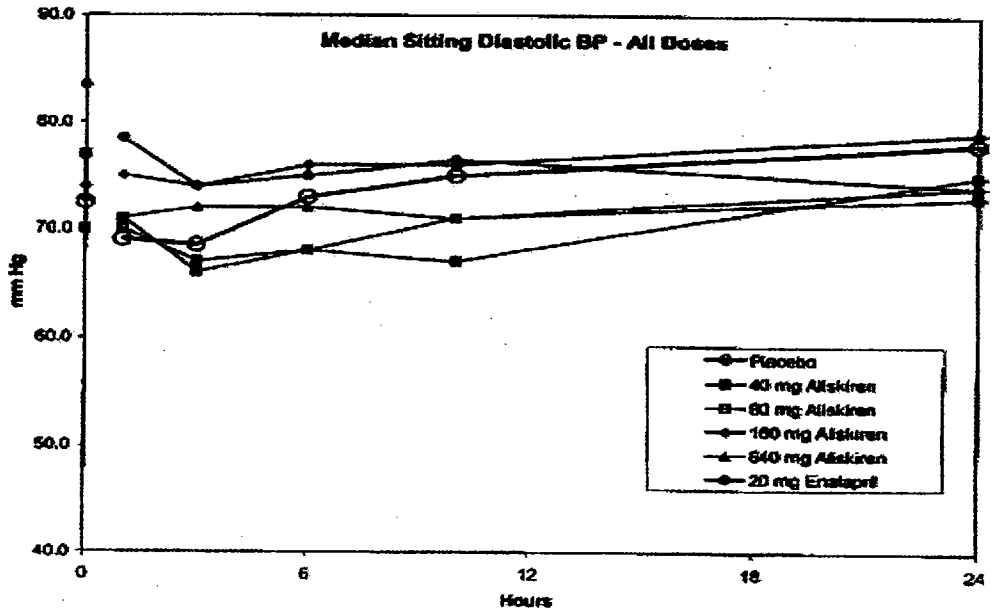
Day 1 top, Day 8 bottom

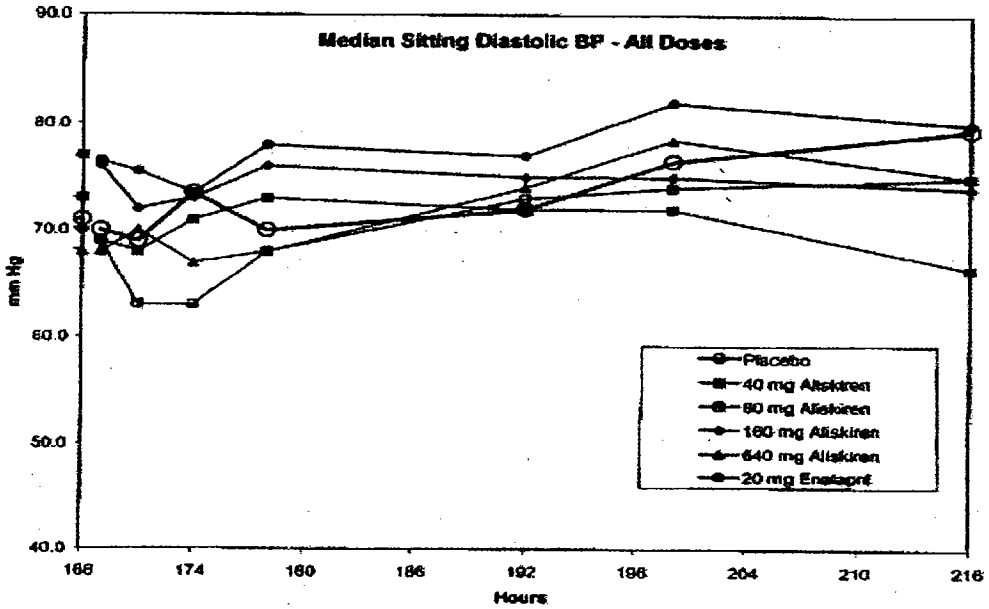




Median Sitting Diastolic Blood Pressure – All Doses

Day 1 top, Day 8 bottom





None of the changes in blood pressure were of statistical significance.

SAFETY:

No serious adverse events were reported throughout the study.

CONCLUSIONS:

Aliskiren is well tolerated after oral administration at doses up to 640 mg over eight days,

Aliskiren inhibits the RAS to an extent comparable to or better than enalapril (20mg)

Aliskiren should be orally effective in lowering blood pressure at doses ≥ 80 mg with the present simple, oral solution formulation

Further improvements in the galenical formulation are likely to decrease the daily dose requirements of Aliskiren in the treatment of hypertension

The near maximal effect of Aliskiren appears to be near 160 mg.

Aliskiren should be effective as an oral, once-a-day treatment regimen for hypertension.

REVIEWER'S COMMENT:

1. Clearly aliskiren is less than dose proportional with increasing doses. Exposures increase about 1.4 to 1.6-fold when doubling the dose.
2. The drug accumulates with repeated dosing as well by about 1.6-fold at dose from 40 to 160 mg and 2.5-fold from 160 to 640 mg.
3. No correlation was made between blood pressure and PD effects. Blood pressure versus concentration of Aliskiren was relatively flat in this study.

APPEARS THIS WAY
ON ORIGINAL

STUDY SPP100A 2202 – AN OPEN-LABEL, PARALLEL-GROUP STUDY TO CHARACTERIZE THE PHARMACOKINETICS AND PHARMACODYNAMICS OF SINGLE AND MULTIPLE DOSES OF SPP100 (ALISKIREN) BETWEEN HEALTHY MALE JAPANESE AND CAUCASIAN SUBJECTS.

STUDY INVESTIGATOR AND SITE:

REPORT # 2202
VOLUMES in EDR, Section 6
STUDY DATES: August 4 – October 10, 2003

OBJECTIVES:

Primary Objective was

- To compare the single and multiple dose pharmacokinetics of SPP100 between healthy male Japanese and Caucasian subjects.

Secondary Objectives were

- To compare preliminary single and multiple dose pharmacodynamics of SPP100 between healthy male Japanese and Caucasian subjects.
- To evaluate safety and tolerability after single and multiple oral doses of SPP100 in healthy male Japanese and Caucasian subjects.

FORMULATION:

SPP100	Aliskiren 300 mg capsules (Batch No: X270 1002; Retest Date: September 31, 2003) by Novartis

STUDY DESIGN:

Design: This was an open label, non-randomized, single center, parallel group single and multiple dose study in healthy male subjects.

Two parallel groups, one Japanese (n = 19), the other Caucasian (n = 19), whose age (± 5 years) and weight matched (± 25%), were enrolled in this study.

Each subject participated in a 21-day screening period, a baseline period (Day -1), a 10 day treatment period (Days 1-10) and a study completion evaluation 4-7 days after the last PK sample.

Each subject received a single 300 mg oral dose of SPP100 (Aliskiren) on Day 1 with 72h blood sampling, followed by multiple (once a day for 7 days) 300 mg oral doses of SPP100 on Days 4 through to 10. All doses of SPP100 were given following a 10 hour fast.

Subjects were domiciled in the study center from Day –1 (baseline) until the 24h post last-dose evaluations were completed on Day 11.

Note: Amendment made to the protocol indicated that subjects were domiciled on day -2 instead of day -1.

A late breakfast was served after the 4 hour post-dose fast, lunch and dinner were served at ~14:30 and ~19:00, respectively, and a large snack was served at ~21:00.

Upon admission to the study centre all subjects commenced a normal sodium diet. All subjects continued to consume a normal sodium diet during the domicile period. Sodium content of meals was not to vary between the two groups.

Duration of treatment: 8 Days (one single dose and 7 days of multiple dosing)

Subjects who drop out at any time after the enrollment were to be replaced by another healthy subject of the same ethnic group from which the subject dropped out. Replacement subjects were also to comply with the age and weight matching requirements of the study.

Criteria for inclusion: Healthy, non- or light-smoking male subjects between 20 and 45 years of age having provided a written informed consent before entering the study. At screening, the subjects must had been in good health as determined by past medical history, physical examination, electrocardiogram, laboratory tests and urinalysis. Subjects must have a minimum body weight of 50 kg and a BMI between 19-29 kg/m^2. Japanese and Caucasian subjects were aged (± 5 years) and weight matched (± 25%).

Japanese subjects were defined as having both parents of Japanese origin and citizenship, as being born in Japan and as living out of Japan for 10 years or less. Caucasian subjects were defined as having both parents of Caucasian origin.

During recruitment, informed consent review, and baseline period, the subjects had to observe the following restrictions:

- No strenuous physical exercise (e.g., weight training, aerobics, football) for 7 days before the first dose in each phase until after the end of study evaluation.
- No alcohol for 72 hours before the first dose in each phase until after the end of study evaluation.
- No grapefruit or grapefruit juice for 72 hours before the first dose in each phase until after the end of study evaluation.
- Intake of xanthine (e.g., caffeine) containing food or beverages must be discontinued 48 hours before the first dose in each period. Consumption of such foods and beverages (i.e., coffee, tea, soda, chocolate) is not permitted at any time while the subjects are domiciled. If a deviation occurs during the domicile period, it must be noted as appropriate in the CRF as a comment.

ANALYTICAL METHODS:

Plasma Aliskiren concentrations:

Analytical Method: by HPLC/MS/MS method with a coefficient of determination > 0.994 and a LLOQ of about 0.5 ng/mL. Within-study assay validation was performed and seems acceptable.

Plasma Active and Total Rennin:

Analytical method: Plasma active and total rennin was measured by immunoradiometric (sandwich technique) assay with a pair of anti-rennin monoclonal antibodies, using a commercially available kit

according to manufacturer's instructions. The first monoclonal specifically recognizes both active and inactive (pro-rennin) forms of rennin (i.e. total rennin). The second monoclonal specifically recognizes the active form only.

Plasma Rennin Activity:

Analytical methodology: Plasma rennin activity (PRA) was measured by an indirect radioimmunoassay in a commercially available kit

according to manufacturer's instructions.

Note: Amendment to the protocol indicates that PRA was measured by an alternative RIA-based method.

Plasma Aldosterone A:
Analytical method: Plasma aldosterone was measured by radioimmunoassay using 125I-aldosterone as a tracer

Plasma Angiotensin I:
Analytical methodology: Plasma angiotensin I was measured by enzyme immunoassay (with chromatographic extraction) in a commercially available kit (Angiotensin I (human) EIA, according to manufacturer's instructions.

Plasma Angiotensin II:
Analytical methodology: Plasma angiotensin II was measured by radioimmunoassay (with extraction) in a commercially available kit (Angiotensin II RIA, according to manufacturer's instructions.

PK Sample Collection/Calculations, PD Assessments/Calculations and Statistical Analysis:

Day 1:	predose and at 0.5. 1, 2, 4, 6, 8, 12, 24, 48 and 72 h postdose
Days 5, 6, 7, 8 and 9:	predose
Day 10:	predose and at 0.5, 1, 2, 4, 6, 8, 12 and 24 h postdose

Pharmacokinetics: Blood samples were collected to determine the pharmacokinetics following a single oral dose of SPP100 300 mg (for 72 hours post-dose) and multiple doses (once daily) of SPP100 300 mg (for 24 hours post-dose). Plasma concentrations of SPP100 were used to determine AUC_{0-24}, AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, t_{max}, and $t_{1/2}$.

Pharmacodynamics: Blood samples were collected to assess various pharmacodynamic parameters (active rennin, total rennin, plasma rennin activity, angiotensin I, angiotensisn II and alsosterone) for 24 hours post-dose following a single dose and daily multiple doses of 300 mg of SPP100. AUC and C_{max} were calculated for the primary parameter active rennin.

- Plasma active and total rennin, plasma rennin activity (PRA) and plasma aldosterone at predose and at 1, 2, 4, 6, 12 and 24 postdose on Day 1 and Day 10.
- Plasma angiotensin I and II at predose and 6, 12 and 24 postdose on Day 1 and Day 10.

Statistical methods: Log-transformed PK parameters AUC and C_{max} were analyzed by an ANOVA model with ethnic group as a fixed factor and matched pair as a random factor. The resulting 90% confidence intervals of the appropriate treatment mean ratios were used to evaluate relative differences between Japanese and Caucasian subjects.

The primary pharmacodynamic variable active renin was compared between two ethnic groups by an ANCOVA (adjusted for baseline) model.

Results:

Number of subjects: A total of 38 subjects (19 Japanese and 19 Caucasians) participated and completed this study.

Demographic data: Demographic data were similar between the two ethnic groups (Japanese and Caucasians). Summary of the demographic data is presented in Table 1.

Table 1 **Summary demographic data**

	Body Weight (kg)		Age (years)	
	Japanese	Caucasian	Japanese	Caucasian
N	19	19	19	19
Mean	64.9	73.4	28	28
sd	5.6	6.5	5	5
Min	54.2	56.8	21	21
Median	64.8	75	27	26
Max	76.1	86.2	40	41

Pharmacokinetics

The mean plasma concentration-time profile of SPP100 following single and multiple doses is presented in Figures 1 and 2, respectively. The plasma concentrations were similar following a single dose in both the ethnic groups. The plasma concentration of SPP100 was slightly higher in Japanese subjects following multiple doses of SPP100. However, there was a wide variability in the plasma concentration profile in both Japanese and Caucasians. The pharmacokinetic parameters following single and multiple doses of SPP100 are presented in Tables 2 and 3, respectively. The AUC_{0-72} of SPP100 in Caucasians was 1124 ng·h/mL and was comparable to that in Japanese (1387 ng·h/mL) following a single dose (23% higher in Japanese). Both t_{max} and $t_{1/2}$ were comparable for both ethnic groups. The rate of absorption, as estimated by a surrogate parameter, C_{max}/AUC, was also similar between the two ethnic groups (0.16 h^{-1} for both the groups). Following a single dose of SPP100, the mean Cmax values were 215 ng/mL and 186 ng/mL, respectively, in Japanese and Caucasians.

Following multiple doses of SPP100, the mean C_{max} values at steady-state were 403 ng/mL and 321 ng/mL, respectively, in Japanese and Caucasians. The AUC during the dosing interval at steady-state (AUCτ) was also comparable (2519 ng·h/mL vs 2135 ng·h/mL in Japanese and Caucasians, respectively). Comparison of the Cmax values following a single and multiple doses indicated an accumulation factor of ~2 for both Japanese as well as Caucasians.

Figure 1 **SPP100 plasma concentrations vs. time following a single dose administration to Japanese and Caucasian subjects**

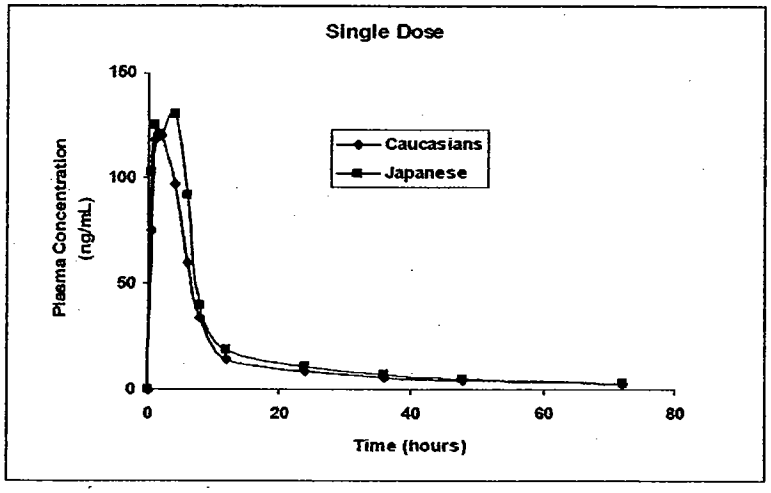
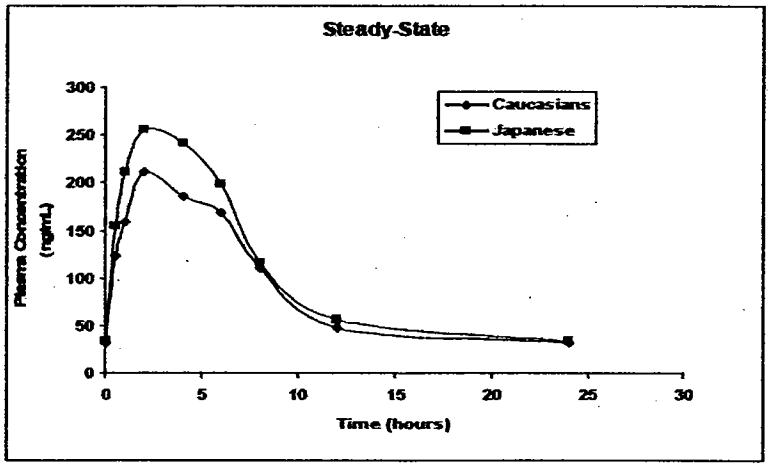


Figure 2 **SPP100 plasma concentrations vs. time at steady state in Japanese and Caucasian subjects**



All the pharmacokinetic parameters were associated with high variability (21-59% for Caucasians and 33-67% for Japanese)

Table 2 **Mean ± SD of pharmacokinetic parameters of SPP100 following single dose administration to Japanese and Caucasian subjects**

	t_{max} * (hr)	C_{max} (ng/ml)	AUC_{0-t} (ng·h/mL)	$t_{1/2}$ (hr)	C_{max}/AUC_{0-t} (h^{-1})
Japanese	2.0 (0.5-4.0)	215±122	1387±615	29.7±10.2	0.16±0.06
Caucasians	2.0 (0.5-6.0)	186±91	1124±339	32.0±6.6	0.16±0.05

* Median (minimum-maximum) was reported for Tmax

Table 3 **Mean ± pharmacokinetic parameters of SPP100 at steady state in Japanese and Caucasian subjects**

	t_{max} * (hr)	C_{max} (ng/ml)	$AUC_{0-\tau}$ (ng·h/mL)	C_{avg} (ng/ml)
Japanese	4.0 (1.0-6.0)	403±193	2519±1179	105±49
Caucasians	2.0 (0.5-8.0)	321±189	2135±791	89±33

* Median (minimum-maximum) was reported for Tmax

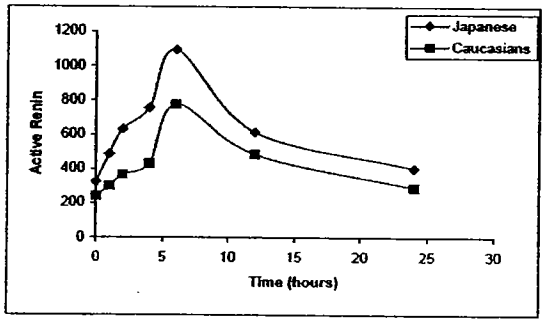
Table 4 **Statistical comparison of pharmacokinetic parameters of SPP100 in Japanese and Caucasian subjects following single and multiple dosing**

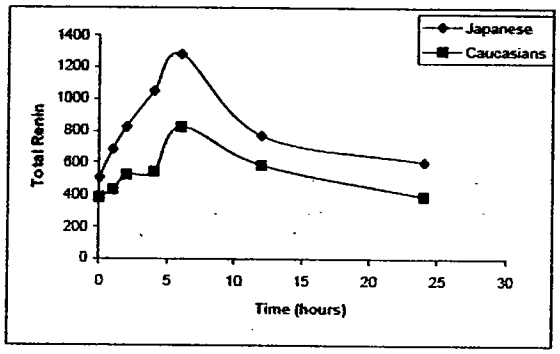
Parameter	Estimated ratio of geometric means	90% CI for ratio of means
	Single Dose	
C_{max}	1.12	(0.88, 1.43)
AUC_{0-72}	1.19	(1.02, 1.39)
	Steady-State	
C_{max}	1.3	(1.00, 1.70)
$AUC_{0-\tau}$	1.16	(0.95, 1.41)

Pharmacodynamics

The pharmacodynamic parameters in general at baseline (Day 1 and Day 10) were slightly higher for Japanese than Caucasians, suggesting an inherent difference in levels of these endogenous parameters. Following administration of SPP100 as a single dose and as multiple doses, there was an increase in active rennin and total rennin. There was also a concurrent reduction in plasma rennin activity. All the parameters showed a tendency to return to baseline at 24 hours post-dose, however, the inherent difference (as seen at baseline) was maintained throughout the time course of observation (Figure 3). Statistical analysis using an ANCOVA model (baseline adjusted values) for AUC and C_{max} of the primary PD parameter, active rennin, showed that although there was a statistical difference between the two ethnic groups following a single dose administration, (p=0.0003 and 0.0075, respectively), this difference was not significant at steady-state (0.80 and 0.64, respectively) following administration of multiple doses of SPP100 (Table 5).

Figure 3 **SPP100 pharmacodynamic profiles at steady state in Japanese and Caucasian subjects**





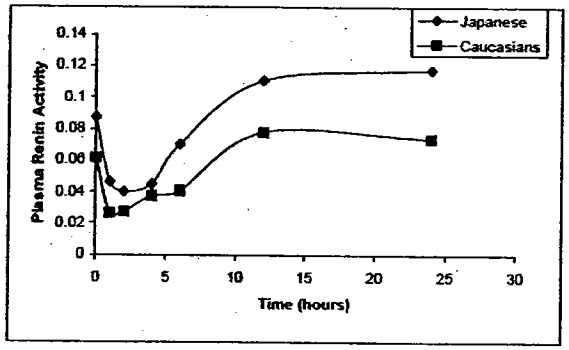


Table 5 **Statistical comparison of active rennin in Japanese and Caucasian subjects following single and multiple dosing of SPP100**

Parameter	Regimen	P Value (Japanese vs Caucasians)
C_{max}	Single Dose	0.0075
AUC	Single Dose	0.0003
C_{max}	Multiple Dose	0.6430
AUC	Multiple Dose	0.7972

SAFETY:

A total of 46 adverse events were reported in 21 subjects. The remaining 17 subjects did not report any AEs. The distribution of adverse events between the two groups was approximately equal. All reported AEs were mild to moderate in severity, with one exception: dizziness, reported in a Caucasian subject which was not suspected to be related to the study drug. In general, no treatment was necessary for reported AEs (only three AEs were treated: one Japanese subject with rash and itching, one with pyrexia and one Caucasian subject with itch). Four AEs were defined by the investigator as suspected to be related to the study drug (3 from Japanese subjects and one from a Caucasian subject).

One of the Japanese subjects developed hair loss after the end of the treatment (reported 10 days post-study). The subject was evaluated by an external dermatologist and the diagnosis was compatible with alopecia areata. This finding was not considered initially as a serious adverse event (SAE) but after the availability of all dermatological evaluations, the investigator decided to redefine it as an SAE. The relationship to the study drug was considered not suspected to be related to study drug by the investigator.

Only one subject had clinical laboratory parameters which were outside the appropriate reference range and considered to be of clinical significance. Subject 5117 had elevated GGT (160 U/L) (normal range: 10 – 60 U/L) and elevated SGPT (ALT) (168 U/L) (normal range: 4 – 45 U/L) values at Study Completion visit on 25-Sep-03; both of which were judged by the Investigator to be clinically significant. GGT and SGPT were repeated on 25-Sep-03, when SGPT levels had fallen to clinical insignificance (68 U/L), but GGT remained elevated and clinically significant (133 U/L). GGT was repeated again on 10-Oct-03 and had fallen to a clinically insignificant level (64 U/L).

CONCLUSIONS:

- Mean plasma concentration versus time profile following single and multiple doses of SPP100 were comparable in Japanese and Caucasian subjects.
- Given the high variability in the pharmacokinetic parameters of SPP100, the pharmacokinetics of SPP100 is comparable between Japanese and Caucasian subjects.
- Single and multiple doses of 300 mg of SPP100 were safe and well tolerated in healthy male Japanese and Caucasian subjects.
- There appears to be an inherent difference in the pre-dose values of the PD parameters in Japanese and Caucasians subjects which is affected after treatment with SPP100 in a similar manner in both Japanese and Caucasian subjects.
- The primary pharmacodynamic parameter (AUC and C_{max} of active renin), when adjusted for pre-dose baseline, is similar in Japanese and Caucasian subjects at steady-state.

REVIEWER'S COMMENT:

1. The drug accumulates with MD by 2-fold after 7 days for both Cmax and AUC.
2. No assay validation for any of the PD methodology used. As a result, there's no way of verifying methods and confirming that the results are accurate.
3. The formulation used in this study was over-encapsulated and may have affected the results of this study.

4. No correlations between PRA, Ang I and II, Aldosterone and blood pressure were made.
5. No correlations between Aliskiren concentrations and any of the PD parameters mentioned above were made.

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STUDY SPP100A 2223 – AN OPEN, SINGLE DOSE STUDY TO INVESTIGATE THE ABSORPTION, DISTRIBUTION, METABOLISM AND ELIMINATION OF 300 MG OF [^{14}C]SPP100 AND ITS METABOLITES IN HEALTHY MALE SUBJECTS.

STUDY INVESTIGATOR AND SITE:

REPORT # 2223
EDR VOLUME 6
STUDY DATES: August 24 – September 7, 2004

Objectives: Primary objectives

- To determine the routes of excretion and the mass balance of total radioactivity in urine and feces following a single oral 300 mg dose of ^{14}C-labeled SPP100 (331.5 mg hemifumarate salt)
- To determine the pharmacokinetics of SPP100, total radioactivity and SPP100 metabolites in blood/plasma
- To identify and quantify the metabolites of SPP100 in plasma, urine and feces
- To elucidate key biotransformation pathways and clearance mechanisms of SPP100 in man
- To evaluate the absorption of unchanged drug and total radioactivity as feasible, from available urinary and fecal excretion data

Secondary objective

- to assess the safety and tolerability of a single 300 mg dose of SPP100 in healthy male subjects

FORMULATION:

Aliskiren (^{14}C-radiolabelled)	300mg oral solution (331.5mg hemifumarate salt, Batch No: E-30585-7; Exp Date: Not provided), Novartis Pharma, Basel, Switzerland.

NOTE: Individual doses provided in plastic bottles each containing 331.5 mg of drug substance salt, dissolved in 50 mL of water as a drink solution ready for use. Drug vial batch number Y0860704.

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Design: This was a single center, open-label study in healthy male subjects.

The study consisted of a 21 day screening period, a baseline period (Day -1), a single dose treatment with a 168 hour in-house observation period (Days 1 – 8), a follow-up observation period of another 7 days (days 8-15), and a study completion evaluation after the last pharmacokinetic sample had been taken.

The subjects were confined to the study center approximately 16 hours prior to dosing. On the evening of admission, subjects received a standardized dinner followed by an evening snack. Thereafter, the subjects fasted overnight for a minimum of 10 hours. On the morning of dosing, pre-dose safety evaluations were conducted and blood, urine and fecal samples were provided by each subject for pharmacokinetic and ADME assessments. Subjects then received a single oral 300 mg dose of [^{14}C]SPP100 as a solution in water to drink. Subjects continued to fast for the next four hours, at which time lunch was served.

Safety, pharmacokinetic, and ADME assessments were performed at pre-specified time-points up to 336 hours post-dose. Following collection of the pharmacokinetic samples (blood, urine and feces) (0-168 h) and upon completion of all safety assessments, subjects were released from the study center. Subjects then returned to the study center to provide single additional samples during the period 168-336 hours post-dose.

Number of subjects:	Planned	4
	Entered	4
	Completed	4

Criteria for inclusion: Healthy, non-smoking, male volunteers between 18 and 55 years of age determined to be in good health by past medical history, physical examination, vital signs, ECG, laboratory tests and urine analysis.

Except for medication required to treat adverse events, no medication other than study drug was allowed from the initial day of screening until all of the final study evaluations had been completed. Administration of paracetamol was acceptable but had to be documented in the CRF). Decisions regarding replacements of subjects requiring concomitant medication had to be discussed with the sponsor on a case by case basis. The administration of any such medication (including over-the-counter medications and vitamins) had to be clearly documented in the CRF.

During recruitment, informed consent review, and baseline period, the subjects were informed and reminded of the following restrictions:

- No strenuous physical exercise (e.g., weight training, aerobics, football) for 7 days before dosing until after the end of study evaluation.
- No alcohol for 72 hours before dosing until after the end of study evaluation.
- Intake of xanthine (e.g., caffeine) containing food or beverages must be discontinued 48 hours before dosing until 48 hours post-dose. Consumption of such foods and beverages (i.e., coffee, tea, soda, chocolate) is not permitted during study Days -2, -1, 1, 2 only
- To avoid interactions with the drug or to avoid technical problems in the analysis of excreta, subjects will not consume asparagus, beetroot, blueberries, cherries with stones, grapes, linseed, or large amounts of carrots.
- Consumption of citrus fruit and fruit juices (grapefruit, orange, lemon, lime) must be discontinued 48 hours before dosing, and is not permitted at any time while the subjects are domiciled. If a deviation occurs during the domicile period, a note should be added to the CRF.

ANALYTICAL METHODS:

- Total radioactivity in blood, plasma, urine and feces by liquid scintillation counting. Counting times: urine, feces: 10 min; blood 60 min; plasma 60 or 180 min. LOQ: blood: 2.8 dpm, 17 ng-eq/mL; plasma: 1.8 dpm, 11 ng-eq/mL; urine, feces: 21-26 dpm, ~0.01% of dose.
- Total radioactivity in plasma (12-144 h) by AMS.
- SPP100 in plasma by LC-MS-MS assay (LLoQ 0.5 ng/mL).
- SPP100 in urine by LC-MS-MS assay (LLoQ 5 ng/mL)
- SPP100 and metabolites in plasma, urine and feces by HPLC with radioactivity detection (radiomonitor or Topcount) with counting time 60 min.
- Characterization of SPP100 metabolites by LC-MS.

PK SAMPLE COLLECTION/CALCULATIONS, PD ASSESSMENTS/CALCULATIONS AND STATISTICAL ANALYSIS:

Sample collection:

- Blood: Blood was collected into heparinized tubes at the following time points: pre-dose, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 48, 72, 96, 120, 144 and 168 h post-dose (23 time points). Three additional 5 mL blood samples were collected on days 10, 12 and 15.
- Urine: Urine was collected quantitatively at pre-dose (blank), 0-6, 6-12, 12-24, 24-48, 48-72, 72-96, 96-120, 120-144, 144-168 h post-dose, in a total of 10 fractions.
- Feces: Pre-dose blank was obtained at screening or baseline, and all complete feces portions from 0-168 h post-dose.
- Vomitus: Not applicable (no vomiting occurred)

PK and ADME Evaluations:

- Descriptive PK parameters, non-compartmental analysis.
- PK parameters: C_{max}, t_{max}, $t_{½}$, AUC_t, AUC, from concentration-time data of ^{14}C-radioactivity (blood, plasma) and SPP100 (plasma).
- Excretion/mass balance of ^{14}C-radioactivity, of SPP100 and of metabolites, in urine and feces
- Metabolite profiles in plasma, urine and feces fractions and fraction pools. AUC of main metabolites
- Identification of metabolites.

Statistical Analysis

Summary statistics (mean ± SD) are provided for demographic and baseline characteristics, safety assessments, pharmacokinetic and ADME measurements. No formal statistical analysis was performed.

RESULTS:

SAFETY:

Two subjects, numbers 5101 & 5104, had out of range laboratory parameters that emerged after dosing. These are all shown in Table 1. The investigator did not consider these to be clinically significant abnormalities.

Table 1 Out of range laboratory parameters emerging post dose

Subject No	Test	Reference Range	SCR	BL	D2	EOS
5101	SGOT (AST)	0-50 U/L	34	35	30	**53**
5101	CPK	0-190 U/L	101	107	66	**692**
5101	Hemoglobin	12.6-17.4 g/dL	13.7	14.4	13.8	**11.8**
5101	Hematocrit	0.39-0.52 L/L	0.46	0.42	0.43	**0.37**
5101	WBC (total)	4-10.5 /NL	7.2	4.8	4.6	**3.7**
5101	Basophils	0-1 %	1	1	1	**2**
5101	Urine ketone dipstick test	-0	-7 (Neg)	-1 (Neg)	2 (Neg)	**15 (+1)**
5104	Bilirubin (total)	0-1.1 mg/dL	0.73	0.92	**1.13**	0.53
5104	Cholesterol (total)	0-200 mg/dL	178	187	186	**206**
5104	Neutrophils	45-75 %	63	69	**79**	64

Adverse Events

No adverse events or serious adverse events were reported during this study.

ECG findings

ECG tracings were all considered as normal.

Pharmacokinetics

Blood and plasma concentrations and pharmacokinetics:

With the aqueous drink solution of [^{14}C]SPP100, C_{max} of ^{14}C in blood and plasma, and of SPP100 in plasma, was reached at 3 hours after dosing (median), indicating a moderate speed of absorption (Figures 1, 2). For individual subjects, the same t_{max} was observed for all analytes, and the concentration-time curves of the analytes were parallel (Figures 3 and 4). The difference between plasma levels of ^{14}C and SPP100 were typically in a range of 10-20%, indicating low exposure to metabolites.

Inter-individual variability of concentrations was substantial (Figures 1, 2). Subject No. 5101 exhibited a particularly low exposure, with levels far lower than in the three other subjects. The main pharmacokinetic parameters are summarized in Table 2.

Radioactivity in plasma was detected typically for 8-12 hours after dose, and was below the LOQ later on. Therefore, samples collected at 16, 48, 96 and 144 hours post dose were reanalyzed using the more sensitive AMS method.

SPP100 was detected typically for 168 hours, except for subject no. 5101 (96 hours). Approximately 81% of the total plasma radioactivity (AUC) was due to unchanged SPP100 (86% of AUC_{0-10h}). Exposure to metabolites was very low.

Early apparent half-lives (by non-compartmental analysis) were 1.8 hours for radioactivity in plasma, and 2.1 hours for SPP100 in plasma. This was observed typically in the time period 4 – 8 hours. In the period 48 – 144 hours, the terminal half-lives of SPP100 and radioactivity in plasma were similar, i.e. 44 and 49 hours (Figure 3).

Figure 1 **Concentrations of total ^{14}C in plasma of 4 subjects**

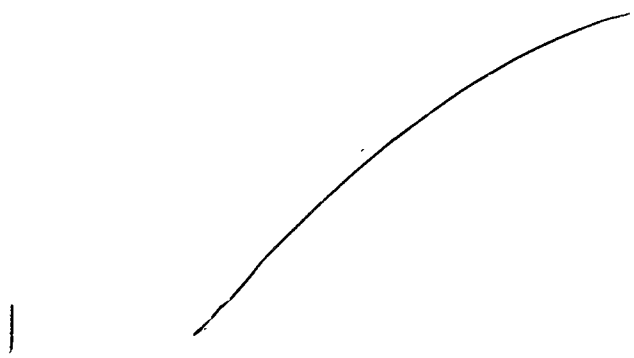
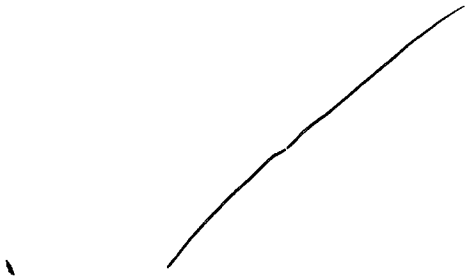
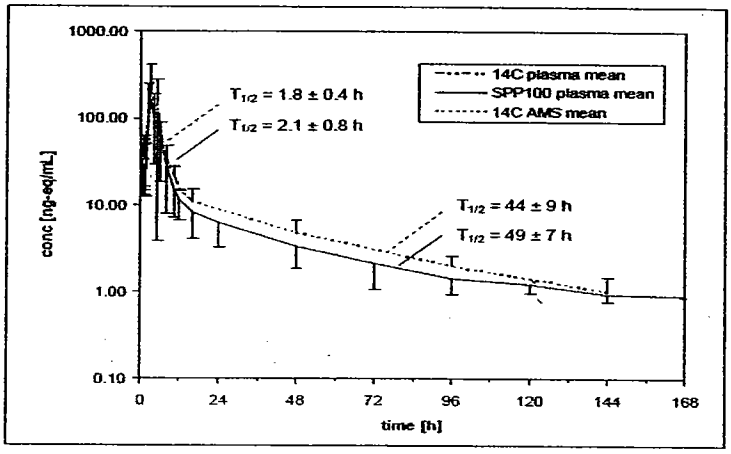


Figure 2 **Plasma concentrations of SPP100 in plasma of 4 subjects**



Subject 5101 had significantly lower plasma concentrations than the other subjects. No reason was provided for such disparity.

Figure 3 **Plasma concentrations of total ^{14}C and SPP100 (N=4, ± SD; semi-log scale)**



Radioactivity was largely present in plasma. The mean ratio of AUC_t blood:plasma was 0.61 (Table 2). About 11% of the radioactivity (F_E, mean) was associated with red blood cells. Though, the AUC_t ratio and the F_E showed large variability which were probably due to the variable hematocrit and the radioactivity statistics at low count rates.

Figure 4 **Concentrations of total ^{14}C in blood and plasma. Representative data, from subject no. 5104.**

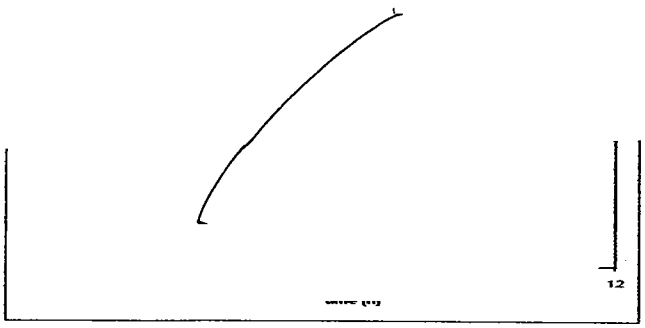


Table 2A: Pharmacokinetic parameters for radioactivity in plasma

Parameter	unit	Subject 5101	5102	5103	5104	Mean (*median)	N	SD
t_{max}	hr					3 *	4	
C_{max}	ng/mL					305.1	4	193.3
$t_{1/2\,el}$	hr					1.78	4	0.39
period from	hr					4	4	
to	hr					9	4	
$AUC_{(0-48h)}$	hr*ng/mL					746.9	4	411.2
t_{last}	hr					144	4	
C_t	ng/mL					1.0	4	0.5
AUC_t	hr*ng/mL					1310.4	4	613.0
$t_{1/2\,el}$	hr					44.30	4	9.38
period from	hr					48	4	
to	hr					144	4	
$AUC_{inf\,obs}$	hr*ng/mL					1373.9	4	637.8
$AUC_{extrap\,obs}$	%					5.2	4	2.0
Vz/F_{obs}	ml	4.73E+07	1.13E+07	1.04E+07	1.02E+07	1.98E+07	4	1.84E+07
Cl/F_{obs}	mL/hr	8.02E+05	1.88E+05	1.50E+05	2.18E+05	2.89E+05	4	2.10E+05

Table 2B; Pharmacokinetic parameters for Aliskiren in plasma

Parameter	unit	Subject 5101	5102	5103	5104	Mean (*median)	N	SD
t_{max}	hr					3 *	4	
C_{max}	ng/mL					253.6	4	163.0
$t_{1/2\,el}$	hr					2.15	4	0.78
period from	hr					4	4	
to	hr					9	4	
$AUC_{(0-48h)}$	hr*ng/mL					649.7	4	361.8
	% of ^{14}C Plasma					86.1	4	3.8
t_{last}	hr					144	4	
C_t	ng/mL					0.3	4	0.1
AUC_t	hr*ng/mL					1051.0	4	546.8
	% of ^{14}C Plasma					81.4	3	3.4
$t_{1/2\,el}$	hr					48.67	4	6.88
period from	hr					48	4	
to	hr					144	4	
$AUC_{inf\,obs}$	hr*ng/mL					1107.8	4	545.9
	% of ^{14}C Plasma					81.4	3	1.9
$AUC_{extrap\,obs}$	%					7.4	4	6.5
Vz/F_{obs}	ml	7.11E+07	1.43E+07	1.23E+07	1.79E+07	2.89E+07	4	2.33E+07
Cl/F_{obs}	mL/hr	8.37E+05	2.23E+05	1.84E+05	2.74E+05	3.79E+05	4	3.07E+05

italicized: data not used for calculation of means

Table 2C: Pharmacokinetic parameters for radioactivity in blood

Parameter	unit	Subject 5101	5102	5103	5104	Mean (*median)	N	SD
t_{max}	hr					2.5 *	4	
C_{max}	ng/mL					170.6	4	88.3
$t_{1/2\,el}$	hr					1.89	3	0.34
period from	hr					4	3	
to	hr					8	3	
$t_{last\,blood}$	hr					8	4	
C_t	ng/mL					21.7	4	6.1
$AUC_{t\,blood}$	hr*ng/mL					26.6	4	225.6
	% of AUC(plasma)*					68.6	3	12.3
AUC(plasma)*	hr*ng/mL					598.2	4	424.9
$t_{1/2\,el}$	hr					1.89	3	0.34
$AUC_{inf\,obs}$	hr*ng/mL					590.6	3	92.6
$AUC_{extrap\,obs}$	%					10.9	3	6.1
Vz/F_{obs}	ml	ns	1.40E+06	1.08E+06	1.74E+06	1.41E+06	3	3.32E+05
Cl/F_{obs}	mL/hr	ns	4.46E+05	4.92E+05	6.12E+05	5.17E+05	3	8.57E+04

ns: not sensible, not calculated
* plasma AUC of ^{14}C for same time period as for blood ($0 - t_{last\,blood}$)
italicized: data not used for calculation of means

Excretion of Radioactivity:

The ^{14}C-radioactivity was excreted almost completely via the biliary/fecal route. Total excretion (mass balance) in 7 days was 91.5 % of dose, with moderate interindividual variability (range 85 – 95%).

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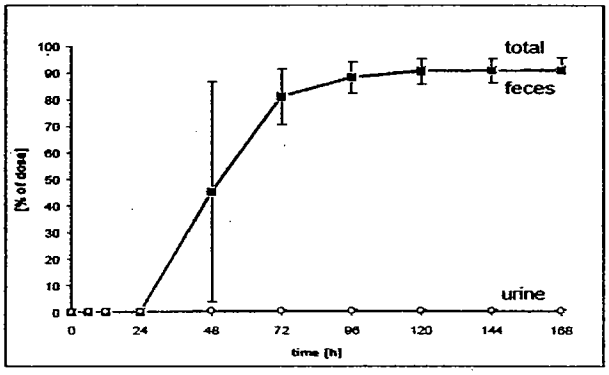
Table 3 **Cumulative excretion of ^{14}C-radioactivity [% dose], 0-168 h (means of N=4, SD)**

Time Period	^{14}C-Excretion [% of dose]					
[h]	Urine		Feces		Total	
0-96	0.57	(0.24)	88.17	(5.44)	88.74	(5.60)
0-168	0.60	(0.25)	90.89	(4.50)	91.50	(4.52)

Figure 5 **Cumulative Excretion of ^{14}C-radioactivity in urine and feces, means of N=4 ± SD [% dose]**



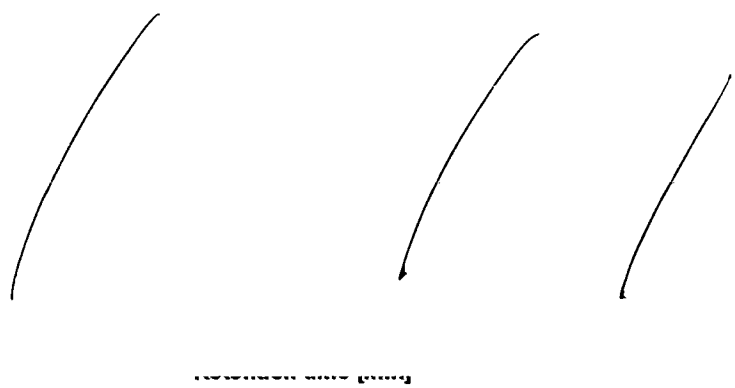
Metabolism:

Metabolite patterns in plasma:

In plasma at t_{max}, most of the radioactivity was associated with unchanged SPP100 (Figure 6). The patterns varied slightly between subjects. Two metabolites M2 and M3 were detected, and a trace of M1. At t_{max}, M2 levels were ≤1% of C_{max} of SPP100. Levels of M3 were in a range of 1-5% of C_{max} of SPP100. SPP100, measured by LC-MS/MS, accounted for approximately 86 % of the radioactivity (based on AUC_{0-10h} of ^{14}C).

Figure 6 **Representative metabolite patterns in plasma at C_{max}**

Representative plasma metabolite pattern at t_{max} (3 h), of subject no. 5103, by HPLC with radiodetection



The AUC fraction of radioactivity represented by these metabolites could not be determined, as patterns were analysed only at t_{max}, and radioactivity at later time points was too low to be quantified. Assuming that the proportion of metabolites remains approximately constant over time, the AUC of M2 and M3 may be estimated roughly to approx. 1% and 3% of the ^{14}C-AUC, respectively.

Metabolite patterns and mass balance in urine and feces:

Due to low renal excretion (0.6%) and low urinary concentrations, analysis of metabolite patterns in urine was feasible only in urine fractions collected in the 1st 12 hours after dose. Only for subject no. 5103, urine collected over 48 hours could be analysed.

In all urine fractions analysed, the metabolite patterns showed a major peak of SPP100. The renal excretion of unchanged SPP100 amounted to approx. 0.4 % of the dose. This is consistent with the result obtained by the (cold) LC-MS/MS assay for urine (0-24 h).

Additionally, the metabolites M3 and M2, and traces of M4 and M6 and others, were detected (Figure 7). The amounts excreted could not be determined accurately. In total, M3 amounted to <0.1% of the dose and all others to traces (<0.03%).

The bulk of the radioactivity was excreted with the feces. For metabolite analysis, feces were extracted with 89-96% extraction yield. Unchanged [^{14}C]SPP100 was the predominant compound in the feces. Metabolites M2 and M3 were found typically in amounts of 0.7-1.2% of the dose (Figure 8). Additionally, traces of M1 and other peaks were detected. Close to the SPP100 peak, an unknown peak P 62 was detected, which accounted for approx. 1 % of the dose.

The metabolites were identified based on mass spectrometry data and HPLC retention time. For metabolite identity and amounts formed see Table 4.

SPP100 in the feces was largely due to unabsorbed drug. The identified metabolites M1 – M6 are thought to be formed upon absorption. As a fecal trace metabolite, M12 was identified as the N-acetyl derivative. Two other fecal metabolites M13 and M14 (in peak P62) were partly characterized as isomers, both containing a $C_3H_4O_2$ moiety. Probably they are formed in the gut, as an artifact by microbial metabolism.

No major differences were observed between subjects in the fecal and urinary patterns of metabolites.

Figure 7 **Representative metabolite patterns in urine**

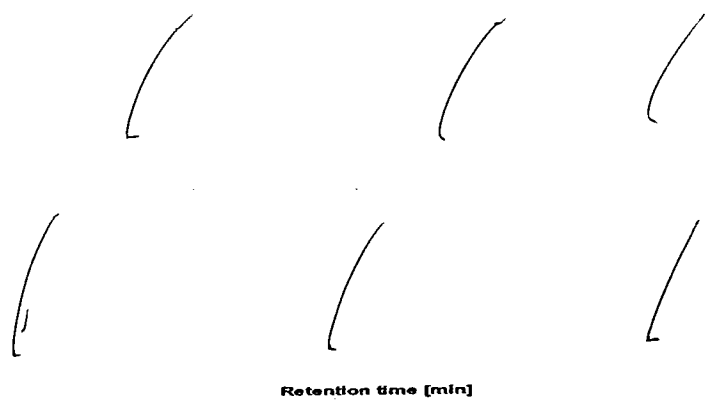
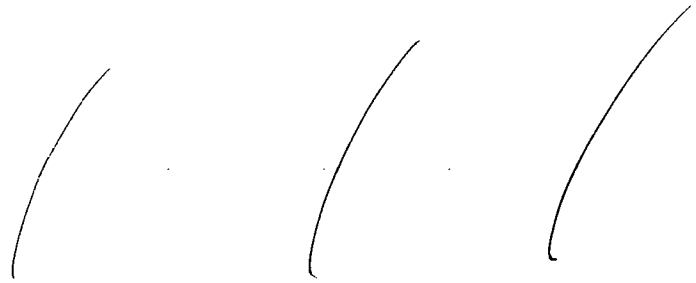


Figure 8 **Representative metabolite patterns in feces**



No major differences were observed between subjects in the fecal and urinary patterns of metabolites.

Metabolism pathways:

A general scheme of the metabolism pathways of SPP100 in human is shown in Figure 9. The metabolites M1 – M4 were all formed by oxidation at the phenol moiety and the attached side chain, by O-demethylation, O-dealkylation and/or alcohol oxidation. The sum of the metabolites M1 - M4 accounted for approx. 1.4 % of the dose. A trace of M4 was excreted in the form of a glucuronic acid conjugate M6. A further trace metabolite (front peak) was formed through hydrolysis of the amino-isobutyramide group.

Table 4 **Metabolites in excreta [% dose] (means of N=4, SD)**

Metabolite		Urine	Feces	Total	SD
Front peak	not identified *	<0.1	0.1	0.1	0.1
M6	O-glucuronide of M4	<0.1		<0.1	
M2	carboxylic acid (oxidized side chain)	<0.1	0.5	0.6	0.5
M4	phenol, O-dealkylated	<0.1		<0.1	
M3	alcohol, O-demethylated	<0.1	0.6	<0.6	0.4
M1	phenol, O-demethylated		0.1	0.1	0.1
SPP100		0.4	77.5	79.8	3.0
P 62	M12: N-acetyl derivative; M13, M14: partly characterized (+$C_3H_4O_2$)		1.0	1.0	0.1
traces	unidentified	<0.1	1.5	1.6	0.7
not analysed			9.7	9.7	3.1
Total ^{14}C		0.6	90.9	91.5	4.5

* not unambiguously identified, possibly 3-amino-2,2-dimethylpropionamide, or a product of hydrolysis

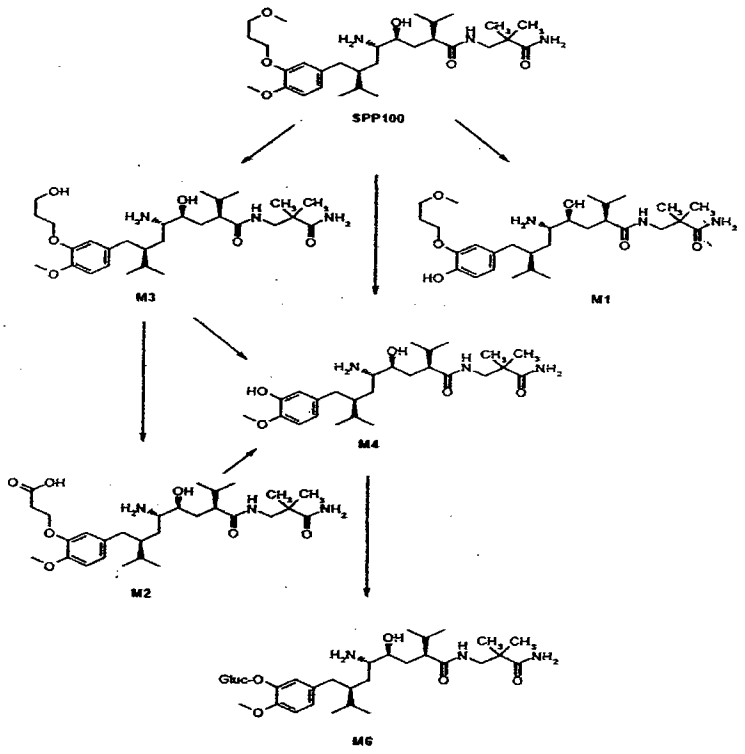



Figure 9 Proposed main biotransformation pathways in man