CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: NDA 20757 AND 20758

PHARMACOLOGY REVIEW(S)

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NDA #20,757; NDA #20,758

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

G. Jagadeesh, Ph.D.

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SPONSOR Sanofi Winthrop, Inc.
Montpellier Cedex, France

AGENT Bristol-Myers Squibb
P. O. Box 4000
Princeton, NJ 08543-4000

NDA 20-757 Irbesartan Tablets

Pharmacological Class: Angiotensin II receptor antagonist

Indication: Hypertension

Formulation and Route of Administration: Each tablet contains 75, 150 or 300 mg of Irbesartan. Inactive ingredients in the tablet are lactose, microcrystalline cellulose, pre-gelatinized starch, croscarmellose sodium, poloxamer, silicon dioxide and magnesium stearate.

Dosage Regimen: The recommended initial dose is 150 mg once daily but for further reduction of blood pressure the dosage may be titrated to 300 mg once daily.

NDA 20-758 Irbesartan/Hydrochlorothiazide Tablets

Pharmacological Class: Angiotensin II receptor antagonist/diuretic drug combination

Indication: Hypertension

Formulation and Route of Administration: Each tablet contains 150 mg of Irbesartan and 12.5 mg of hydrochlorothiazide. Inactive ingredients include lactose monohydrate, microcrystalline cellulose, pre-gelatinized starch, croscarmellose sodium, ferric oxide red, ferric oxide yellow, silicon dioxide and magnesium stearate.

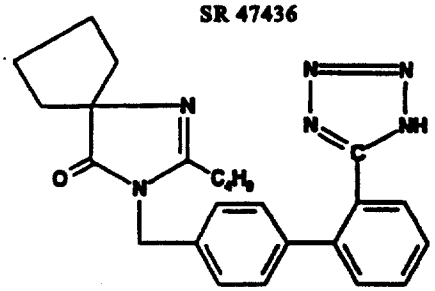
Dosage Regimen: The recommended initial dose is 150/12.5 mg once daily but for further reduction of blood pressure the dosage may be titrated to 300/25 mg once daily (two tablets).

RELATED INDs Clinical trials for Irbesartan were conducted under B-M Squibb IND
Clinical trials for Irbesartan/HCTZ were conducted under B-M Squibb IND

DRUG CHEMISTRY

Irbesartan
Code Names: SR 47436; BMS 186295; BMS-186295-01
Chemical Name: 2-butyl-3-[(2'-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl]-1,3-diazaspiro[4,4]non-1-en-4-one]
CAS Registry No: 138402-11-6

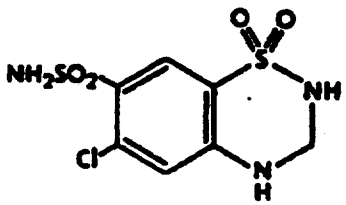
SR 47436



M. WT. 428.5

Hydrochlorothiazide
Code Names:None
Chemical Name: 2H-1,2,4-benzothiadiazine-7-sulfonamide,6-chloro-3,4-dihydro-1,1-dioxide
CAS Registry No: 58-93-5

HCTZ



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NOTE: For summary and evaluation of reproductive toxicity studies, see review of Dr. S. Stolzenberg.

INTRODUCTION

Angiotensin II, a potent vasoconstrictor and a component of the renin-angiotensin-aldosterone system (RAAS), is important in the pathophysiology of hypertension and sodium homeostasis.

Angiotensin-converting enzyme inhibitors (ACEIs) have been established as a class of highly effective and safe agents for the treatment of hypertension. However, ACEIs do not specifically affect the RAAS, but rather inhibit the hydrolysis of many peptides by converting enzyme. Cough, a common and troublesome side effect with ACEIs, is due to potentiation of bradykinin's action (*via* inhibition of kininase). An alternative approach is to use drugs that block the actions of angiotensin II at its target sites. The effects of angiotensin are exerted through specific cell surface receptors, characterized pharmacologically into two subtypes, AT_1 and AT_2, which are distributed in varying proportions in different tissue beds.

Stimulation of AT_1 receptors can result in or contribute to a prolonged increase in arterial blood pressure by a direct vasoconstrictor action on blood vessels; potentiation of peripheral sympathetic transmission into the hypertensive range; stimulation of aldosterone secretion by the adrenal cortex; and stimulation of smooth muscle cell growth. Angiotensin II promotes salt and water retention by stimulating adrenal production of aldosterone, by acting in the CNS to cause the release of ADH and to stimulate thirst and salt appetite, and by directly stimulating sodium reabsorption in the kidney. Angiotensin II also acts on the renal juxtaglomerular cells that secrete renin to inhibit renin secretion. In CHF, reduced cardiac output leads to activation of several vasoconstrictor systems, including the RAS, to maintain cardiovascular hemostasis. This compensatory increase in angiotensin II brings about unwanted consequences such as an increased loading to the failing heart, increased salt and water retention and exacerbation of cardiac arrhythmias. Again, these effects are probably mediated by the AT_1 receptors. Recent evidence suggests that there are at least structural, if not functional, differences in the AT_1 receptor subtypes (AT_{1a} and AT_{1b}). The functions of the AT_2 receptor are not fully understood, but that receptor may be involved in neuronal ion channel modulation and in fibroblast collagen metabolism.

Current research has focused on the development of orally active nonpeptide angiotensin II receptor antagonists as new antihypertensive agents. This review summarizes the results of the preclinical evaluation of one such antagonist, SR 47436 (synthesized at Sanofi Recherche, France), alone and in combination with hydrochlorothiazide (HCTZ). SR 47436 is a potent specific antagonist of angiotensin II at the AT_1 receptor. The sponsor's NDA contains extensive preclinical data supporting this classification. Hydrochlorothiazide is a diuretic and antihypertensive. The fixed combination of SR 47436 and HCTZ, with different pharmacologic mechanisms and additive effects, provides potential benefits in terms of efficacy, compliance, and convenience to appropriate patients.

1. PHARMACODYNAMICS

1.1. Studies Related to Proposed Therapeutic Indication

SR 47436 is practically insoluble in water. For *in vitro* studies, it was dissolved in methyl/ethyl alcohol or dimethylsulfoxide and further dilutions were made in physiological salt solution. For studies with oral and intravenous administration, solutions of SR 47436 with stoichiometrically equivalent amounts of potassium hydroxide or L-arginine (thereby creating the potassium- or arginine-salt of SR 47436) were prepared in distilled water. (In this review, all doses of test agent are expressed in terms of SR 47436 rather than its potassium or arginine salt.) Suspensions of SR 47436 in 5 or 10% solutions of gum arabic also were used for oral administration studies.

1.1.1. *In Vitro:*

1.1.1.1. Receptor Specific Studies

The selectivity of SR 47436 for angiotensin II receptors was evaluated by receptor binding techniques using the AT_1 receptor predominant rat adrenal cortical membrane. The receptor subtype specificity was determined in the presence and absence of dithiothreitol (DTT) and WL 13, which are known to block AT_1 and AT_2 receptors, respectively. In the presence of the two receptor subtypes, SR 47436 displaced only 60% of the specific binding of [^{125}I]angiotensin II in the range of concentrations used (10^{-10} to 10^{-6} M), corresponding to AT_1 receptor subtype. When the inhibition of specific binding by SR 47436 was repeated in the presence of 1 µM of WL 13 (blocking all available AT_2 receptors), the displacement curve was in good agreement with a one site model. The calculated IC_{50} was 1.17 nM. On the other hand, in the presence of DTT (5 mM), where the specificity for AT_1 receptor subtype is lost, the calculated IC_{50} was over 10 µM. DuP 753, another AT_1-receptor selective antagonist was used as a reference compound. SR 47436 was shown to be approximately 16-fold more potent than DuP 753 for binding to the AT_1 receptor.

The specificity of SR 47436 for angiotensin II receptors was evaluated in another study, using rat liver cell membranes, which are known to contain primarily AT_1 receptors. This study compared the binding characteristics of test drug, the potassium salt of test drug (SR 47436A), and the reference compounds DuP 753 and saralasin. Both SR 47436 and its potassium salt exhibited high affinity for the AT_1 receptor, displaying similar IC_{50s} (1.28 and 1.32 nM, respectively). SR 47436 had approximately 10-fold and 2-fold higher affinity for the AT_1 receptor than DuP 753 and saralasin, respectively. Furthermore, the saturation binding data revealed that the antagonism is competitive in nature. The Scatchard analysis of the binding data revealed that in the presence of 3 nM SR 47436 the apparent K_D of [^{125}I]angiotensin II binding was increased from 0.87 nM to 4.25 nM, whereas the B_{max} was practically unchanged.

The affinity of SR 47436 for angiotensin II receptors of aortic smooth muscle was determined by measuring the displacement of [^{125}I]angiotensin II bound to cultured rat aortic smooth muscle cell membranes. In this study [^{125}I]angiotensin II identified a single population of high affinity

binding sites and SR 47436 displaced 100% of the specific binding with an IC_{50} value of 1.58 ± 0.34 nM. SR 47436 was approximately nine-fold more potent than DuP 753 (IC_{50} 13.98 ± 2.14 nM) for binding to AT_1 receptor. This study further confirms that SR 47436 is a potent antagonist of angiotensin II at the AT_1 receptor, as was demonstrated in preceding reports on rat liver and adrenal cortex membranes.

The affinity of SR 47436 for human AT_1 receptors from aortic smooth muscle membranes was compared with that of 12 of its metabolites. M1, M2, M3, M4, M5, M6 and M7 were SR47436 metabolites isolated from human urine and the rest were synthesized in the laboratory. SR90149A, M5 and M7 were as active as SR47436. The affinities of SR90150 (SR47436 glucuronide) and of M6 were about ten-fold less than that of SR47436. SR90148, M2and M4 were forty fold less potent than SR47436. The other metabolites (SR49498, SR 90145A , SR 90146A , M1 and M3) showed little or no activity (Table 1.1.1).

TABLE 1.1.1

INHIBITORY EFFECTS OF SR 47436 AND ITS METABOLITES ON SPECIFIC BINDING OF [^{125}I]-ANGIOTENSIN II

	IC50 (nM)	n_H
SR47436	0.54 ± 0.053	0.86 ± 0.07
SR49498	3000	
SR 90145A	3000	
SR 90146A	588 ± 57	0.84 ± 0.08
SR90148	22.1 ± 1.0	0.81 ± 0.03
SR90149A	0.195 ± 0.011	0.95 ± 0.04
SR90150	4.55 ± 0.91	1.00 ± 0.18
M1	119 ± 7.2	0.94 ± 0.05
M2	20.9 ± 6.6	0.91 ± 0.17
M3	761 ± 99	0.83 ± 0.14
M4	31.6 ± 3.1	0.80 ± 0.06
M5	0.44 ± 0.012	1.01 ± 0.03
M6	6.45 ± 0.65	1.08 ± 0.11
M7	0.631 ± 0.046	1.05 ± 0.05

n_H : Hill coefficient or slope of the line.

SR 47436 was also evaluated for binding activity at various neurotransmitter receptor sites, ion channels and antiports. SR 47436 had no appreciable affinity (>10 μM) for binding to receptor sites for α_1- or α_2-adrenergic ligands, M_1- or M_2-muscarinic ligands, histamine, serotonin, bombesin, bradykinin, endothelin 1, neurotensin, vasopressin, neuropeptide Y, imidazolines, calcitonin gene-related peptide, phencyclidine, or cannabis. Further, SR 47436 had no appreciable affinity for calcium channels or ion transport mechanisms (sodium/calcium pump, sodium/hydrogen pump) and had no appreciable activity in tests of platelet agglutination and fibrinogen clotting mechanisms. These results suggest that SR 47436 has very specific affinity for angiotensin II receptors.

1.1.1.2. Studies in Isolated Tissues

Angiotensin II-induced contractions of isolated rabbit aortic rings were inhibited by SR 47436 in a concentration-dependent manner. The rightward shift occurred in a parallel fashion with a decrease in maximal effect significant at 30 nM (Fig. 1.1.1). This is characterized as a competitive effect of the unsurmountable type. pA_2 as determined at 40% of the dose response curve was 8.64 ± 0.08. SR 47436 produced no marked smooth muscle contraction (no agonist activity) and no marked inhibition of norepinephrine- or potassium-induced contractions.

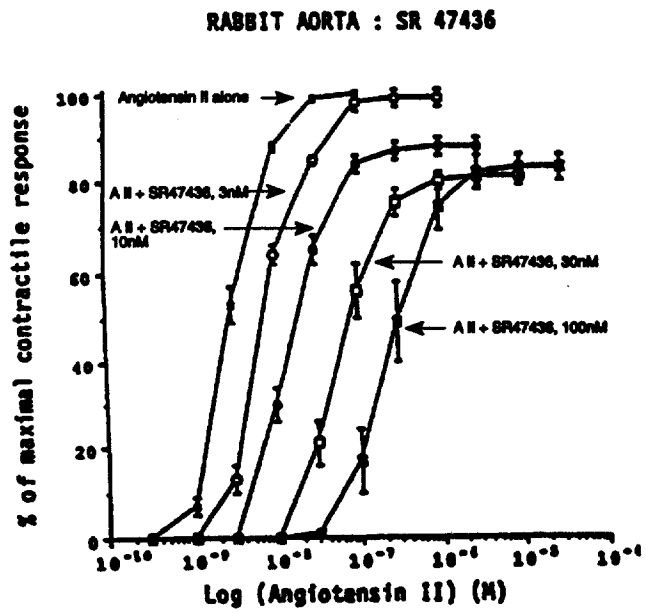

Figure 1.1.1.: Concentration response curve of angiotensin II in the absence and presence of varying concentrations of SR 47436 in isolated rabbit aortic rings. Note parallel shift to the right side and decrease in the maximal effect.

1.1.2. *In Vivo:*

1.1.2.1. Studies in Rats (male Sprague-Dawley from Charles River unless otherwise noted)

A. Normotensive Rats

a) ***Per se* effects:** Since angiotensin II does not normally contribute to the maintenance of arterial pressure, a receptor antagonist for the AT_1 angiotensin II receptor would not be expected to affect

arterial pressure in normotensive animals. Such was the case when SR 47436 (arginine salt) was administered intravenously to conscious normotensive rats at a dose of 10 or 30 mg/kg. SR 47436 produced no statistically significant changes in mean arterial pressure compared to pretreatment values; heart rate was slightly (12 to 15%) but statistically significantly increased after administration of SR 47436.

b) **Angiotensin II antagonism:** The ability of SR 47436 to selectively antagonize angiotensin II-induced pressor response was evaluated in conscious rats following oral and i.v. administration.

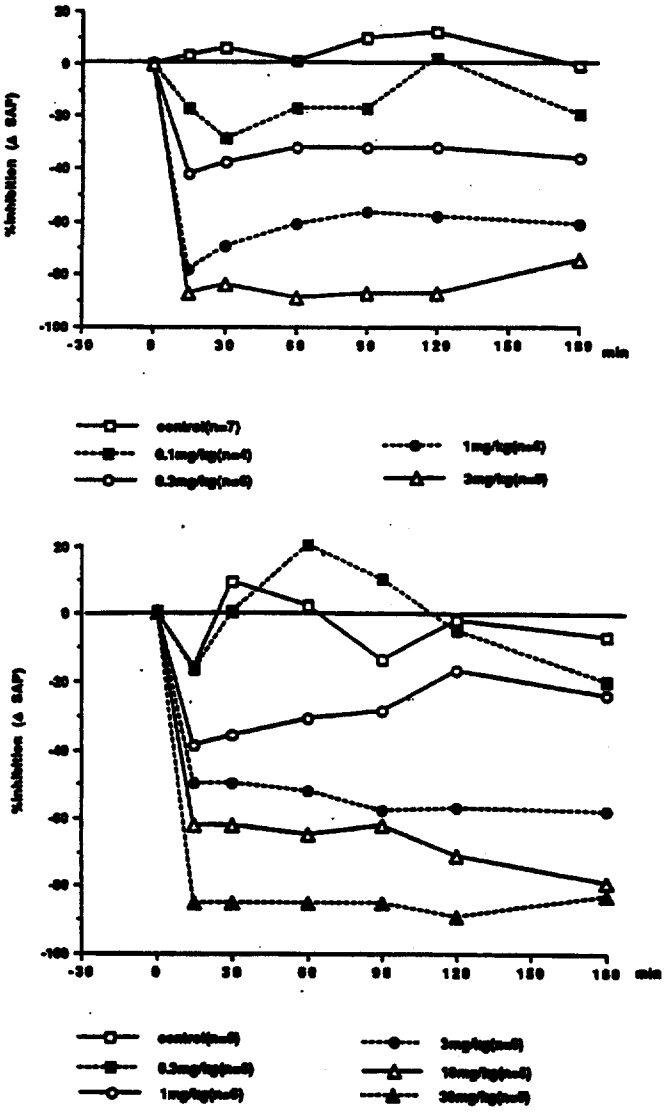

Figure 1.1.2.: Inhibitory effect of SR 47436 (i.v., upper panel; oral, lower panel) on the angiotensin II-induced pressor response in normotensive rats.

Rats were prepared surgically with indwelling vascular cannulas; each rat had a cannula in a carotid artery for recording b.p. and a second cannula in a jugular vein for injection of drugs. Twentyfour hours later, the rats were divided into groups and given SR 47436 orally (0.3, 1, 3, 10, or 30 mg/kg; potassium salt of SR 47436) or i.v. (0.1, 0.3, 1, or 3 mg/kg; arginine salt of SR 47436). Vehicle control rats received distilled water orally or i.v. Each rat received i.v. administration of angiotensin II before and at predetermined times from 15 to 180 minutes after administration of SR 47436; the angiotensin II dose was adjusted to produce approximately 40 mm Hg increase in systolic b.p. (30 to 50 ng/kg). Both i.v. (Fig. 1.1.2 upper panel) and oral (Fig. 1.1.2 lower panel) administration of SR 47436 induced dose-dependent inhibitory effects on the angiotensin II-induced pressor response at doses of 0.3 or more mg/kg. The onset of activity was fast with effects noted within 15 minutes after oral dosing; the time to maximum inhibition ranged from 15 minutes after administration of 1 mg/kg to 180 min after adminstration of 10 mg/kg SR 47436. At the highest dose (30 mg/kg) the maximal inhibition occurred at 15 min postdose and it lasted at least until 180 min postdose.

In a related study, male Wistar Janssen rats were prepared for acute recording of b.p. Rats were anesthetized and pithed. Pressor responses to i.v. administration of 0.3 µg/kg angiotensin II were recorded before and at predetermined intervals after i.v. administration of vehicle or SR 47436. SR 47436 (arginine salt of) produced no agonistic activity (no change in baseline diastolic b.p.) and inhibited angiotensin II-induced pressor responses for at least 70 min (the last observation time).

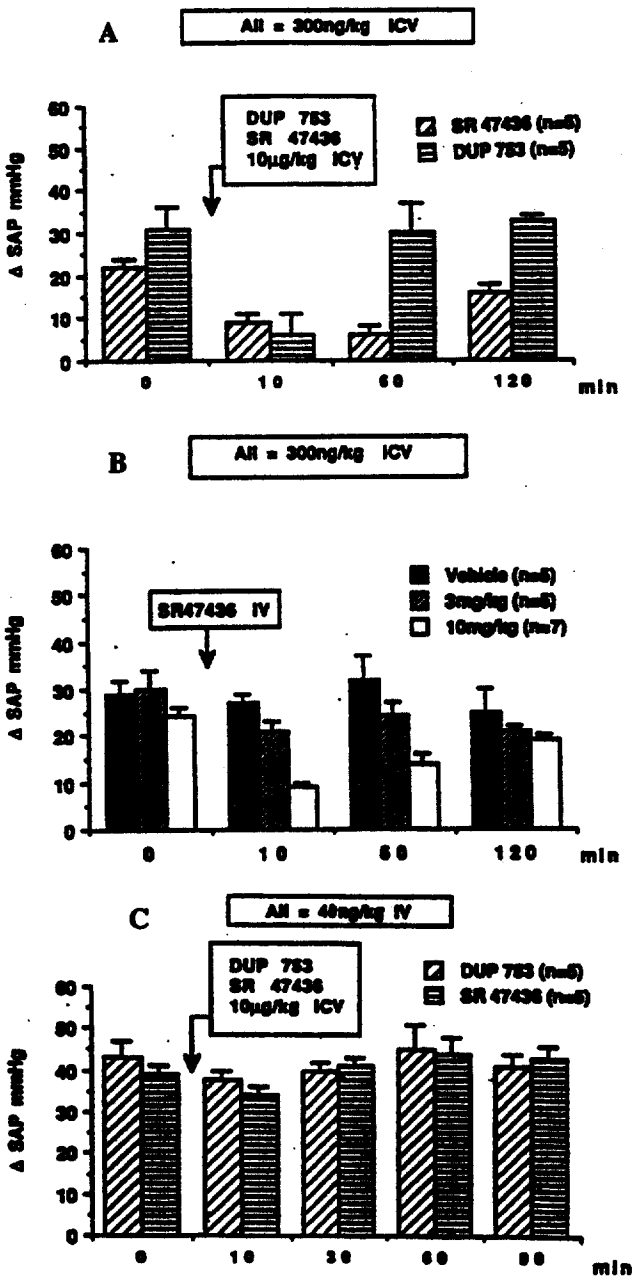

Figure 1.1.3.: Effect of i.c.v. (A, C) or i.v. (B) administration of SR 47436 or DuP 753 on the pressor response to i.c.v (A, B) or i.v. (C) administration of angiotensin II.

Comparison of effects of i.v. and intracerebroventricular (i.c.v.) administration of SR 47436 were made against pressor responses to angiotensin II administered i.v. or by i.c.v. injection in rats. Rats were prepared surgically with an indwelling cannula for administration of drugs into the lateral ventricle. Six or seven days later the rats were surgically prepared with indwelling catheters for recording b.p. (carotid artery) and for i.v. (jugular vein) injection of compounds. Pressor responses to angiotensin II administered i.v. or by i.c.v. injection were measured before and at predetermined times from 10 to 120 min after i.v. administration of vehicle, or 3 or 10 mg/kg SR 47436 (dissolved in KOH); separate group received i.c.v. administration of 10 µg/kg SR 47436. I.c.v. (300 ng/kg) administration of angiotensin II produced a transient increase in systemic b.p. This rise was inhibited to an extent of 73% ($p < 0.05$) after i.c.v. administration of SR 47436 (Fig. 1.1.3A). After i.v. administration at 3 and 10 mg/kg, SR 47436 blocked in a dose-dependent manner (30% and 60%, respectively, $p < 0.05$) the pressor response to i.c.v. administration of angiotensin II. In contrast, i.c.v. administration of SR 47436 produced only a slight (13%, not significant) inhibition of the pressor response to i.v. administered angiotensin II (Fig. 1.1.3C). The results suggest that SR 47436 penetrates the brain to affect central AII receptors. Also, a ten times higher dose of SR 47436 (10 mg/kg, i.v.) is needed to elicit a 60% inhibition of the pressor response to angiotensin II

administered i.c.v. (Fig. 1.1.3B).

A study was undertaken to investigate the effect of SR 47436 on the pressor effect and on the aldosterone secretion induced by a perfusion of angiotensin II in conscious rats. The day before the experiment, anesthetized rats were prepared with indwelling catheters for recording arterial pressure and for drug administration. On the day of the test, arterial pressure was recorded for 30 minutes before and for 30 minutes after the start of angiotensin II infusion (0.1 µg/min/rat at a rate of 3 µl/min for 30 min). SR 47436 was solubilized in KOH solution and administered intravenously fifteen minutes before the onset of angiotensin II infusion. Arterial blood samples were collected at the end of the infusion for measurement of aldosterone concentration.

Intravenous infusion of angiotensin II increased arterial blood pressure 54% and increased plasma aldosterone concentration 520% (compared to vehicle control values). SR 47436 inhibited both parameters in a dose-dependent manner. Thus, at the doses of 1, 3, and 10 mg/kg SR 47436, the effects were of 4%, 16% and 36% ($P < 0.05$) inhibition of blood pressure and of 52%, 84% ($P < 0.05$) and 96% ($P < 0.05$) inhibition of aldosterone secretion, respectively. Though a good correlation was observed between the reduction in b.p. and the inhibition of aldosterone secretion, the inhibition of 50% aldosterone secretion with SR 47436 was obtained at a dose (1 mg/kg) which did not modify arterial pressure at all (Table 1.1.2).

TABLE 1.1.2
EFFECTS OF SR 47436 ON THE PRESSOR EFFECT AND ON THE ALDOSTERONE (PA) SECRETION INDUCED BY A PERFUSION OF ANGIOTENSIN II IN CONSCIOUS RATS

Groups	Doses	SAP		PA	
	(mg/kg)	mmHg	% Var	pg/ml	% Var
Control vehicle	0	131 ± 4		345 ± 48	
Control AII	0	202 ± 3	+54♦	2142 ± 202	+520♦
AII+SR 47436	1	194 ± 10	-4 NS	1022 ± 192	-52 NS
AII+SR 47436	3	169 ± 12	-16 NS	335 ± 42	-84*
AII+SR 47436	10	130 ± 4	-36*	296 ± 53	-96*

NS : Not Significant; ♦ : $p < 0.05$ versus control vehicle;
* : $p < 0.05$ versus control AII

c). **Selectivity and specificity for angiotensin II receptors**

The following three studies investigated the activity of SR 47436 on pressor reponses in pithed rats to evaluate the selectivity and specificity of action for angiotensin II receptors and also delineated the nature of the antagonism.

In the first study, male Wistar Janssen rats were anesthetized, pithed and ventilated with air. The left carotid artery and the dorsal vein of the penis were cannulated respectively for measuring arterial diastolic b.p. and for i.v. injection of drugs. After the preparation was allowed to stabilize for 15 min, NaCl (0.9%) or SR 47436 (solubilized in arginine, 3 mg/kg) was administered

intravenously. At the end of 15 min, a cumulative dose response curve for vasopressin was constructed. Vasopressin dose-dependently induced a pressor response. SR 47436 did not inhibit the pressor response to vasopressin.

In the second study, male Sprague-Dawley rats were prepared surgically with indwelling vascular cannulas; each rat had a cannula in a carotid artery for recording b.p. and a second cannula in a jugular vein for injection of drugs. The pulsatile diastolic and systolic b.p. were continuously recorded in each conscious rat over one hour before and one hour after the administration of cumulative doses (1 + 3 mg/kg at an interval of 30 min) of SR 47436 (solubilized in arginine). The doses of two agonists, norepinephrine (0.1 to 1 µg/kg) and vasopressin (20 to 60 ng/kg), were chosen to elicit a rise in the systolic arterial pressure higher than 30 mm Hg. These agonists were injected before the administration of SR 47436 and, subsequently, 15 min after each administration of test drug. SR 47436 did not significantly reduce the pressor response to norepineprhrine or vasopressin.

In the third study, the nature of angiotensin II antagonism by SR 47436 was evaluated in pithed male Wistar Janssen rats. Further, this activity was compared with two other angiotensin II receptor antagonists, DuP 753 and saralasin. The left carotid artery and the dorsal vein of the penis were cannulated respectively for measuring arterial diastolic pressure and i.v. injection of drugs. Separate groups of rats received i.v. doses of SR 47436 (0.3, 0.6, 1, 3, 6, or 10 mg/kg), DuP 753 (1, 3, 6, or 10 mg/kg)(both solubilized with arginine) or saralasin (0.03, 0.1, 0.3 or 1 mg/kg). Cumulative intravenous doses of angiotensin II were administered beginning 15 min after dosing with test compounds.

SR 47436 dose-dependently decreased the pressor responses to angiotensin II. Increasing doses of SR 47436 reduced both the maximal amplitude of the angiotensin II dose-pressor response curve and also the slope of this dose response curve (Fig. 1.1.4A). The nature of this effect was similar to that observed *in vitro* in the rabbit aorta preparation (see section 1.1.1.2). Reduction of the maximal effect and non-parallel shift to the right of the dose respone curve suggest that this antagonism was of the noncompetitive, unsurmountable type. Because of the nature of the antagonism, *in vivo* pD'_2 (and not *in vivo* pA_2, a quantitative measure for competitive antagonists) is a valid parameter. Calculation of *in vivo* pD'_2 (a quantitative measure for noncompetitive antagonists) values for each tested dose of SR 47436 did not change with the maximal pressor response to angiotensin II. The

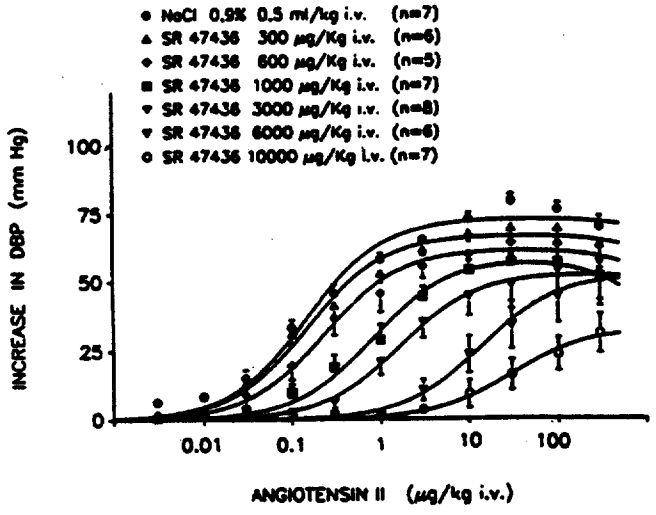

Figure 1.1.4A: Cumulative dose-response curves to ang II in the absence and presence of different doses of SR 47436 in pithed rats. Drugs were administered i.v.

calculated *in vivo* pD'_2 value averaged 4.98 ± 0.1. In contrast, the activity of the reference compound DuP 753 was characterized as competitive antagonism. Cumulative doses of DuP 753 did not reduce the maximal amplitude of the angiotensin II dose response curve, and shifted the curve to the right in a parallel manner (Fig. 1.1.4B). Thus, the *in vivo* pA_2 calculated for each dose of DuP 753 was independent of the dose tested and the mean value was 5.85 ± 0.12. Saralasin on the other hand, while inhibiting the pressor response to angiotensin II in a dose-dependent manner, tended to reduce both the maximal response as well as the slope of the dose response curve (Fig. 1.1.4C). The calculated *in vivo* pD'_2 value for each tested dose of saralasin changed with the maximal pressor response to angiotensin II, suggesting 'atypical' (i.e. neither competitive nor noncompetitive) antagonism.

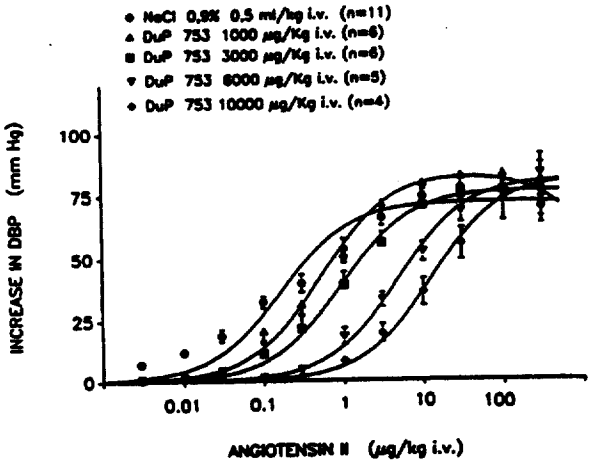

Figure 1.1.4B: Dose-response curves for inhibition of angiotensin-II induced pressor response in pithed rat: comparison of DuP.

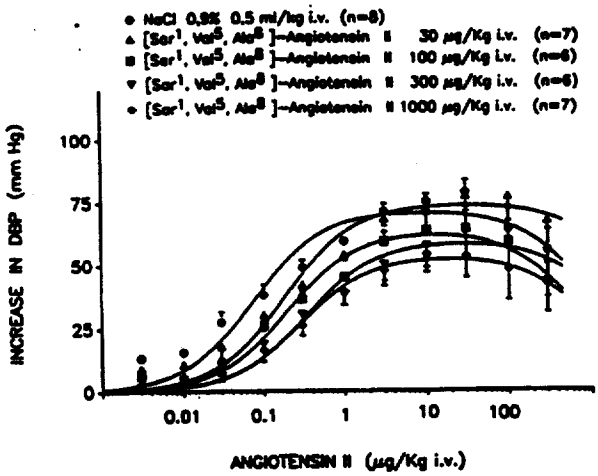

Figure 1.1.4C: Dose-response curves for inhibition of angiotensin-II-induced pressor response in pthed rat: comparison of Sar^1,Val^5, Ala^8 (sarlasin).

B. Hypertension Models

a) **High Renin Hypertension:** The dose-response effects of SR 47436, administered orally, on b.p. and heart rate were studied in the 2 kidney, 1 clip (2K-1C, Goldblatt method), renal hypertensive rat (n=7). Renin-dependent hypertension was established in rats by constriction of one renal artery. Blood pressure was recorded from conscious rats beginning the day after implantation of the catheters. Arterial pressure was recorded for 30 min before and for 24 hr after oral administration of 10 mg/kg SR 47436 (in KOH) or vehicle. SR 47436 induced a marked (maximum 29% at 10 hr, $P < 0.01$) and sustained hypotensive effect (> 24 hr); the differences between vehicle- and SR 47436-treated groups were statistically significant 1 hr and 3 to 10 hr after dosing. The onset of activity was delayed by 60 min. Twenty-four hours later, the mean blood pressure was still 17% lower than vehicle control but the difference was not significant ($P > 0.05$). The heart rate slightly increased (5 to 16%) throughout the experiment but the heart rate effects were not significant except at the 4 and 10 hr observations.

In another study, experimental renin-dependent hypertension was induced in rats by ligation of the left renal artery (two kidney, one ligation model). Five days later, two catheters were implanted in each rat, one for recording b.p. from the abdominal aorta and another for i.v. administration of drugs into the jugular vein. Arterial pressure was recorded from these animals soon after their return to conscious state. On the day of experiment, b.p. was recorded for 30 min before and for 3 hr after administration of SR 47436 or vehicle. One group of rats received single oral doses of 1, 3, or 10 mg/kg SR 47436 (dissolved in KOH) by gavage. In these animals, SR 47436 produced a dose-related, progressive decline in mean arterial pressure. At 1 and 3 mg/kg, the onset of the hypotensive effect was delayed (150 and 120 min, respectively). It was more rapid (30 min) at 10 mg/kg. At all three doses the peak activity occurred at 6 hr; it was 14 (P >0.05), 23 (P<0.05), and 32% (P <0.01) (Fig. 1.1.5A, Table 1.1.3). Twenty-four hours later, the mean arterial pressure was still below that of the control group (14%, P <0.05) in those rats given 10 mg/kg SR 47436. The hypotensive effects were not accompanied by an increase in heart rate although a slight nonsignificant increase was obtained at 10 mg/kg. A light bradycardia of 10% (P <0.05) was observed at 1 mg/kg at 5 and 6 h (Table 1.1.3).

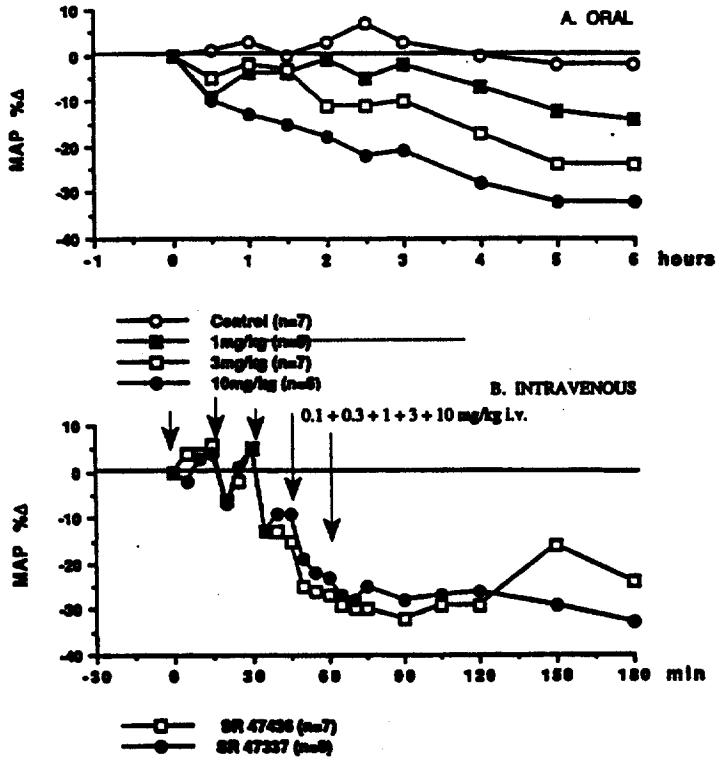

Figure 1.1.5.: Effects of SR 47436 on mean arterial pressure (MAP) in conscious 2K-1L hypertensive rats. A: Oral route; B: Cumulative intravenous doses as indicated above for SR 47436 and SR 47337.

In the same study, a second group of rats received cumulative intravenous doses of SR 47436 or SR 47337 (DuP 753) (0.1, 0.3, 1, 3, and 10 mg/kg at intervals of 15 min). Hypotensive effects for both drugs were observed at 0.3 or more mg/kg and the effects reached a peak between 5 and 30 min postinjection. Reductions in mean arterial pressure for SR 47436 were 6%, 15%, 27%, and 32% (all P <0.01) at doses of 0.3, 1, 3, and 10 mg/kg, respectively. SR 47337-induced effects were similar to those of SR 47436 in magnitude (7%, 13%, 23%, and 33% (all P <0.01) at doses of 0.3, 1, 3, and 10 mg/kg, respectively) and duration (Fig. 1.1.5B). A concomitant significant increase in heart rate accompanied the fall in mean arterial pressure (Table 1.1.3).

TABLE 1.1.3.
EFFECTS OF ORAL AND IV ADMINISTRATION OF SR 47436 ON MEAN ARTERIAL BLOOD PRESSURE AND HEART RATE IN 2K-1L HYPERTENSIVE RATS (N = 7 TO 8)

Dose (mg/kg)	After oral administration						After I.V. administration	
	Mean arterial pressure			Heart rate			MAP	Heart rate
	Maximal effect (variation %)	Time of maximal effect (h)	Duration (h)	Maximal effect (variation %)	Time of maximal effect (h)	Duration (h)	Maximal effect (variation %)	Maximal effect (variation %)
Control	-9*			+6**				
0.1	Doses not studied orally						+6*	7NS
0.3							-6**	+10**
1	-14NS	6		-11*	6	>6	-15*	+21**
3	-23**	6	>6	+6NS			-27**	+30**
10¶	-32**	6 to 8	>24	+15*		2	-32**	+34**

NS: not significant; *: $p \leq 0.05$; **: $p \leq 0.01$; ¶: dose studied for 24 hours.

b) Low-Renin Hypertension: (i) *Genetic Hypertension*: Acute Studies: These studies investigated the antihypertensive effects of SR 47436 in conscious SHR, an experimental model for non-renin-dependent hypertension. A day before the experiment, male SHR (Wistar) were anesthetized and two catheters were implanted in each rat, one catheter for recording arterial b.p. from the abdominal aorta and another one for intravenous administration of SR 47436 into the jugular vein. Mean arterial pressure and heart rate were recorded in conscious rats before and at predetermined intervals for 3 hr after administration of SR 47436 or vehicle. Two studies were conducted, one study with the arginine salt of SR 47436 and another study with the potassium salt of SR 47436. Intravenous administration of the arginine salt of SR 47436 at 10 mg/kg produced a slight hypotensive effect reaching a maximum of 7% at 15 min with a duration of approximately 120 min ($P < 0.01$). The intensity and duration of these effects were very slightly enhanced at 30 mg/kg. Maximum intensity (12%, $P < 0.01$) was reached

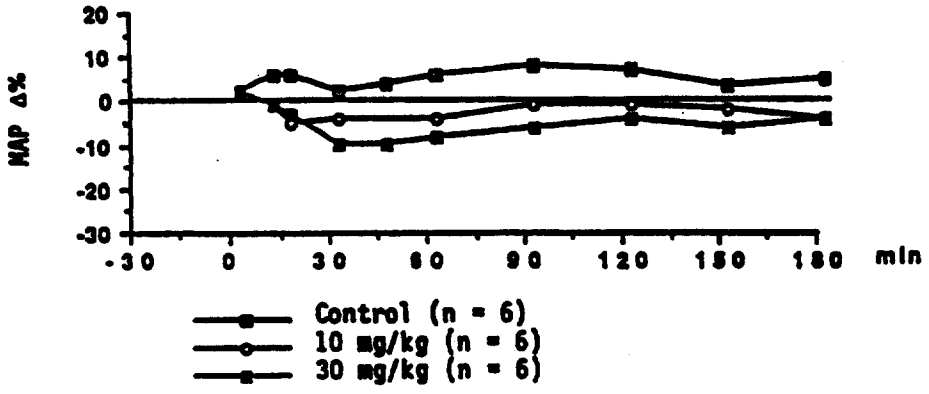

Figure 1.1.6.: Effects of SR 47436 administered intravenously on mean arterial pressure in the unanesthetized 13-week SH rat.

at 30 min while duration of effect lasted approximately 120 min (Fig. 1.1.6). Heart rate was unchanged at 10 mg/kg. However, at 30 mg/kg, an increase ranging from 14% (not significant) to 26% ($P<0.05$) was observed throughout the experiment (150 min).

In the second study, in which the potassium salt of SR 47436 (SR 47436A) was used, 30 mg/kg induced a slight hypotensive effect ranging from 10% to 12% (significant at $p<0.01$ level). The duration of this effect was 180 min. Heart rate was slightly increased ($p \leq 0.01$) between 45 and 180 min.; the amplitude of effect was small in contrast to that observed with the arginine salt of SR 47436. The findings thus suggest that the SHR model does not seem to be a suitable model for the acute study of angiotensin II antagonists.

Chronic Study: The effects of SR 47436 on the early development of genetic hypertension (GH) in SH rats were studied during the treatment of the animals from the age of 4 weeks until the age of 20 weeks and after treatment interruption until the age of 28 weeks (i.e., 8 week recovery period). Hemodynamic and histomorphometric parameters were measured at the ages of 8, 14, 20 and 28 weeks. Systolic arterial pressures (SAP), heart rates (HR) and bodyweights were determined weekly. Test substance was formulated as a suspension in distilled water containing a few drops of a 20% gum arabic solution and administered orally (gavage) at a dose of 60 mg/kg/day (0.1 ml/kg).

Two experimental protocols were conducted in parallel. One was designed to study the effects of SR 47436 on GH and on changes in hemodynamic parameters (treated group B compared with control group A); the other one was designed to study the effects of SR 47436 on the histomorphometric parameters (treated group D compared with control group C).

SR 47436, chronically administered to the young SHR at the daily oral dose of 60 mg/kg from the age of 4 weeks to the age of 20 weeks, repressed the development of GH. On the last day of treatment, the arterial pressures of the treated animals were significantly lower than those of the control animals. After treatment interruption, the arterial pressures of the animals previously treated with SR 47436 increased progressively but remained significantly lower than those of the control animals, at least until the age of 27 weeks (Fig. 1.1.7), which demonstrates that the preventive effect of SR 47436 against GH persisted after the end of its administration.

During treatment, the preventive effect of SR 47436 against GH was explained at the hemodynamic level by the limitation of the increase, usually observed as a function of age in the SHR, in total peripheral resistance and in all the regional vascular resistances, particularly those of the muscles, the skin, the kidneys and the heart. Simultaneously, the inhibition by SR 47436 of vascular remodeling (prevention of the development of the vascular smooth muscle of hypertrophy and of fibrosis), and (b) cardiac remodeling (prevention of myocytic hypertrophy and of fibrosis), resulted in the interruption of the vascular and cardiac amplifiers and thus greatly contributed to the prevention of GH in a context of improved vascular compliance and myocardial oxygenation. After treatment interruption, at the time when the hemodynamic effects of SR 47436 had disappeared, the durable persistence of the inhibition of vascular remodeling,

thus of the interruption of the vascular amplifier, permitted the prolonged maintenance of the preventive effect against GH, despite the slow and progressive development of left ventricular hypertrophy. The overall results suggest that the mechanism responsible for these preventative effects is the interruption of the renin-angiotensin system at a crucial time of development of GH.

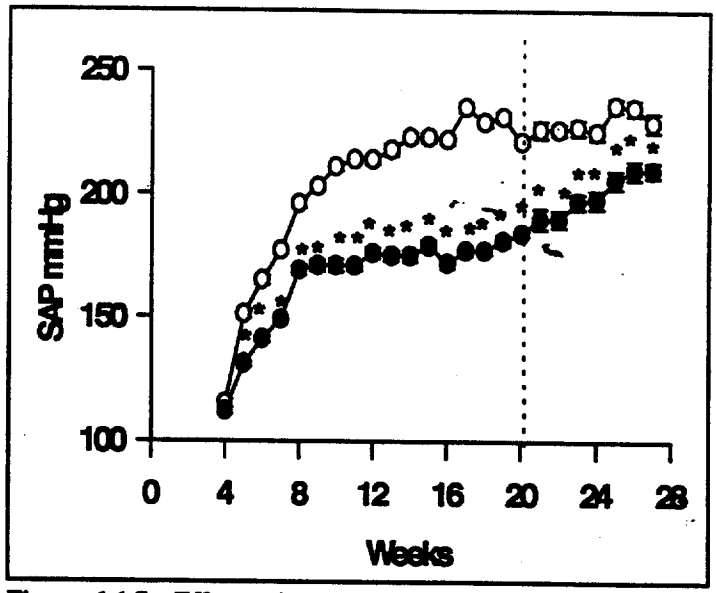

Figure 1.1.7.: Effects of age and treatment on systolic arterial pressure (SAP) in conscious control SHRs (○) or in SHRs treated with SR 47436 (●). *: value significantly different from the corresponding control value, $p < 0.05$. The vertical dotted line indicates treatment interruption.

(ii) ***DOCA-salt hypertensive rats***: The antihypertensive effect of SR 47436 was investigated in the desoxycorticosterone acetate (DOCA)-salt hypertensive rat, a low-renin model of hypertension. In this study, SR 47436 (30 mg/kg, p.o.) did not decrease either mean blood pressure or heart rate. However, it increased both plasma renin activity and plasma angiotensin II levels. Thus, the results suggest that SR 47436 blocks juxtaglomerular angiotensin II receptors and consequently induces a release of renin (through interruption of negative feedback mechanisms).

1.1.1.2.2. Studies in Mongrel Dogs

A. Normotensive

a) **Inhibition of angiotensin II-induced pressor responses:** Under anesthetic conditions, chronically indwelling catheters, one for recording aortic blood pressure and another for intravenous infusion (in the cephalic vein) of angiotensin II, were implanted in one male and seven female dogs. Experiments were conducted in conscious state. Repeated challenge doses of 10 or 20 ng/kg angiotensin II were administered (as bolus in volume of 0.5 ml), initally at 10-min intervals until a consistent pressor response to angiotensin II was noted and then at 30-min intervals after administration of the test compound. In a cross over design, the dogs received 1, 3, and 10 mg/kg SR 47436 and 3, 10, and 30 mg/kg DuP 753 in capsules (not all dogs received all doses); a 7-day treatment-free interval separated successive tests for each dog.

SR 47436 inhibited angiotensin II-induced pressor responses at all dose levels; the intensity of inhibition was related to SR 47436 dose. At 1 mg/kg, the effect began 2 hr after administration and lasted for 2 hr 30 min with a maximum inhibition of angiotensinII-induced diastolic blood pressure response of 18% ($P > 0.05$). A higher dose of 3 mg/kg decreased the elevation of

diastolic b.p. induced by angiotensin II by a maximum of 45% (P <0.01). This activity started at 1 hr after the administration of the drug and lasted for 5 hr. The highest dose tested, 10 mg/kg, inhibited the angiotensin II-induced response by a maximum of 82% (P <.001) with onset of activity at 30 min, reaching its maximal value at 1 hr 30 min and ending 5 hr after administration (Table 1.1.4). SR 47436 produced no marked alterations in baseline diastolic arterial pressure or heart rate. DuP 753 produced qualitatively similar but less intense inhibition of angiotensin II activity and table 1.4 compares it with SR 47436.

TABLE 1.1.4
INHIBITION BY SR 47436 AND DUP 753 OF DIASTOLIC HYPERTENSIVE EFFECT INDUCED BY 10 NG/KG I.V. ANGIOTENSIN II.

Compound	Dose (mg/kg)	Maximal inhibition (%)	Maximal inhibition (hours)	Total duration (hours)
SR 47436	1	-18 NS	2.5	2.5
	3	-45 **	2.5	4.0
	10	-82 ***	1.5	4.5
DUP 753	3	-18 NS	1.0	0.5
	10	-36 ***	3.0	4.0
	30	-45 NS	1.5	4.0

Ns : Not Significant ; ** : $p < 0.01$; *** : $p < 0.001$ (Student's 't' test on paired samples)

b) **Hemodynamic study:** The hemodynamic profile of SR 47436 was evaluated in anesthetized, normotensive dogs (seven males and one female). The parameters measured were systolic and diastolic blood pressure, pulmonary arterial diastolic pressure, left ventricular end diastolic pressure (LVEDP), heart rate, cardiac output, blood flow in carotid, renal and femoral arteries, and lead II ECG. SR 47436 (dissolved in KOH) was administered intravenously in five successive doses of 0.2, 0.5, 1, 2, and 5 mg/kg at 30 or 60 min intervals.

No alterations in hemodynamic values attributable to SR 47436 were noted at doses up to and including 2 mg/kg. Small increases in pulmonary arterial diastolic pressure, and small decreases in left ventricular end diastolic pressure and total peripheral resistance were noted following administration of 5 mg/kg SR 47436; these values, however, remained within the normal range of variability for this animal model. There were no changes in ECG values attributed to SR 47436.

c) **Cardiovascular and respiratory tolerance study:** The effects of SR 47436 on hemodynamics and myocardial function and on the respiratory system were assessed after single intraduodenal administration in anesthetized dogs (2 males and 8 females). Separate groups received 30 and 60 mg/kg SR 47436 (suspended in a 10% gum arabic suspension) by the intraduodenal route. Hemodynamic, cardiac, and respiratory measures were recorded at predetermined intervals from 10 to 150 min after SR 47436 administration.

At 30 mg/kg, only left ventricular contractility was slightly and transiently increased. All other hemodynamic, cardiac and respiratory parameters were unchanged or remained within the limits of spontaneous variation compatible with the experimental model. Principal findings after

administration of 60 mg/kg SR 47436 were: decrease in b.p. (23 mm Hg) due to decrease in total peripheral vascular resistance (maximum 20 mm Hg), moderate increase followed by decrease in cardiac output (19% and -21%) and left ventricular stroke volume (16% and -20%), decrease in left ventricular pressure (10%), and transient increase (14%) followed by slight decrease (16%) in left ventricular contractility. A slight improvement in the respiratory flow and tidal volume was observed at the high dose level only.

B. Sodium-depleted Dogs

The effects of orally administered SR 47436 on blood pressure, heart rate and plasma concentrations of angiotensin II and aldosterone were measured in conscious sodium depleted dogs. Fourteen male and 2 female mongrel dogs weighing between 17 and 23 kg were used. Sodium depletion was achieved by the combined use of a low sodium diet (dog food containing 0.1 g Na/kg given at the rate of 30 g/kg/day) on days 1 to 7 and 10 mg/kg/day i.m. of furosemide on days 2 to 5. A single oral (by gavage) dose of 10 mg SR 47436 (in capsule form)/kg was administered on day 8 and parameters were studied up to 6 hr after treatment.

Sodium depletion increased heart rate but left arterial pressure unaffected. Baseline values for plasma angiotensin II (7 pg/ml) and aldosterone (29 pg/ml) before furosemide treatment were very low. After treatment, there were striking increases in both of these parameters (Table 1.1.5). The administration of an empty capsule (placebo group) did not induce any change in the different parameters recorded. The administration of SR 47435 (10 mg/kg p.o.) induced a marked decrease in diastolic b.p., from 78 ± 4 mm Hg to 48 ± 5 mm Hg with maximal effect (39%) at 210 minutes. This effect on diastolic pressure first appeared 60 min after SR 47436 administration and was significant between 90 and 345 minutes. A similar effect was observed on systolic arterial pressure, with values decreasing from 123 ± 6 mm Hg to 93 ± 7 mm Hg at 180 min (the time of maximal effect) (Table 1.1.5). This effect on systolic arterial pressure first appeared 75 min after SR 47436 administration and was significant between 150 and 360 minutes. The hypotensive effect of SR 47436 was accompanied by a slight increase in heart rate, from 88 ± 5 bpm to 117 ± 17 bpm at 90 min ($P>0.05$). Additionally, SR 47436 markedly increased plasma angiotensin II concentration, particularly at 90 and 180 min (164%, Table 1.1.5). A rapid and long lasting decrease in aldosterone secretion was also observed in dogs treated with SR 47436.

TABLE 1.1.5
EFFECTS OF ORALLY ADMINISTERED SR 47436 IN SODIUM- DEPLETED CONSCIOUS DOG.

Treatment	Parameters	Basal values After Na depletion	Effects after Treatment of Sodium-depleted dogs (D8)		
		Maximal effects, %, (D0 - D8)	Maximal effects, %	Time of maximal effect, hr	Duration of effects, hr
CONTROL	HR	+ 10	- 11 NS	2.5	-
	DAP	+ 6 NS	- 6 NS	5.5	-
	SAP	- 3 NS	- 7 NS	4.5	-
	MAP	+ 1 NS	- 6 NS	5.5	-
	AII	+ 3771	- 12 NS	3.0	-
	ALDO	+ 25107	+ 4 NS	1.5	-
SR 47436	HR	+ 29	+ 34 NS	1.5	4.5
	DAP	+ 4 NS	- 39*	3.5	> 6
	SAP	- 2 NS	- 24*	3.0	> 6
	MAP	+ 1 NS	- 32*	3.0	> 6
	AII	+ 3917	+ 164 ●	1.5	3
	ALDO.	+ 8926	- 40 NS	1.5	-

* : $p < 0.05$; ** : $p < 0.01$ « U » test (Mann/Whitney) versus basal valuesO ; $p < 0.05$ Dunnett's *versus* control group values ; ●: $p < 0.05$ Student's test *versus* control group values; n=6 in all groups. D0: day 0, before furosemide treatment; D8: 8 days after initiation of sodium depletion regimen.

1.1.1.2.3. Studies in Monkeys (*Macaca fascicularis*)

A. Normotensive

a) **Inhibition of angiotensin II-induced pressor responses: Single dose study:** Under anesthetic conditions, a chronic indwelling catheter was implanted into the thoracic aorta for recording arterial pressure. After a recovery period of 3 weeks, monkeys were trained to sit quietly in restraining chairs and b.p. was recorded in conscious state. Angiotensin II (50 to 100 ng/kg, 0.5 ml/kg) administered by i.v. bolus injection into a saphenous vein produced an approximately 30 mmHg increase in mean arterial pressure. The challenge doses of angiotensin II were repeated 15 min before and at predetermined intervals after administration of SR 47436. SR 47436 (as L-arginine salt) was administered orally by gavage at 1 and 3 mg/kg or intravenously at 1 mg/kg. Mean arterial pressure and heart rate were monitored 30 min before and for 3 (i.v. study) or 5 hr (oral study) post-administration.

SR 47436 given at 1 mg/kg intravenously caused a maximal inhibitory effect on the angiotensin II response (89%) at 15 min postinjection. The effect decreased gradually with time and was still present (28%) at 3 hr after the injection. SR 47436 reduced baseline mean arterial pressure by 7% and heart rate by 9%. Oral administration of 1 mg/kg SR 47436 induced a maximal decrease of the angiotensin II pressor response at 30 min post-administration (66%)(Table 1.1.6). This effect decreased gradually and was only 10% at 5 hr post-administration. Baseline arterial pressure and

heart rate were decreased by 7% and 14%, respectively. At 3 mg/kg, p.o., SR 47436 produced a maximal inhibition (84%) of the angiotensin II pressor response at 30 min post-administration (Table 1.1.6). The inhibition was 55% at 2 hr and 20% at 5 hr. This dose also caused a fall in baseline b.p. of 9% within 1 hr of treatment; and a decrease in heart rate, which was less pronounced than that obtained at the lower dose (3 to 8%).

TABLE 1.1.6
INHIBITORY EFFECT OF SR 47436 ON THE PRESSOR RESPONSE TO ANGIOTENSIN II IN CONSCIOUS MONKEYS

		Effect on MAP			*Effect on AII pressor response*		
Route	Dose (mg/kg)	Maximal effect (%)	Time of maximal effect (hours)	Duration (hours)	Maximal inhibition (%)	Time of maximal inhibition (hours)	Duration (hours)
P.O	1	- 7	1.0	5	- 66	0.5	2 to 3
P.O	3	- 13	2.5	5	- 84	0.5	4
I.V	1	- 7	0.25	2	- 89	0.25	3

b) **Repeated dosing study:** In a related study, mean arterial pressure, heart rate, plasma renin activity (PRA), and plasma concentrations of active renin (AR), angiotensin II, and aldosterone were measured before, during, and after daily administration of SR 47436 to conscious monkeys (one male and nine females) for 6 days. Arterial blood pressure was measured from a chronically implanted aortic catheter. SR 47436 (in KOH) was administered orally by gavage for six consecutive days (day 1 to day 6) at 1, 3, and 10 mg/kg/day. Arterial pressure and heart rate were monitored before the beginning of treatment (day 1) for 30 min; during treatment on days 1, 3 and 6 from 30 min before to 45 min after each administration, then at the fifth hour postdose over 30 minutes; and after withdrawal of treatment on days 7, 8 and 10 for 30 min. Blood samples were collected through the arterial catheter at the corresponding times of b.p. measurements.

Administration of the vehicle did not induce any notable variation either in mean arterial pressure (-3 to -4%) or heart rate (-4 to -7%) throughout the period of treatment. On day 1, SR 47436 induced a dose-related decrease in mean arterial pressure. It was maximal at 45 min post administration (12, 14, and 17% for doses of 1, 3, and 10 mg/kg, respectively, all Ps <0.01) and the effect was still observable at 5 hr (9% to 10% for all doses, all Ps <0.05)(Table 1.1.7 part 1). For groups given 3 or 10 mg/kg/day SR 47436, the intensity of the hypotensive activity was greater after the third (23 and 22%, respectively, P <0.01) and sixth doses (25 and 24%, P <0.01) than after the first dose and the duration of the hypotensive activity was prolonged (>24 hr). The hypotensive activity persisted for more than 48 hr after the sixth dose and returned to approximately pretreatment mean arterial pressure values by 96 hr after the the last administration. Heart rate was still slightly decreased 45 min after each treatment at all three doses. However, heart rate effects were not dose-related (Table 1.1.7 part 1).

The hypotensive effects induced by SR 47436 were associated with changes of the renin-

angiotensin-aldosterone system, where PRA, AR and angiotensin II increased, and aldosterone decreased. The concentrations of PRA, AR (Table 1.1.7 part 2), and angiotensin II (Table 1.1.7 part 3) increased for the groups given 3 and 10 mg/kg/day from day 1 through day 6. The increases were dose-related and peaked out 45 min post-administration throughout the treatment period. After cessation of dosing, PRA and plasma concentrations of AR and angiotensin II slowly declined to near pretreatment values at a slower rate compared with return of b.p. values. Aldosterone was inhibited throughout the 6-day treatment at all three doses. The values after the sixth dose were 52, 73, and 89% when compared with pretreatment values for groups given 1, 3, and 10 mg/kg SR 47436, respectively (Table 1.1.7 part 3). On day seven, the inhibition of plasma aldosterone levels ranged from approximately 40 to 60% in the three dose groups and it disappeared almost entirely 48 hr postadministration. The findings demonstrate that in the conscious monkey, daily oral administration of SR 47436 for 6 days results in a sustained hypotension without any rebound effect in spite of the activation of the RAAS.

TABLE 1.1.7

DOSE EFFECTS OF A DAILY ORAL ADMINISTRATION OF SR 47436 IN THE MONKEY OVER A 6-DAY PERIOD.

1. Maximal effect (% var) on MAP and HR at 45 minutes

	Before	During					After
Doses		D1		D3		D6	
(mg/kg)		MAP	HR	MAP	HR	MAP	HR
0		-2°	-4	-2°	-7	-4	-5
1		-12**	-10*	-11**	-14**	-12**	-10*
3		-14**	-11*	-23**	-11**	-25**	-14**
10		-17**	-9*	-22**	-8	-24**	-4

2. Maximal effect (% var) on PRA and AR at 45 minutes

	Before	During					After
Doses		D1		D3		D6	
(mg/kg)		PRA	AR	PRA	AR	PRA	AR
0		-13°	-5°	-35	-22°	-36°	-37°
1		+244	+184	+194	+202°	+180	+193
3		+86	+60	+356	+335	+367	+439
10		+120	+127	+294	+510	+517	+1496

3. Maximal effect (% var) on AII and aldosterone (Ald) at 45 min.

	Before	During					After
Doses		D1		D3		D6	
(mg/kg)		AII	Ald	AII	Ald	AII	Ald
0		-18	/	-36°	/	-64°	/
1		+122	-61	+200°	-51	+289	-52
3		+136	-70	+457	-76	+900	-73
10		+182°	-81	+353	-90	+747	-89

Student's paired sample t-test : * : $p \leq 0.05$; ** : $p \leq 0.01$; ° : Effect at 5 hours

B. Sodium-depleted monkeys

a) **Single dose, oral:** Under anesthetic conditions, a chronic catheter was implanted into the thoracic aorta of nine female and one male monkeys for recording aortic b.p. After a recovery period of 3 weeks, the animals were trained to sit quietly in a primate restraining chair. The 8 animals were sodium depleted by administering furosemide (10 mg/kg, i.m.) for five consecutive days prior to the study. This resulted in volume depletion and a corresponding activation of the RAS; arterial pressure of the animals remained within the physiological limits of normal but b.p. regulation was more dependent on renin than in untreated animals. Mean arterial pressure and heart rate were recorded for 30 min before and 3, 5, and 7 hr after the administration of SR 47436. Blood samples were collected from the arterial catheter for measurement of plasma renin activity (PRA), active renin (AR) and angiotensin II. Single oral doses of 0.3, 1, or 3 mg/kg SR 47436 (solubilized in L-arginine solution) were administered by gavage (5 ml/kg volume).

The oral administration of SR 47436 at the doses of 0.3, 1 and 3 mg/kg induced hypotensive effects which were dose-related both in intensity and duration. These effects were rapid in onset (15-30 min) and reached their peaks of 11% ($P < 0.05$, at 90 min) at the dose of 0.3 mg/kg, 23% ($P < 0.01$, at 60 min) at the dose of 1 mg/kg, and 30% ($P < 0.01$, at 45 and 60 min) at the dose of 3 mg/kg. Their respective durations were 180, 300 and ≥420 min (Table 1.1.8 part 1). These hypotensive effects were correlated with increases in PRA, AR, and angiotensin II (Table 1.1.8 part 2). There were no marked changes in heart rate.

TABLE 1.1.8
EFFECTS OF ORALLY ADMINISTERED SR 47436 IN THE SODIUM DEPLETED MONKEYS

1. Maximal effect on MAP and Heart rate (HR)

Dose(mg/kg)	MAP (var %)	Maximal effect (h)[1]	Duration of effect (h)	HR (var %)	Maximal effect (h)	Duration of effect (h)
0.3	-11*	1.50	3	-6 NS	3.00	-
1	-23**	1.00	5	-5 NS	1.00	-
3	-30**		>7	-4 NS	1.25	-

2. Maximal effect on plasma renin activity (PRA), active renin (AR) and Heart rate (HR)

Dose (mg/kg)	PRA (var %)	Maximal effect (h)[1]	Duration of effect (h)	AR (var %)	Maximal effect (h)	Duration of effect (h)	AII (var %)	Maximal effect (h)	Duration of effect (h)
0.3	+33*	1.5	1.5	+ 59*	1.5	3	+56*	1.5	1.5 to 3
1	+79*	1.5	5	+155*	1.5	>7	+180*	1.5	5 to 7
3	+84NS	1.5	>7	+113*	1.5	>7	+151°	0.5	7

Student's paired sample t-test : NS : Not Significant ; * : $p < 0.05$; ** : $p < 0.01$; ° : $n = 1$
1: Time of maximal effect in hours

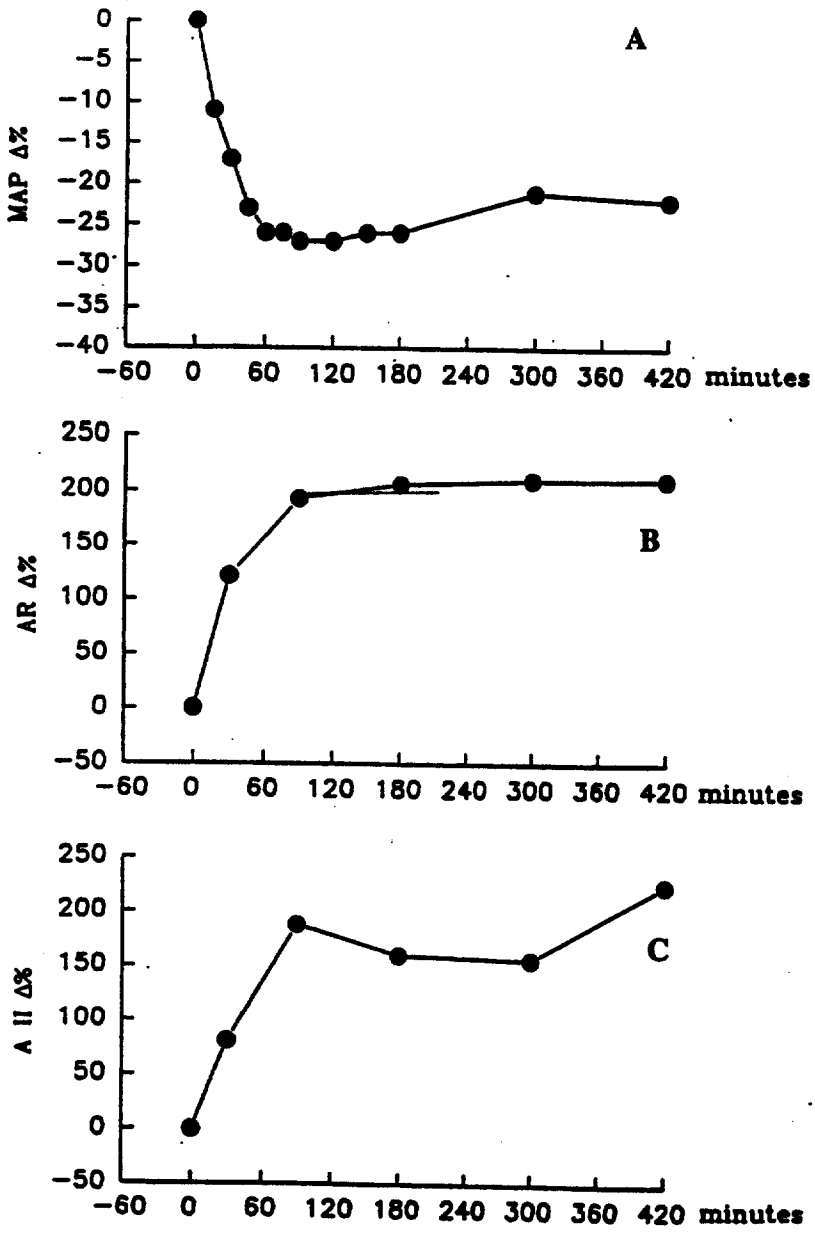

Figure 1.1.8.: Effects of orally administered suspension of SR 47436 at 3 mg/kg on MAP (panel A), active renin (AR)(panel B) and angioteinsin II (panel C) in sodium-depleted macaque.

In a related study, the activity of SR 47436 administered orally by gavage in an insoluble form (suspension in 5% gum arabic) was evaluated under conditions similar to those described in the previous paragraph. Single oral doses of 3 mg/kg SR 47436 caused a decrease in mean arterial pressure. The maximal effect (26-27% decrease compared with pretreatment values) was reached 60 min postdosing. Five and seven hours after dosing, b.p. was reduced by 21% and 22%, respectively (Fig. 1.1.8A). There were no marked changes in heart rate attributed to SR 47436. Plasma AR (Fig. 1.1.8B) and angiotensin II (Fig. 1.1.8C) increased markedly by 90 min postdosing and maximal effect was observed at 7 hr: 209% and 225%, repectively, compared to pretreatment values. The results show that, although SR 47436 was administered in an insoluble form, its efficacy was as good as that previously observed when it was given in solution (see above).

b) **Single dose, intravenous:** The pretreatment, recording procedures, and blood sampling procedures were similar to the experimental design described above (1.1.1.2.3. B (a)). One male and seven female sodium-depleted monkeys received single intravenous doses of 0.1, 0.3, or 1 mg/kg SR 47436 (solubilized in L-arginine)(n=4 males or females/dose). All doses induced a

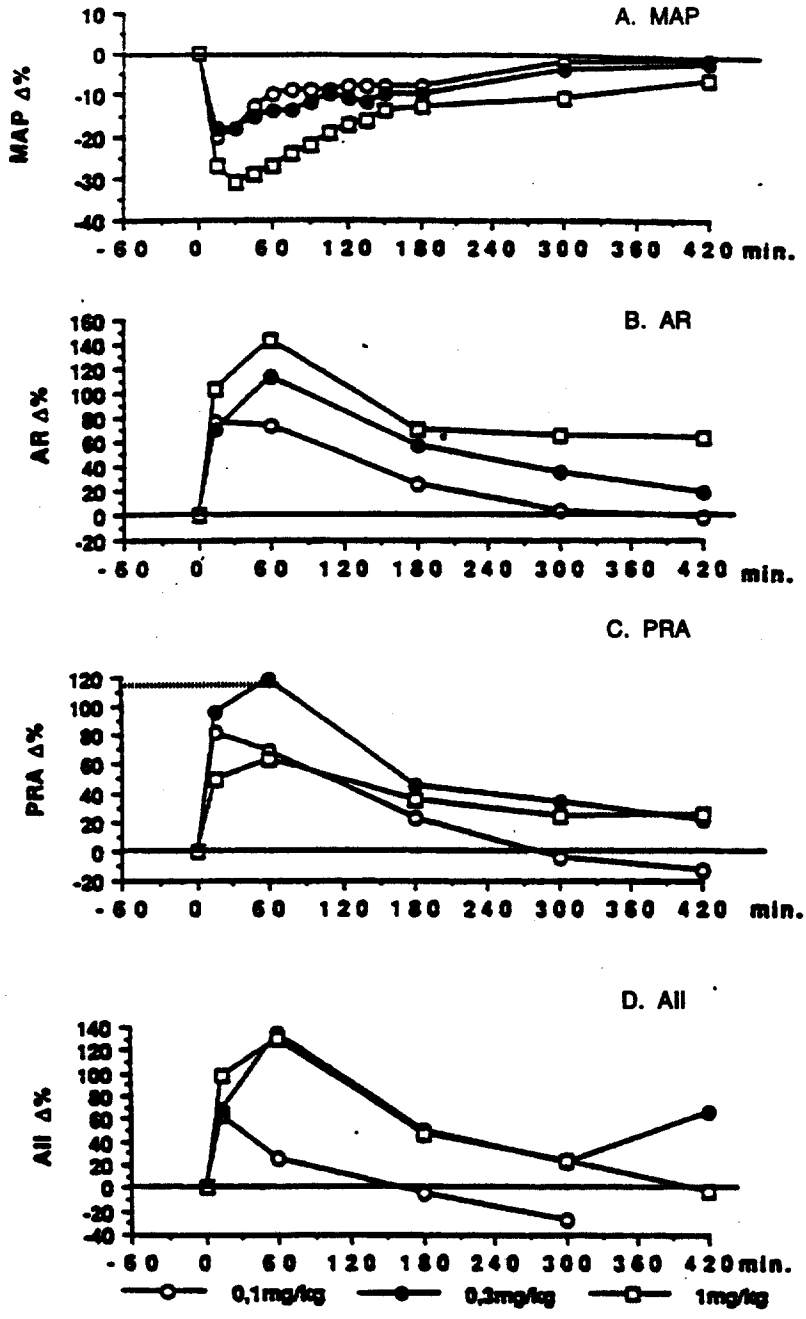

Figure 1.1.9.: Effects of SR 47436 on MAP (A), active renin (B), plasma renin activity (C) and angiotensin II (D) in the conscious sodium-depleted macaca (n=4/dose).

decrease in mean arterial pressure. These hypotensive effects reached their peaks (15-30 min of dosing) of 20% ($P < 0.01$) at the dose of 0.1 mg/kg, 18% ($P < 0.01$) at the dose of 0.3 mg/kg and 31% ($P < 0.01$) at the dose of 1.0 mg/kg. Onset of activity was similar at all dose levels; the duration of action, but not the intensity, was proportional to SR 47436 dose (2, 3 and 7 hours at doses 0.1, 0.3 and 1 mg/kg, respectively, Fig. 1.1.9A). These hypotensive effects were accompanied by an increase in PRA and angiotensin II and with the release of renin (AR). The increase in PRA was significant at 60 min postdosing, and the values were 69%, 118%, and 64%, respectively, at doses of 0.1, 0.3 and 1 mg/kg (Fig. 1.1.9C). Although the release of active renin was dose-dependent, the peak effects at 0.1 and 0.3 mg/kg were not significantly different from pretreatment values. The effects were significantly higher (peak 60 min and lasting more than 7 hr) at the dose of 1 mg/kg for all the time points measured (Fig. 1.1.9B). The increase in angiotensin II was at its peak of 62% ($P < 0.01$) at 15 min at the dose of 0.1 mg/kg (duration of effect 1 hr), 135% ($P < 0.05$) at 60 min at the dose of 0.3 mg/kg (duration of effect 5 hr), and 130% ($P > 0.05$) at the dose of 1 mg/kg (duration of effect 5 hr) (Fig. 1.1.9D). At the two lowest doses, slight and brief decreases in heart rate of -8% ($P<0.05$, lasting 75 min) and -7% ($P<0.01$, lasting 45 min), respectively, were observed.

1.2. General Pharmacology (Secondary Activities)

1.2.1. *In Vitro:*

SR 47436 was evaluated for agonist or antagonist effects at acetylcholine, bradykinin, histamine and angiotensin II receptors in guinea pig ileum at concentrations up to 10 µM. The test compound did not show any agonist activity. At concentrations of 3 µM, it produced no statistically significant inhibition of acetylcholine-, histamine,- or bradykinin-induced contractions. However, a statistically significant inhibition of the acetylcholine- and the bradykinin-elicited contractions was observed after exposure to SR 47436 at 10 µM. This may represent a nonspecific spasmolytic effect. On the other hand, SR 47436 produced a dose-related inhibition of angiotensin II-induced contractions, which is suggestive of the specific pharmacological activity of the test compound.

The interaction of SR 47436 with the adrenergic system was studied in the isolated rat vas deferens. SR 47436 produced no statistically significant inhibition of norepinephrine-induced contractions of the vas deferens up to a concentration of 1 µM; 10 µM SR 47436 did produce significant inhibition (-42%, $P \leq 0.05$). The same concentration did not significantly inhibit electrically-induced contractions of the vas deferens preparations. This suggests that SR 47436 is unlikely to interact with the adrenergic system. SR 47436 produced no marked effect on any of these preparations suggesting absence of agonist activity.

SR 47436 was investigated for the possible inhibition of renin angiotensin system enzymes (renin and angiotensin converting enzymes) and other aspartyl proteases, like pepsin, cathepsin D and HIV-1 proteases. The results showed that SR 47436 neither inhibits baboon and human renins, nor other aspartyl proteases, thus confirming the specificity of action of SR 47436.

1.2.2. *In Vivo:*

Test substance was evaluated for activity on central or autonomic nervous systems of male mice. Groups of 10 mice each received single oral doses of 0, 30, 60, or 120 mg/kg SR 47436 (suspended in 5% gum arabic soultion) by gavage. No mortality was noted over the 7-day follow-up period for any groups given SR 47436. Nor were there effects on overt behavior (modified Irwin method), body weight gain, rectal temperature, muscle tone or motor coordination. At the highest dose tested (120 mg/kg), spontaneous activity was lower (24 to 29%) for the group given SR 47436 than for the group given vehicle but the difference was not statistically significant.

The potential effect of SR 47436 on intestinal transit was assessed in mice using the charcoal meal test. Groups of ten male mice each received single oral doses of vehicle (5% gum arabic solution) or 60 mg/kg SR 47436 by gavage. Thirty minutes after treatment mice were given orally 0.25 ml of a 4% vegetal charcoal suspension in 0.2% CMC. The mice were sacrificed 30 min later and intestinal transit of the charcoal was measured. Intestinal transit, expressed as a percentage, was measured as the ratio of the distance covered by the charcoal meal to the total

length of the intestine. SR 47436 at a dose of 60 mg/kg did not modify intestinal charcoal transit in mice.

A study was performed to assess the potential effect of SR 47436 on gastric emptying in female rats. SR 47436 was given orally in single doses of 30 or 60 mg/kg by gavage as a suspension in a 5% gum arabic solution. Sixty minutes after treatment, rats were given orally 1 ml of phenol red as a 0.07% suspension in a 1.5% CMC solution. The animals were sacrificed 10 min later and gastric emptying (total amount of phenol red administered minus amount remaining in stomach contents) was measured. No significant effects on gastric emptying were noted after administration of 30 mg SR 47436/kg. However, at 60 mg/kg SR 47436 induced a moderate and significant ($P < 0.05$) inhibition (-38%) of gastric emptying.

The effect of SR 47436 on gastric acid secretion was determined in female OFA rats. SR 47436 was given orally (gavage) as a suspension in a 5% gum arabic solution at dosage levels of 30 or 60 mg/kg. Sixty minutes after dosing, each rat was anesthetized and the pylorus was ligated; rats were killed 1 hr later and the stomach contents were collected. No significant effects were noted on gastric acid secretion (volume, pH and hydrogen ion concentration) in any of the groups given SR 47436.

A study was performed to assess the effect of SR 47436 on the "hydroelectric balance" of male and female OFA rats after a single oral administration at 30, 60 or 120 mg/kg. Test compound was administered by gavage as a suspension in a 10% gum arabic solution. Urinalysis (biochemistry, and electrolyte and creatinine excretion) was performed on urine collected over the 16-hour period postdosing. According to the sponsor, no marked abnormalities attributed to SR 47436 were noted for urine specific gravity, proteins, glucose, ketone bodies, bilirubin, urobilinogen, or leukocyte count. Urine pH remained within the normal range in males, while in females the values were higher at all dose levels (8.5, 8.4 and 8.2, respectively, at doses of 30, 60 and 120 mg/kg) than for the vehicle treated females (7.7) but the differences (statistically significant at 30 and 60 mg/kg) were non-dose related. The sponsor suggests that these differences stem from the abnormally low urine pH for control females (7.7) in comparison to control males (8.5). Urine volume was slightly lower for males given 30 (9.1 ± 9.43 ml), 60 (8.5 ± 0.6 ml), or 120 (7.9 ± 0.5 ml) mg/kg SR 47436 and for females given 30 (6.5 ± 0.37 ml) or 60 (6.95 ± 0.6 ml) mg/kg SR 47436 but the differences were not statistically significant compared to control-group values (males: 9.8 ± 0.5 ml, females: 7.75 ± 0.42 ml).

Concentration and amount of chloride excreted in urine were lower for both sexes from 30 mg/kg without enhancement of the effect at the higher dose levels. Variations in chloride concentration were statistically significant only in females at 30 (27 ± 3.8 mM, $P<0.001$) and 120 mg/kg (32.31 ± 3.1 mM, $P < 0.027$) when compared to control group values (46 ± 3.6 mM). With regard to excreted quantities, variations were statistically significant or close to significant in both sexes at 30 mg/kg ($P=0.055$ in males, $P=0.004$ in females), and in males at 120 mg/kg ($P=0.031$). Potassium concentration in the urine was significantly lower for the group of females given 120 mg/kg SR 47436 (74.1 mM, $P < 0.004$) than for the group given vehicle (101.2 mM) although the

amount of potassium excreted in urine was similar for both groups. No significant variation for potassium was noted in males. No marked variations in calcium concentration or excretion in urine attributed to SR 47436 were observed. A dose-related increase in creatinine concentration was noted in males at all dose levels (3, N.S.; 3.3, N.S.; and 3.6 mM, $P < 0.01$, respectively, at doses 30, 60 and 120 mg/kg versus 2.8 mM, control), whereas, a slight and nonsignificant decrease was observed in females at 60 and 120 mg/kg. These findings are indicative of changes in kidney function produced by SR 47436 in long term toxicity studies (see section 3.0).

In the same study, blood samples were collected on day 2 (16 hr postadministration) from the retro-orbital sinus to measure hematocrit and assay electrolytes. No marked varations attributed to SR 47436 were noted for: hematocrit; plasma concentrations of sodium potassium, chloride, and calcium; and plasma creatinine concentration. Endogenous creatinine clearance (calculated from creatinine concentrations in urine and plasma, and 16-hour urine volume) values for groups of males given SR 47436 were not significantly different from control group values. In females, the values were slightly lower for groups given 60 (-13%) or 120 mg/kg SR 47436 (-17%) than for control group but the differences were not statistically significant.

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2. DRUG DISPOSITION (ADME)

For all pharmacokinetic studies, the formulation of SR 47436 for oral administration was a suspension in a 10% aqueous solution of gum arabic. The formulation for intravenous administration was a solution of SR 47436 in 0.04 - 0.1% L-arginine in saline. For all *in vitro* studies, SR 47436 was dissolved in methyl or ethyl alcohol, or dimethylsulfoxide.

2.1. Absorption and Pharmacokinetics

The non-compartmental parameters reported in this subsection were defined as follows:

Cmax: maximum plasma concentration observed in the dosage interval
Cmin: minimum plasma concentration observed in the dosage interval
C_0: plasma concentration observed before daily administration (in i.v. studies, it is the zero intercept value calculated from the first data points by linear regression after a log transformation on the concentration observed)
Tmax: time of Cmax observed
C_{24h}: plasma concentration observed 24 hours after administration
Cmean: mean plasma concentration value in the dosage interval
T½ ß: apparent terminal half-life calculated from last data points by the peeling algorithm (T½ ß = log 2/ß, where ß is the slope of the curve in semi-logarithmic scale)
$AUC_{(0\text{-}24)}$: area under the plasma concentration curve between time 0 and 24 hours post administration.
$AUC_{(0\text{-}C\ last)}$: area under the plasma concentration curve between time 0 and time of C last (last null concentration observed).
$AUC_{(0\text{-}Inf)}$: area under the plasma curve extrapolated from time 0 to infinity = AUC (0-C last) + C last/ß
$AUC_{(obs)}$: area under the plasma concentration curve between the first and the last sampling time
AUC_{ext} : area under the plasma concentration curve, extrapolated
Cl/F: plasma clearance extrapolated to infinity/bioavailability factor = Dose PO/AUC(0-Inf)
MRT: Mean residence time extrapolated to infinity calculated from AUC(0-Inf)
Vd/F or Vd: distribution volume/bioavailability factor calculated from the terminal phase =[Cl/F]/ß
PTF: peak trough fluctuation = (Cmax-Cmin)/Cmean
R: accumulation factor = AUC(0-24)(day *last* (e.g., 29)) / AUC(0-24)(day *first* (day 1))
F: bioavailability, %

2.1.1. Single I.V. and Single and Repeated Oral Administration of SR 47436 in Rats (Report # RS 0005920618/01, Study #TPK0009), Vol. 70

This non GLP study was conducted by the department of Metabolism and Pharmacokinetics of Sanofi Recherche, Montpellier Cedex, France between January and June 1992. The objective of the study was to determine the pharmacokinetic profiles of SR 47436 after single 1 mg/kg i.v. and repeated 10 mg/kg oral administrations in the Sprague-Dawley (CD(SD)BR) rat.

Male (320 gm) and female (230 gm) rats (8 weeks old) received single 10 mg/kg oral (n=21♂+21♀) or 1 mg/kg intravenous (n=24♂+24♀) doses of SR 47436 (batch 91.01). Intravenously, the 5 ml/kg volume was injected in a caudal vein as a bolus. Orally, the same volume was administered by gavage. Additional groups of 21 male and 21 female rats received oral doses of 10 mg/kg/day SR 47436 for 8 or 28 days (n=21/sex/dose day). Blood was collected from the abdominal aorta from 3 animals/sex/sampling time point. Samples were collected at 0 (before dosing), 0.083, 0.5, 1, 2, 4, 8, and 24 hr following single i.v. dose administration. For oral route, samples were collected at: 0 (before dosing), 0.5, 1, 2, 4, 8, and 24 hr following administration on dosing days 1, 8 and 28. Animals were not fasted before treatment.

Results

The pharmacokinetic values following intravenous administration were different for males and females. In males, pharmacokinetics were characterized by a long terminal half-life, a low plasma clearance, a long mean residence time and a large distribution volume. In females, no assessment of the terminal half-life was possible since no appreciable decrease in SR 47436 plasma concentration was observed between 0.5 h and 24 hr post-administration. This is presumably because of slow elimination of SR 47436 from plasma (Table 2.1.1.1).

TABLE 2.1.1.1
SR 47436 PHARMACOKINETIC PARAMETERS AFTER SINGLE 1 MG/KG INTRAVENOUS ADMINISTRATION IN RATS

PARAMETERS	MALES	FEMALES
C_0 (mg/l)*	2.444	1.701
TERMINAL SLOPE (1/h)	0.0592	-
TERMINAL HALF-LIFE (h)	11.7	-
AUC(0-24h) (mg.h/l)	9.03	15.79
AUC(0-Inf) (mg.h/l)	12.05	-
AUCext (%)	26.6	-
Cl(0-24h) (l/h.kg)	0.111	0.063
Cl(0-Inf) (l/h.kg)	0.083	-
MRT(0-Inf) (h)	16.2	-
Vd (l/kg)	1.4	-

* the zero intercept concentration value extrapolated by linear regression

After single or daily repeated 10 mg/kg oral administrations of SR 47436, no major inter-sex differences in SR 47436 plasma concentration profiles were observed. Elimination of SR 47436 was slow and appreciable amounts of SR 47436 persisted in plasma 24 hr after dosing and thus the apparent terminal half-life was not calculable. SR 47436 pharmacokinetics were similar on days 1, 8 and 28 with no accumulation and low peak trough fluctuations (Table 2.1.1.2). The estimated bioavailability of SR 47436 after single administration was 22% in males and 11% in females. However, the sponsor does not consider these values as accurate estimations due to incomplete drug elimination during the sampling interval.

TABLE 2.1.1.2
SR 47436 PHARMACOKINETIC PARAMETERS DURING REPEATED 10 MG/KG/DAY ORAL ADMINISTRATIONS IN RATS

Parameters	MALES			FEMALES		
	Day 1	Day 8	Day 28	Day 1	Day 8	Day 28
Tmax (h)	24	1	1	1	2	24
Cmax (mg/l)	0.892	1.324	0.823	0.919	0.921	0.974
Cmin (mg/l)	0	0.443	0.363	0	0.570	0.551
C0 (mg/l)	0	0.443	0.363	0	0.570	0.551
C24h (mg/l)	0.892	0.557	0.550	0.632	0.721	0.974
Cmean (mg/l)	0.827	0.671	0.630	0.748	0.676	0.825
AUC (0-24h) (mg.h/l)	19.85	16.11	15.13	17.95	16.22	19.79
C1/F (0-24h) (1/h.kg)	0.504	0.621	0.661	0.557	0.616	0.505
PTF	-	-	0.73	-	-	0.51
R	-	-	0.76	-	-	1.10
F(0-24h) (%)	22.0	-	-	11.4	-	-

2.1.2. Single Oral Administration of SR 47436 in Male Rats (Report # RS 0005930623/01, Study #ABS0127). Vol. 70

This non GLP study was conducted by the Toxicology department of Sanofi Recherche, Montpellier Cedex, France between December 15, 1992 and January 15, 1993. The objective of the study was to determine the pharmacokinetic profiles of SR 47436 after single 10, 30 and 90 mg/kg oral administrations in male Sprague-Dawley rats.

A single dose of 10, 30 or 90 mg SR 74436 (batch 92.02)/kg was given orally by gavage (5 ml/kg) to three groups of 30 males each. The rats (Sprague-Dawley, CD(SD)BR)) were 7 weeks old and weighed 183 - 213 gm. The animals were dosed in a non-fasted status. Blood was collected from the abdominal aorta from 3 animals/dose/sampling time point. Samples were collected at: 1, 2, 3, 4, 8, 24, 32, 48, 56 and 72 hr after dosing.

Results

No clinical abnosrmality was observed following single oral adminstration of test substance. SR 47436 was quantified in all plasma samples except in two samples collected at 56 and 72 hr post dose in the low dose group. A moderate inter-individual variablility in SR 47436 plasma levels was observed at each sampling time during the first 48 hr post dose. Irrespective of the dose administered, SR 47436 pharmacokinetics were characterized by a rapid absorption and a long apparent half-life (14-19 hr). Cmax and AUC increased less than expected by dose proportionality (Table 2.1.2.1).

TABLE 2.1.2.1
SR 47436 PHARMACOKINETIC PARAMETERS IN PLASMA AFTER SINGLE 10, 30 AND 90 MG/KG ORAL ADMINSTRATION IN MALE RATS

Parameters	Dose (mg/kg)		
	10	30	90
Cmax (mg/ml)	0.578	1.145	1.451
Tmax (h)	2.000	4.000	4.000
T½β (h)	13.500	19.000	17.300
$AUC_{(0\text{-}24h)}$ (mg.h/l)	8.830	14.270	19.290
$AUC_{(0\text{-}\infty)}$ (mg.h/l)	15.830	24.440	31.120
$MRT_{(0\text{-}\infty)}$ (h)	22.400	26.000	24.300

In a related study (Report # RS 0005950502/02, Study #DPK0039), blood and plasma kinetics were measured in two strains of rats (Wistar and Sprague-Dawley) after oral administration of a single 5 mg/kg dose of [^{14}C]-SR 47435 (batch 4SNP006) and in Wistar rats after oral administration of a single 500 mg/kg dose. After dosing with 5 mg/kg, T½ (hour) in plasma was

longer in females than in males (49 *vs.* 13 for Wistar rats and 72 *vs.* 22 for S-D rats). Cmax in plasma was approximately the same for all the animals treated with 5 mg/kg; values were more than 100-fold increased for (Wistar) rats treated with 500 mg/kg.

2.1.3. Blood Distribution, Pharmacokinetics and Urinary Excretion of Radioactivity Following Single Oral Administration of [^{14}C]-SR 47436 in Female Rabbits (Report # RS 0005951031/02, Study #LPR0066), Vol. 71

This non GLP study was conducted by the Preclinical Metabolism and Pharmacokinetics Department of Sanofi Recherche, Montpellier Cedex, France (study period January 1995). The objective of the study was to determine the blood distribution and pharmacokinetic profiles of SR 47436 after single 10 mg/kg oral administrations in female New Zealand hybrid rabbits.

Suspensions of [^{14}C]-SR 47436 (batch 93.06) were administered orally by gavage (2 ml/kg) at a single dose of 10 mg/kg (n=9). The animals were fasted overnight. The rabbits were 18 weeks old and weighed 3.24 - 3.73 kg. The animals were dosed in a non-fasted status and fed 4 hours after administration. Blood was collected from the jugular vein from 3 animals/sampling time (2-3 sampling times/animal). Samples were collected at: 0, 1, 2, 4, 8, 24, 48 and 72 hr after dosing. Urine samples were collected from a pool of 3 animals at: 0-8 h, 8-24 h and 24-48 h after administration of test substance.

Results

The concentration of radioactivity in plasma was higher than in blood at all investigated time points. The maximal blood and plasma concentrations of total radioactivity were found 8 hr after drug intake (Table 2.1.3.1). A large inter-individual variability was observed in blood concentration and urinary excretion data. Urinary excretion was low, accounting for 3.33, 15.33 and 22% of the administered dose over 0-8, 0-24 and 0-48 hrs, respectively.

TABLE 2.1.3.1
PHARMACOKINETIC PARAMETERS (MEAN ± SD) OF TOTAL BLOOD AND PLASMA RADIOACTIVITY AFTER SINGLE ORAL ADMINSTRATION OF 10 MG/KG [^{14}C]-SR 47436 TO FEMALE RABBITS

Parameters	Blood	Plasma
Cmax (mg Eq/kg or l)*	0.291 ± 0.04	0.42 ± 0.09
Tmax (hour)	8.00	8.00
T½ (hour)	13.10	13.40
$AUC_{0-\infty}$ (mg Eq.h/kg or l)*	7.50	10.76

*: blood radioactivity is expressed as mg Eq/kg or mg Eq.h/kg and plasma radioactivity is expressed as mg Eq/l or mg Eq.h/l

2.1.4. Preliminary Pharmacokinetic Profile of SR 47436 After Single Oral and Intravenous Administration to Monkeys (Report #RS0005920408/01, Study #MPK133), Vol. 71

This non GLP study was conducted by the department of Metabolism and Pharmacokinetics of Sanofi Recherche, Montpellier Cedex, France between January and March 1992. The objective of the study was to determine the pharmacokinetic profiles and absolute bioavailability of SR 47436 after single 1 mg/kg i.v. and repeated 10 mg/kg oral administrations in the monkey.

Two groups of two male (6.64-7.45 kg) *Macaca fascicularis* monkeys each received single oral doses of 10 mg SR 47436 (batch 91.01)/kg by gavage (5 ml/kg) (n=4) and bolus (1 ml/kg) intravenous doses of 1 mg/kg (n=4). A cross-over design with 15 days of washout was used. Animals were fasted overnight the day before administration, dosed in the morning and then fed in the afternoon. Blood was collected from the femoral vein at: 0 (predose), 0.083 (in i.v. dosed animals only), 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 24, 30, 48, 54, 72, 96, 120 and 144 hr following administration.

Results

Following i.v. administration, the plasma SR 47436 concentration declined exponentially during the first 4 to 6 hr of dosing, followed by an increase reaching a secondary maximum concentration, then again declining. The pharmacokinetics was characterized by a long terminal half-life, low plasma clearance, long mean residence time and large distribution volume (Table 2.1.4.1). After oral administration, SR 47436 appeared in plasma at 0.25 hr after dosing, the earliest sampling interval. The time to maximum plasma SR 47436 concentration (T_{max}) as well as C_{max} varied considerably among the four monkeys. The coefficient of variation of the pharmacokinetic parameters was in the range of 12 to 77% (Table 2.1.4.1). Several secondary peaks appeared in the concentration versus time curve. The investigator noted that the origin of these variations was unknown but may be due to enterohepatic recirculation. The calculated mean absolute bioavailability of SR 47436 was 78 ± 38% and ranged from 45 to 120% (Table 2.1.4.1). This suggests that oral absorption of SR 47436 was good but variable.

TABLE 2.1.4.1

SR 47436 PHARMACOKINETIC PARAMETERS AFTER SINGLE 1 MG/KG INTRAVENOUS AND 10 MG/KG ORAL ADMINISTRATIONS TO THE MACACA.

PARAMETERS	MALE 1	MALE 2	MALE 3	MALE 4	MEAN	SD	CV %
A. INTRAVENOUS ROUTE							
DOSE (mg/kg)	1	1	1	1			
C_0 (mg/l)	6.059	5.344	10.488	8.853	7.686	2.405	31
TERMINAL SLOPE (1/h)	0.0239	0.0407	0.0310	-	0.0319	0.0084	26
TERMINAL HALF-LIFE (h)	29.0	17.0	22.4	-	22.8	6.0	26
AUC (0-Clast) (mg.h/l)	5.530	3.436	6.353	3.464	4.696	1.477	31
AUC (0-Inf) (mg.h/l)	6.241	4.123	7.031	-	5.798	1.504	26
AUCext (%)	17.6	26.5	21.6	-	21.9	4.5	20
Cl(0-Clast) (l/h.kg)	0.181	0.291	0.157	0.289	0.230	0.071	31
Cl(0-Inf) (l/h.kg)	0.160	0.243	0.142	-	0.182	0.054	30
MRT (0-Inf) (h)	30.5	25.6	25.8	-	27.3	2.8	10
Vd (l/kg)	6.7	6.0	4.6	-	5.8	1.1	19
B. ORAL ROUTE							
DOSE (mg/kg)	10	10	10	10			
Cmax (mg/l)	2.656	0.441	2.408	0.948	1.613	1.086	67
Tmax (h)	4.0	6.0	2.0	0.5	3.1	2.4	77
TERMINAL SLOPE (1/h)	0.0388	0.0291	0.0334	0.0460	0.0368	0.0073	20
TERMINAL HALF-LIFE (h)	17.9	23.8	20.7	15.1	19.4	3.7	19
AUC (0-Clast) (mg.h/l)	74.054	18.073	48.092	12.015	38.059	28.717	75
AUC (0-Inf) (mg.h/l)	74.569	18.657	49.079	12.732	38.759	28.696	74
AUCext (%)	0.7	3.3	2.1	5.9	3.0	2.2	74
Cl/F(0-Clast) (l/h.kg)	0.135	0.553	0.208	0.832	0.432	0.323	75
Cl/F (0-Inf) (l/h.kg)	0.134	0.536	0.204	0.785	0.415	0.303	73
MRT (0-Inf) (h)	32.7	37.0	30.6	27.5	32.0	4.0	12
MAT (0-Inf) (h)	2.2	11.4	4.8	-	6.1	4.7	77
Vd/F (l/kg)	3.5	18.4	6.1	17.1	11.3	7.6	67
F (0-Clast) (%)	133.9	52.6	75.7	34.7	74.2	43.2	58
F (0-Inf) (%)	119.5	45.3	69.8	-	78.2	37.8	48

2.1.5. Pharmacokinetic Profiles of SR 47436 in Monkeys Following Escalating Oral doses (Report #RS0005920902/01, Study #ABS0100) Vol. 71

This non GLP study was conducted by the department of Metabolism and Pharmacokinetics of Sanofi Recherche, Montpellier Cedex, France between January and July 1992. The objective of the study was to determine the pharmacokinetic profiles of SR 47436 after single 10, 30 and 90 mg/kg oral administrations in the male monkey.

Three male (4.4-5.6 kg) *Macaca fascicularis* monkeys each received single oral doses of 10, 30, and 90 mg/kg SR 47436 (batch 91.01) by gavage (5 ml/kg) in a randomized cross-over design with 15 days of washout (n=3/dose level). Animals were fasted overnight the day before administration, dosed in the morning and then fed in the afternoon. Blood samples were collected from a femoral vein at: 0 (predose), 0.5, 1, 2, 4, 6, 8, 24, 30, 48, 54, 72, 96, 120 and 144 hr following administration.

Results

As noted in the previous study (see section 2.1.4), the inter-individual variability of SR 47436 pharmacokinetics in the monkeys was large. The coefficient of variation range of the pharmacokinetic parameters was large, especially for the group given 30 mg/kg SR 47436 (3 to 110%). This variability did not allow an accurate assessment of dose-proportionality in AUC, C_{max} and T_{max}. The maximum SR 47436 concentration in plasma was not linearly related to dose (mean values of 1.3, 4.4, and 3.5 mg/l for groups given 10, 30, and 100 mg/kg SR 47436, respectively). For the two higher doses, C_{max} did not increase with the dose administered. However, AUC values and the plasma concentration 24 hr after dosing increased with increasing doses of SR 47436. SR 47436 pharmacokinetics were characterized by a T_{max} observed in the 0.5 to 4 hr range, a long apparent terminal half-life (12-26 hr), a variable oral clearance (0.2 to 1.3 l/hr.kg) and a long mean residence time (13 to 41 hr, Table 2.1.5.1). The pharmacokinetics observed in this study were consistent with those obtained after single 10 mg/kg oral administration (section 2.1.4) and after 10 mg/kg/day repeated dose administration (see below, section 2.1.6).

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TABLE 2.1.5.1

SR 47436 PHARMACOKINETIC PARAMETERS (Mean ± SD) IN PLASMA AFTER SINGLE 10, 30 AND 90 MG/KG ORAL ADMINSTRATION IN MALE MONKEYS

Parameters	Dose (mg/kg)		
	10	30	90
Cmax (mg/l)	1.30 ± 0.3	4.40 ± 4.8	3.50 ± 1.1
Tmax (h)	2.50 ± 1.8	0.80 ±0.3	3.00 ± 1.0
C24h (mg/l)	0.19 ± 0.068	0.69 ± 0.67	1.34 ± 0.41
T½β (h)	18.80 ± 6.6	13.60 ± 1.4	15.70 ± 0.5
$AUC_{(0\text{-}C\,last)}$ (mg.h/l)	15.40 ± 5.9	70.10 ± 69.8	88.80 ± 21.5
$AUC_{(0\text{-}inf)}$ (mg.h/l)	16.60 ± 6.7	70.90 ± 69.4	90.00 ± 20.7
Cl/F (l/h.kg)	0.70 ± 0.36	0.74 ± 0.51	1.04 ± 0.24
MRT (h)	26.20 ± 11.1	23.70 ± 15.3	31.50 ± 4.9
Vd/F (l/kg)	17.2 ± 4.8	15.1 ± 11.1	23.5 ± 5.6

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2.1.6. Pharmacokinetic Profiles of SR 47436 After Multiple (1 month) Oral Dosing in Monkeys (Report #RS0005920529/02, Study #TPK0008) Vol. 72

This non GLP study was conducted by the department of Metabolism and Pharmacokinetics of Sanofi Recherche, Montpellier Cedex, France between January and April 1992. The objective of the study was to determine the pharmacokinetic profile of SR 47436 during one month of repeated daily 10 mg/kg oral administrations in the male monkey.

Four male (3.95-6.19 kg) *Macaca fascicularis* monkeys each received oral doses of 10 mg/kg/day SR 47436 (batch 91.01) by gavage (5 ml/kg) daily for 29 days. Diet was distributed at 1 p.m. and withdrawn 1 to 2 hours later. Drug administrations were performed in the morning. Blood samples were collected from a femoral vein before and at preset intervals from 0.25 to 24 hr after the first and eighth doses and from 0.25 to 144 hr after the 29th dose.

Results

The inter-individual variability of SR 47436 plasma levels was large (CV, 16-97%). SR 47436 plasma levels, T_{max}, C_{max} and C_{24hr} observed on day 1 were consistent with those observed in an earlier study (see section 2.1.4) after single dose administration at the same dose level (AUC much lower in the present study). Since the pharmacokinetic parameters values on days 8 and 29 were generally similar (Table 2.1.6.1), it is concluded that steady-state was reached within 8 days of daily dosing with SR 47436.

TABLE 2.1.6.1
SR 47436 PHARMACOKINETIC PARAMETERS (Mean ± SD) IN PLASMA AFTER REPEATED ORAL ADMINSTRATION (10 mg/kg/day) TO MALE MONKEYS

Tmax (h)	4.00 ± 1.40	1.40 ± 1.10	3.50 ± 0.60
Cmin (mg/l)	0	0.17 ± 0.10	0.14 ± 0.12
C_{24h} (mg/l)	0.16 ± 0.13	0.24 ± 0.18	0.20 ± 0.14
T½β (h)	-	-	20.00 ± 6.40
$Cl/F_{(0-24h)}$ (l/h.kg)	-	1.22 ± 0.39	1.52 ± 0.63
PTF	-	-	2.00 ± 0.20

2.1.7. Plasma Levels of SR 47436 after Single Dose Administration to Rats, Monkeys and Humans

Pharmacokinetic profiles of SR 47436 following single dose administration in rat, rabbit, monkey and human are summarized in Table 2.1.7.1.

TABLE 2.1.7.1
SUMMARY OF PRECLINICAL AND CLINICAL PHARMACOKINETIC PARAMETERS (MEAN ± SD) FOR SR 47436 (SINGLE DOSE) - PLASMA DATA

Animal species	Dose (mg/kg)	Route	Sex	Feed*	Cmax (mg/l)	tmax (h)	t½β (h)	AUC (mg.h/l) 0-24 h	AUC (mg.h/l) 0-Inf	Vd (l/kg)	Cl (l/h.kg)	F (%)	Ref Sec #
Rat	1	IV	M	nf	2.4	0	12	9.0	12.1	1.4	0.11		2.1.1
			F	nf	1.7	0		15.8			0.06		
	10	PO	M	nf	0.9	24		19.9				22	2.1.1
			F	nf	0.9	1		18.0				11	
	10	PO	M	nf	0.6	2	14	8.8	15.8				2.1.2
	30	PO	M	nf	1.2	4	19	14.3	24.4				2.1.2
	90	PO	M	nf	1.5	4	17	19.3	31.1				2.1.2
Macaque	1	IV	M	f	7.7 ± 2.4	0	23 ± 6		5.8 ± 1.5	5.8 ± 1.1	0.2 ± 0.1		2.1.4
	10	PO	M	f	1.6 ± 1.1	3 ± 2	19 ± 4		38.8 ± 28.7			78 ± 38	2.1.4
	10	PO	M	nf	0.4 ± 0.2	4 ± 1		5.8 ± 2.7					2.1.6
	10	PO	M	f	0.6 ± 0.2	3 ± 1	14 ± 8		5.5 ± 1.4				-
	10	PO	M	f	1.3 ± 0.3	3 ± 2	19 ± 7		17 ± 7				2.1.5
	30	PO	M	f	4.4 ± 4.8	1 ± 0	14 ± 1		71 ± 69				2.1.5
	90	PO	M	f	3.5 ± 1.1	3 ± 1	16 ± 0.5		90 ± 21				2.1.5
Human	50(mg)	IV	M	f	4.4 ± 0.9		13 ± 6		5.6 ± 1.2	0.8 ± 0.2d	0.13 ± 0.03d		-
	50 (mg)	IV	M	f	3.8 ± 0.6		16 ± 10		5.1 ± 1.6	1.3 ± 0.8d	0.15 ± 0.05d		-
	50(mg)	POa	M	f	2.1 ± 0.6	0.3c	12 ± 5		4.6 ± 1.3			82 ± 17	-
	50(mg)	POb	M	f	1.3 ± 0.4	1.3c	14 ± 9		5.0 ± 1.9			88 ± 20	-
	150(mg)	POb	M	f	1.8 ± 0.4	1.5c	16 ± 7		9.7 ± 3.0				-
	150(mg)	POa	M	f	3.3 ± 1.0	0.3c	20 ± 12		9.5 ± 4.1			61 ± 10	-
	300(mg)	POb	M	f	3.0 ± 0.9	1.5c	13 ± 7		20.0 ± 5.2				-

Cmax following intravenous injection corresponds to the back extrapolated concentration at the time of injection (C_0 value)
*: Feeding conditions, nf: non fasting condition, f: fasting condition, PO: oral route, IV: intravenous route,
a) administered as a tablet, b) administered as a solution, c) median value, d) calculation assuming an average body weight of 70 kg.

2.1.8. Plasma Levels of SR 47436 after Chronic Administration to Mice, Rats and Monkeys

Toxicokinetic profiles of SR 47436 after repeated administration in mice, rats and monkeys are discussed under toxicology studies (see studies in section 3.1). The plasma concentration profile documented in each of these studies is sumarized separately for each species on the following pages (Tables 2.1.8.1 through 2.1.8.4).

TABLE 2.1.8.1
SR 47436 PLASMA LEVELS IN CD1 MICE

Oral studies*		Dose (mg/kg/d)	Cmax or C1h¶ (mg/l)		C3h (mg/l)		AUC0-24h (mg.h/l)	
			Males	Females	Males	Females	Males	Females
4-week study[a]	Day 28	100	2.0	10.4	-	-	2.4	11.9
		300	25.2	18.5	-	-	48.1	48.0
		1000	38.1	47.1	-	-	76.1	122.4
13-week studies	Week 13[b]	15	-	0.3	-	-	-	0.4
		50	0.1	0.3	-	-	0.3	0.7
		150	3.9	22.0	-	-	5	25
		500	13.7	38.0	-	-	20	94
	Week 14[c]	1000	28.3	61.3	-	-	105	136
		2000	46.5	92.5	-	-	227	269
2-year[d] study	Week 13	100	18.1	9.8	0.1	0.2	-	-
		300	6.7	14.0	2.3	2.5	-	-
		1000	58.5	52.6	9.9	12.8	-	-
	Week 27	100	0.4	13.4	0.1	0.2	-	-
		300	9.5	30.6	2.4	0.5	-	-
		1000	10.6	38.0	11.6	4.1	-	-
	Week 53	100	2.2	4.3	0.1	0.4	-	-
		300	20.0	28.1	2.8	2.0	-	-
		1000	20.4	53.4	9.9	12.7	-	-

- : Not determined
*: drug administered orally by gavage
¶: C_{1h} in case of 2 year study
a : not reviewed
b : see section 3.4.1
c : see section 3.4.2
d : see section 3.4.3

TABLE 2.1.7.2
SR 47436 PLASMA LEVELS IN SPRAGUE DAWLEY RATS

Studies*	Interval	Dose (mg/kg/d)	Cmin (mg/l)		Cmax (mg/l)		AUC0-24h (mg.h/l)	
			Males	Females	Males	Females	Males	Females
Oral	Day 1	10	0	0	0.89	0.92	19.85	17.95
4-week study[a]	Day 8	10	0.44	0.57	1.23	0.92	16.11	16.22
(1st study)	Day 28	10	0.36	0.55	0.82	0.97	15.13	19.79
Oral		30	[0.50-0.78]	[0.63-0.91]	-	-	-	-
1-month study[b]	Day 37	70	[0.60-0.95]	[0.54-0.71]	-	-	-	-
(2nd study)		150	[0.68-0.85]	[0.57-0.73]	-	-	-	-
Oral		10	0.33	0.54	1.1	1.1	16.2	18.2
	Week 5	30	0.50	0.54	1.2	0.6	14.2	14.3
6-month study[c]		90	0.63	0.59	1.9	4.1	23.9	25.7
(1st study)		10	0.44	0.44	1.1	0.9	18.4	14.1
	Week 26	30	0.66	0.34	1.3	2.0	18.3	15.6
		90	0.62	0.38	1.6	19.3	23.4	54.1
Oral		control	0.07	0.07	0.14	0.11	2.5	2.1
		250	0.65	0.68	2.1	5.3	25.1	30.6
	Week 5	500	0.94	0.74	5.7	37.0	43.1	95.8
		1000	1.28	0.64	14.1	55.4	76.4	140.3
		control	0.08	0.03	0.17	0.08	3.2	1.6
		250	0.65	0.69	2.4	13.0	26.3	48.3
6-month study[d]	Week 13	500	1.14	0.54	9.8	51.5	57.5	255.5
(2nd study)		1000	1.36	0.79	57.5	103.9	311.1	239.8
		control	0.08	0.04	0.21	0.06	3.2	1.1
		250	0.37	0.24	2.5	43.4	23.3	127.2
	Week 26	500	0.85	0.20	5.8	2.8	41.2	26.8
		1000	0.60	0.43	58.6	88.3	147.8	226.5
Oral		10/10			0.7	1.1	13.0	16.2
	Day 1	90/90			3.1	3.0	22.4	27.2
		90			2.9	4.6	27.5	33.6
6-month study[e]		10/10			1.9	1.6	29.2	27.0
with	Month 3	90/90			2.9	6.1	37.2	49.2
SR 47436/HCTZ		90			4.1	6.8	41.6	85.3
		10/10			1.7	2.9	24.1	46.0
	Month 6	90/90			4.8	10.1	58.3	57.4
		90			3.2	9.0	36.2	54.6

IV study		Dose	Range of sampling times (min.)		Range of concentrations (mg/l)	
		(mg/kg)	Males	Females	Males	Females
		0.8	[22-312]	[22-306]	[0.42-0.83]	[0.36-0.72]
2-week study[f]	Day 16	2	[20-145]	[37-131]	[0.40-0.88]	[0.50-1.40]
		5	[28-132]	[47-245]	[0.61-1.79]	[0.72-1.13]

-: Not determined; *: in all oral studies drug was administered by gavage.
a: section 2.1.1; b: section 3.2.1 (drug administered for 36 days); c: study not reviewed; d: section 3.2.4; e: section 3.2.7; f: study not reviewed

TABLE 2.1.7.3
SR 47436 PLASMA LEVELS IN WISTAR RATS

Oral Studies*	Duration	Dose (mg/kg/d)	Cmin (mg/l)		Cmax or C2h (mg/l)		AUC0-24h (mg.h/l)	
			Males	Females	Males	Females	Males	Females
4-week study[a]	Day 28	5	0.35	0.40	0.7	0.7	11.5	11.4
		50	0.62	0.65	1.4	5.9	18.6	29.5
		500	0.66	0.87	22.3	75.8	71.3	255.8
		1000	-	1.66	-	214.2	-	495.6
13-week study[b]	Week 13	Control	0.20	0.20	0.31	0.30		
		15	0.53	0.40	1.10	1.10	17.1	16.3
		50	0.74	0.70	1.46	2.27	22.3	23.6
		150	0.70	0.66	3.36	19.4	25.7	56.9
		500	1.04	0.93	20.6	258.8	60.5	473.4
2-year study[c]	Week 5	Control	0.03	0.04	0.02	0.03	-	-
		5	0.38	0.42	0.58	0.56	-	-
		50	0.42	0.65	1.16	3.14	-	-
		500	0.97	2.03	16.4	91.3	-	-
		1000		3.91		129.2	-	-
		2000		8.84		498.6	-	-
	Week 13	Control	0.07	0.03	0.04	0.02	-	-
		5	0.49	0.65	0.59	0.65	-	-
		50	0.58	0.60	1.42	3.32	-	-
		500	0.90	1.11	25.9	87.3	-	-
		1000		1.89		78.1	-	-
		2000		3.78		298.7	-	-
	Week 27	Control	0.02	0.04	0.01	0.07	-	-
		5	0.46	0.48	0.70	0.54	-	-
		50	0.61	0.96	2.88	6.01	-	-
		500	1.17	1.80	103.1	255.4	-	-
		1000		3.45		217.8	-	-
	Week 53	Control	0.02	0.10	0.01	0.05	-	-
		5	0.99	0.42	0.80	0.72	-	-
		50	1.22	0.72	4.56	9.08	-	-
		500	1.31	4.83	35.0	141.7	-	-
		1000		4.84		171.5	-	-

- : Not determined; *: in all oral studies drug was administered by gavage.
a : study not reviewed; b: see section 3.4.4; c: see section 3.4.6

TABLE 2.1.7.4
SR 47436 PLASMA LEVELS IN MONKEYS

Oral studies*	Interval	Dose (mg/kg/d)	Cmin (mg/l)	C1h (mg/l)	C2h (mg/l)	Cmax (mg/l)	AUC_{0-24h} (mg.h/l)
4-week study[a] (1st study)	Day 1	10	0	-	-	0.42	5.75
	Day 8	10	0.17	-	-	1.04	9.24
	Day 29	10	0.14	-	-	0.77	7.51
4-week study[b] (2nd study)	Day 10	10	0.07	0.7	-	-	-
		30	0.25	1.7	-	-	-
		90	0.39	4.4	-	-	-
	Day 30	10	0.07	0.7	-	-	-
		30	0.26	2.7	-	-	-
		90	0.76	2.7	-	-	-
4-week study[c] (3rd study)	Day 33	250	1.05	6.2	17.8	28.1	107.7
		500	1.47	12.6	41.9	57.1	329.4
		1000	1.49	14.2	66.4	106.4	570.4
6-month study[d]	Day 190	10	0.12	-	0.80	0.80	6.9
		30	0.17	-	1.1	1.1	11.4
		90	0.64	-	2.3	2.3	23.3
1-year study[e]	Week 4	20	0.09	-	0.62	-	-
		100	0.57	-	2.3	-	-
		500	0.62	-	23.4	-	-
	Week 27	20	0.10	-	0.62	-	-
		100	0.45	-	1.6	-	-
		500	0.41	-	31.1	-	-
	Week 52	20	0.21	-	1.0	-	-
		100	0.37	-	1.6	-	-
		500	1.09	-	20.6	-	-
6-month study[f] with SR 47436/HCTZ	Day 1	10/10	-			0.84	11.2
		90/90	-			5.18	48.5
		90	-			8.64	33.1
	Month 3	10/10	-			0.64	5.2
		90/90	-			4.53	22.3
		90	-			4.74	20.7
	Month 6	10/10	-			0.54	7.9
		90/90	-			4.38	30.6
		90	-			11.72	30.5
IV study 2-week study[g]	Day 15	0.8	0.07		-	7.5	4.0
		2	0.26		-	12.0	6.5
		5	0.76		-	37.8	20.7

- : Not determined; *: in all oral studies drug was administered by gavage.
a: section 2.1.6; b: section 3.2.2; c: section 3.2.3; d: section 3.2.5; e: section 3.2.6; f: section 3.2.8; g: not reviewed

2.2. Distribution (Blood and Tissue distribution, and Plasma Protein Binding)

2.2.1. Blood Distribution of Labelled SR 47436 in the Rat, the Monkey (Report #RS0005920410/02, Study #LPR114) and the Human (Report #RS0005920409/01 Study #LPR217), Vol. 74

These non GLP studies were conducted by the department of Metabolism and Pharmacokinetics of Sanofi Recherche, Montpellier Cedex, France between February and March 1992. The objective of the studies was to determine, *in vitro*, the erythroplasmatic distribution of SR 47436 from spiked rat, monkey and human blood samples. Since erythrocytes act as a slow release carrier system, the sponsor felt that it would be useful to establish possible correlations of pharmacodynamics/toxicity and pharmacokinetics. The studies thus investigated the partitioning of SR 47436 between RBC and plasma in all three species.

Fresh blood samples (1 ml) from male Sprague Dawley rats, a male *Macaca fascicularis* (Mauritius) monkey and a healthy male volunteer were incubated with increasing amounts (0.01 to 20 µg) of SR 47436 (^{3}H-SR 47436 diluted with unlabeled SR 47436, batch 91.01) at 37°C for 30 min. The range of concentrations used here overlaps the concentrations found in animals during toxicology studies or encountered in humans during clinical trials. Plasma and cells were separated by centrifugation of blood. Erythrocytes were washed with normal saline and, after centrifugation, they were shattered in distilled water. Drug concentrations in plasma, washes and erythrocytes were measured by liquid scintillation counting. The results are expressed as:

$$\text{The percentage of drug in plasma, P\%} = \frac{\text{DPM plasma + DPM washes}}{\text{Total DPM recovered*}} \times 100$$

(*DPM plasma +DPM washes + DPM erythrocytes)
(DPM= disintegration per minute)

Within the range of blood concentrations investigated, the percentage of radioactivity in plasma was higher than in red blood cells. Plasma radioactivity ranged from 85 to 92% in rats, 81 to 90% in the monkey, and 91 to 96% in human. The washing of erythrocytes contributed another 12% (rat and human) or 18% (monkey). In conclusion, SR 47436 showed no significant affinity for RBC and more than 90, 80 and 80% of the whole blood radioactivity was recovered in plasma of human, rat and monkey, respectively.

2.2.2. Binding of Labeled SR 47436 to Rat, Monkey (Report #RS0005920428/01, Study #LPR514) and Human (Report #RS0005920410/03, Study #LPR412) Plasma proteins or Mouse and Rabbit (Report #RS0005951212/01, Study #LPR0525) Serum Proteins, Vol. 73

These non GLP studies were conducted by the department of Metabolism and Pharmacokinetics of Sanofi Recherche, Montpellier Cedex, France from February to March 1992 (first two studies); and September to December 1995 (the last study).

Serum samples were prepared from male Sprague-Dawley CD rats, the male *Macaca fascicularis* monkey and the human; and plasma samples were prepared from male CD1 mouse and male New Zealand rabbit. Binding of SR 47436 (batch 91.01) to plasma or serum proteins was measured using an equilibrium dialysis technique. The diffusion of SR 47436 (^{3}H-SR 47436 in case of rat, monkey and human; ^{14}C-SR 47436 in case of mouse and rabbit) through the membrane was studied at concentrations ranging from 1 to 150 mg/l in mouse and rabbit, and 0.01 to 200 mg/l in the other species. The radioactivity (measured as dpm) in the protein cell represents the free plus bound drug. The difference in dpm between the protein cell and the buffer cell represents the bound drug.

The binding percentage of the drug remained fairly constant in the rat (92-96%), in the monkey (90-94%), and in the human (90-92%) at concentrations up to 50 mg/l, then decreased to 86% in the rat and in the human, and to 84% in the monkey as concentration of SR 47436 increased to 200 mg/l. Thus, the binding of SR 47436 to serum proteins from all three species appears to be fairly high and saturable at SR 47436 concentrations higher than 50 mg/l. On the other hand, in the mouse and rabbit over the concentration range 1 to 150 mg/l, the mean fraction of SR 47436 bound to plasma proteins was 84.60 ± 1.90% and 68.44 ± 2.26%, respectively. In the rabbit, the binding appeared to be not saturable while a saturable process might take place at 150 mg/l in the mouse.

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2.2.3. Tissue Distribution Following a Single Oral Administration of ^{14}C-SR 47436 to the Male Mouse (Report #RS0005950404/01, Study #DIS0176) Vol. 74

This non GLP study was conducted by the department of Preclinical Metabolism and Pharmacokinetics of Sanofi Recherche, Montpellier Cedex, France from October 1994 to March 1995.

Suspensions of ^{14}C-SR 47436 (batch 4SNP017) were administered orally by gavage (5 ml/kg) at a single dose of 300 mg/kg. The animals were fasted overnight (to 4 hours after dosing) with free access to water. The mice (CD1) were approximately 20-28 days old and weighed 19.2 to 21.0 g. Groups of three mice (except for sampling time 168 hours where it was two) were sacrificed by diethyl ether anesthesia at 2, 8, 24, 48, 96 and 168 hours after oral administration. The animals were deep frozen and 30 µm thick 30 sagittal cross sections were obtained at different levels of anatomical interest. During sectioning, aliquots of selected organs and tissues (blood, brain and liver) were excised. The radioactivity trapped in these tissues was measured by liquid scintillation spectrometry. Each sagittal section was subjected to nuclear image analysis for qualitative whole body radioluminography. The nuclear image analysis was further submitted to a software package to generate quantitative distribution of total radioactivity in various tissues.

Results

Nuclear image processing for qualitative whole body radioluminography gave the following results:

Time	Tissues
2 hours:	bile > gut content > gastric content > liver > kidney > Harder's gland > blood > lung > heart > pancreas > skin > brown fat > spleen > salivary glands > hypophysis > bone marrow
8 hours:	liver > blood > kidney > lung > Harder's gland > skin > brown fat > heart > pancreas > spleen > salivary glands > bone marrow > thymus > muscle > testis > eye > brain > spinal cord
24 hours:	gut content > gastric content > oesophagus > liver > heart > bone marrow > blood > skin > lung > kidney> hypophysis > brown fat > thymus > muscle > pancreas > spleen > testis > eye > brain > spinal cord
48 hours:	urine > gut content > liver > kidney
96 hours:	no trace of radioactivity detected
168 hours:	no trace of radioactivity detected

The ^{14}C concentrations in the organs and tissues calculated from the nuclear image analysis (qualitative radioluminography) is given in Table 2.2.3.1. Blood, brain and liver concentrations obtained by liquid scintillation counting were well correlated with those obtained by quantitative radioluminography.

In conclusion, highest tissue concentrations were observed at the first sampling time. The radioactivity was mainly located in organs and tissues involved in absorption, metabolism and excretion. The pattern of distribution was similar over the 2 to 24 hr sampling period, but the radioactivity had decreased considerably by 24 hours. No radioactivity was detected at 96 hours after dosing.

TABLE 2.2.3.1
QUANTITATIVE TISSUE DISTRIBUTION BY RADIOLUMINOGRAPHY
MEAN ± SD ^{14}C CONCENTRATIONS (MG EQ./KG OF TISSUE) IN ORGANS AND TISSUES OF MALE MICE AFTER A SINGLE ORAL DOSE OF 300 MG/KG [^{14}C]-SR 47436

Tissues	Sampling time		
	2h	8h	24h
Adrenal gland	ND	ND	112.58 (n=1)
Bile	2373.61 (n=2)	ND	4382.92 (n=1)
Blood	221.02 ±32.19	148.30 ±92.84	139.27 (n=2)
Bone marrow	111.02 ±14.77	71.60 ±61.62	149.80 (n=1)
Brain	11.62 ±1.45	9.46(n=2) (UQL)	7.88 (n=2) (UQL)
Brown fat	150.32 ±40.42	98.71 ±84.73	69.91 (n=2)
Eye	33.98 ±4.06	18.68 ±6.72	15.59 (n=2)
Harder's gland	221.03 (n=2)	121.38 (n=2)	128.42 (n=1)
Heart	177.78 ±19.55	96.58 ±88.21	150.67 (n=1)
Hypophysis	141.31 (n=1)	ND	111.13 (n=1)
Kidney	252.91 ±16.91	141.43 ±100.81	116.58 ±103.25
Liver	433.25 ±26.58	372.25 ±109.13	206.43 ±165.92
Lung	210.45 ±24.33	129.26 ±98.47	119.24 (n=2)
Muscle	94.18 ±6.50	56.08 ±59.53	61.78 (n=2)
Pancreas	165.46 ±37.05	91.12 ±77.10	58.83 ±49.54
Salivary gland	144.69 ±33.84	80.36 ±81.50	81.89 (n=2)
Skin	160.23 ±36.75	109.99 ±91.72	115.77 (n=2)
Spinal cord	11.74 (n=2)	4.45 (n=1) (UQL)	8.18 (n=2) (UQL)
Spleen	145.23 ±11.52	86.11 ±77.77	80.94 (n=2)
Testis	38.43 ±19.23	52.19 (n=2)	49.13 (n=2)
Thymus	95.53 ±12.55	62.29 ±48.71	69.05 (n=2)

n=3 animals per time point excepted when indicated between brackets
Limit of detection: 3.06 mg Eq./kg; Limit of quantification: 10.21 mg Eq./kg; ND: Not detected; UQL: Under quantification limit

2.2.4. Excretion Balance and Tissue Distribution Following a Single (30 mg/kg) Oral Administration of ^{14}C-SR 47436 to Male (Report #RS0005930414/01, Study #DIS0037) and Female (Report #RS0005930405/01, Study #DIS0143) Rats, Vol. 75

These GLP studies were conducted by the department of Metabolism and Pharmacokinetics of Sanofi Recherche, Montpellier Cedex, France from December 16, 1992 to March 9, 1993 (males) or January 15, 1993 to February 25, 1993 (females).

Suspensions of ^{14}C-SR 47436 (batch 92.02) were administered orally by gavage (5 ml/kg) at a single dose of 30 mg/kg. The (20) animals were fasted from 12 hours before to 4 hours after dosing with free access to water. The male and female rats (Sprague-Dawley) were approximately 7-8 weeks and 9-10 weeks old, respectively, and weighed 256 ± 5 g (male) and 238 ± 4.3 g (female). Groups of three mice (except 5 animals for last sampling time, 168 hours) were sacrificed by exsanguination after ether anesthesia at 2, 8, 24, 48, 96 and 168 hours post-drug administration. Whole blood was collected and a part of it was used to prepare plasma. Major tissues and organs were collected from each animal, blotted dry, weighed, and the radioactivity trapped in these tissues was measured by liquid scintillation spectrometer. Excretion of ^{14}C-SR 47436 was determined by measuring the radioactivity present in urine and feces samples at 168 hours after dosing (n=5). Biological samples were collected at 0-6, 6-24, 24-48, 48-72, 72-96, 96-120, 120-144 and 144-168 hours post-drug administration. The metabolism cages were washed with water at 168 hours after treatment and the radioactivity present in the washing-water samples was measured.

Results

The uptake of radioactivity by the tissues and organs was poor. Most of the radioactivity was observed in the tissues and the contents corresponding to the gastrointestinal tractus and liver. Absorption of drug from small intestine was rapid with peak blood and tissue levels appearing 2 hours after dosing. The appearance of radioactivity in large intestine and its contents, the brown fat and the carcass did not occur until 8 hours after administration of drug (Table 2.2.4.1 and 2.2.4.2). The distribution of drug in tissues fell into three groups:

(a) the liver, kidneys, and GI tract with higher levels than in blood,
(b) lungs with almost equal concentration and
(c) adrenal, heart, salivary gland, spleen, testis, pancreas, ovary, prostate, eyes, bone marrow, Harder's gland, thymus, thyroid, hypophysis, skin, muscle, spinal cord, fat, bone and brain with much lower concentrations.

By 48 hours, the radioactivity had been essentially eliminated, with 99% (females) or 102% (males) of the dose recovered in urine and feces (Table 2.2.4.3), 0.5 to 2% remaining in carcass.

TABLE 2.2.4.1

MEAN TOTAL RADIOACTIVITY (AS % OF THE ADMINISTERED DOSE) OBSERVED IN WHOLE ORGANS AFTER 30 MG/KG SINGLE ORAL ADMINISTRATION OF ^{14}C-SR 47436 TO MALE RATS

TIME (hr)	2	8	24	48	96	168
BRAIN	0.01	0.002	ILQ	ILQ	ILQ	ILQ
LUNGS	0.02	0.01	0.001	ILQ	ILQ	ILQ
LIVER	5.47	2.93	0.59	0.05	0.02	0.01
HEART	0.02	0.003	0.001	ILQ	ILQ	ILQ
KIDNEYS	0.15	0.03	0.005	0.003	0.001	ILQ
TESTICLES	0.05	0.01	ILQ	ILQ	ILQ	ILQ
STOMACH	0.65	0.03	0.01	0.002	ILQ	ILQ
SMALL INT.	7.22	0.32	0.02	ILQ	ILQ	ILQ
LARGE INT.	0.54	2.65	0.09	0.01	ILQ	ILQ
SALIVARY GL	0.01	0.002	ILQ	ILQ	ILQ	ILQ
THYMUS	0.01	0.002	ILQ	ILQ	ILQ	ILQ
PANCREAS	0.01	0.003	0.001	ILQ	ILQ	ILQ
SPLEEN	0.01	0.002	0.0005	ILQ	ILQ	ILQ
PROSTATE	0.04	0.01	0.0002	0.0002	ILQ	ILQ
BLADDER	0.02	0.003	0.0004	ILQ	ILQ	ILQ
EYES	0.003	0.002	0.0001	ILQ	ILQ	ILQ
THYROID	0.0002	ILQ	ILQ	ILQ	ILQ	ILQ
ADRENALS	0.001	0.0003	ILQ	ILQ	ILQ	ILQ
MEDULLA	0.003	0.001	0.0002	ILQ	ILQ	ILQ
HYPOPHYS	0.0002	ILQ	ILQ	ILQ	ILQ	ILQ
HARDER GL	0.005	0.001	0.0004	0.0002	0.0001	ILQ
GAST. CT	1.93	0.10	0.03	0.006	ILQ	ILQ
S. INT. CT	62.12	18.46	0.41	0.02	0.005	ILQ
L. INT. CT	9.42	30.97	2.13	0.19	0.007	ILQ
CARCASS	3.40	27.15	2.11	0.18	ILQ	ILQ
CAGE WASH	NP	NP	NP	NP	NP	0.04

CT: contents, ILQ: Inferior to the limit of quantitation taken equal to 100 dpm for RAD, NP: Not performed.
RAD: radioactivity measured

TABLE 2.2.4.2
MEAN TOTAL RADIOACTIVITY (AS % OF THE ADMINISTERED DOSE) OBSERVED IN WHOLE ORGANS AFTER 30 MG/KG SINGLE ORAL ADMINISTRATION OF ^{14}C-SR 47436 TO FEMALE RATS

TIME (h)	2.00	8.00	24.00	48.00	96.00	168.00
BRAIN	0.004	ILQ	ILQ	ILQ	ILQ	ILQ
LUNGS	0.04	0.005	0.002	0.002	ILQ	ILQ
LIVER	25.13	5.43	3.70	0.94	0.14	0.01
HEART	0.02	0.002	0.001	0.001	ILQ	ILQ
KIDNEYS	0.63	0.05	0.01	0.01	0.002	ILQ
OVARIES	0.005	ILQ	ILQ	ILQ	ILQ	ILQ
STOMACH	1.40	0.03	0.01	0.01	ILQ	ILQ
SMALL INT.	4.51	0.31	0.07	0.03	ILQ	ILQ
LARGE INT.	0.51	2.13	0.20	0.02	0.00	ILQ
SALIVARY GL	0.01	0.001	0.0005	ILQ	ILQ	ILQ
THYMUS	0.01	0.002	ILQ	ILQ	ILQ	ILQ
PANCREAS	0.04	0.003	0.001	0.001	ILQ	ILQ
SPLEEN	0.02	0.001	0.001	ILQ	ILQ	ILQ
BLADDER	0.01	0.001	0.0004	0.0002	0.0001	ILQ
EYES	0.002	0.0005	0.0002	0.0001	ILQ	ILQ
THYROID	0.0005	ILQ	ILQ	ILQ	ILQ	ILQ
ADRENALS	0.0033	0.0003	0.0001	ILQ	ILQ	ILQ
HYPOPHYS	0.0003	ILQ	ILQ	ILQ	ILQ	ILQ
HARDER GL	0.01	0.001	0.0005	0.0003	0.0001	ILQ
GAST.CT	4.82	0.07	0.04	0.74	0.06	ILQ
S.INT.CT	32.87	5.19	1.17	0.32	0.08	ILQ
L.INT.CT	12.05	38.37	6.47	0.86	0.08	ILQ
CARCASS	3.94	23.81	5.83	0.43	0.11	ILQ

CT: contents, ILQ: Inferior to the limit of quantitation: 100 dpm for RAD

TABLE 2.2.4.3.
TOTAL EXCRETION OF THE RADIOACTIVITY (% OF THE DOSE ADMINISTERED ± SD) AFTER 30 MG/KG SINGLE ORAL ADMINSTRATION OF ^{14}C-SR 47436 TO FIVE FEMALE S-D RATS

	Urine	Faeces	Cage W-W	Urine	Faeces	Cage W-W
0-48 hours	3.5 ± 1.0	98.5 ± 4.0		4.5 ± 0.7	95.0 ± 3.8	
TR recovered	102.6 ± 3.32			102.0 ± 3.04		

Cage W-W: Cage Washing-Water; TR recovered: Total radioactivity recovered

2.3. **Metabolism** *The proposed metabolic pathways of SR 47436 are presented at the end of this section (Figure 2.3.13)*

2.3.1. Profile and Identification of Plasma and Urinary Metabolites Following a Single Oral Administration of ^{14}C-SR 47436 to Male Mice (Report #RS0005940202/02; Study #MET0231). (Vol 70)

This non GLP study was conducted by the department of Metabolism and Pharmacokinetics of Sanofi Recherche, Montpellier Cedex, France from May 1993 to November 1993.

Ninety male mice (COBS CD-1 (ICR) BR) each received a single oral dose of 10 mg/kg ^{14}C-SR 47436 (batch 92.02) by gavage (10 ml/kg). Pretest conditions such as fasting are not given in the report. The mice were approximately 5-6 weeks old and weighed 27-36 g.

Blood samples were collected from abdominal vein after anesthesia at 5, 15, 30 min, and 1, 2, 4, 6, 10 and 24 hours post-drug administration (n=10 animals/timepoint). Excretion of ^{14}C-SR 47436 was determined by measuring the radioactivity present in urine and feces samples from the 24 hours post-drug administration group. Biological samples were collected at 0-6 and 6-24 hours post-drug administration. The radioactivity trapped in samples was measured by liquid scintillation spectrometer.

Results

Following a single oral (10 mg/kg) administration of ^{14}C-SR 47436 to male mice, most of the radioactivity over the first 24 hr after dosing was found in plasma. Peak concentrations were attained within 0.5 to 1 hr after dosing (Table 2.3.1.1). In plasma, the parent drug was detected only in the early sampling time as a minor compound and accounted for 12.5, 8.6 and 2.6% of plasma radioactivity at 5, 15 and 30 min after dosing, respectively. Analysis of plasma radiochromatograms confirmed that the main radioactive peaks detected in urine exhibited the same HPLC retention times as observed in plasma samples (between 14 and 18 minutes). The main identified compound in plasma was a N-dealkyl derivative of parent compound (metabolite F or SR 48001, retention time 19.57 min). Two isomers of hydroxy-N-dealkyl SR 47436 (retention time 15 min) were also identified.

The radioactivity excreted in urine and feces represented 37.7 and 37.1%, respectively, of the administered dose. The parent compound detected in plasma was not excreted in urine. It was extensively metabolized. The main identified compounds in urine were four isomers of monohydroxy-N-dealkyl SR 47436 (metabolites G) and one N-dealkyl-oxo derivative of SR 47436.

TABLE 2.3.1.1

Mean blood and plasma concentrations of radioactivity following a single oral (10 mg/kg) administration of ^{14}C-SR 47436 to male mice

Group n°	Sampling time[1] (hours)	Blood (mg Eq/kg)	Plasma (mg Eq/l)	Haematocrit	% in plasma*
0	0.083	0.779	1.215	0.47	82.7
1	0.25	2.031	2.812	0.46	74.8
2	0.50	2.852	3.737	0.45	72.1
3	1	3.042	3.440	0.45	62.2
4	2	1.552	1.696	0.43	62.3
5	4	0.759	0.895	0.45	64.9
6	6	0.324	0.361	0.47	59.1
7	10	0.186	0.220	0.48	61.5
8	24	0.029	0.030	0.47	54.8

* Percentage of radioactivity in plasma = $\frac{\text{Plasma radioactivity (mg.Eq/l)} \times (1-h) \times 100}{\text{Blood radioactivity (mg.Eq/kg)}}$ Where h = haematocrit

[1]: n = 10 at each time point

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2.3.2. Profile and Identification of Plasma Metabolites Following Single and Repeated Oral Administration of ^{14}C-SR 47436 to Male Rats (Report #RS0005951109/02, Study #MET0240). (Vol 79)

This non GLP study was conducted by the Preclinical Metabolism and Pharmacokinetics department of Sanofi Recherche, Montpellier Cedex, France from June 1995 to November 1995.

Thirtynine male rats (Sprague-Dawley, CD (SD) BR) each received, orally by gavage (5 ml/kg), 10 mg/kg ^{14}C-SR 47436 (batch 4SNP006) once daily for 14 days. Two suspensions were prepared, one for administrations occurring between days 1 and 7, and the second for administrations occurring between days 8 and 14. The animals were fasted overnight before dosing to 4 hours after dosing. Normal diet was provided thereafter. The rats were approximately 8 weeks old and weighed 228 to 256 gm. Blood samples were collected from the abdominal vein after anesthesia, before treatment and 1, 2, 4, 8, and 24 hours after drug administration on days 1 and 14; before treatment on day 7; and 168 hr after drug administration on day 14 (n=3 animals/timepoint). Total radioactivity and plasma circulating compounds were analyzed by HPLC with UV, fluorescence, radioactivity and mass spectrometry detections.

Results

Following single and repeated oral administration of ^{14}C-SR 47436 to male rats, the maximum plasma concentration of SR 47436 (mean ±S.D.) was 1.109 ± 0.058 mg/l (Tmax, 2 hr) and 0.80 ± 0.273 mg/l (Tmax, 1 hr) on days 1 and 14, respectively. The observed $AUC_{0\text{-}24h}$ of SR 47436 on days 1 and 14 was 12.3 and 15 mg.h/l, respectively. The ratios of unchanged SR 47436 AUC/total radioactivity AUC were in the same range on days 1 and 14 (80.2 and 73.3%, respectively).

Whatever the sampling time or the period of observation, SR 47436 was the main circulating compound (73-91% of detected radioactivity). The residual radioactivity was associated with several other quantitatively minor metabolites, two of which were characterized as an N_2-glucuronide (SR 90150, metabolite E) and an SR 47436 derivative corresponding to the opening of the imidazoline ring (SR 49498, metabolite J).

2.3.3 Profile and Identification of Urinary Metabolites Following Single Oral or Intravenous Administration of ^{14}C-SR 47436 to Rats (Report #RS0005931210/02, Study #MET0169). (Vol 79)

This non GLP study was conducted by the Department of Metabolism and Pharmacokinetics of Sanofi Recherche, Montpellier Cedex, France from August 1992 to November 1993.

Two groups of eight male (270-340 gm) and eight female (250-310 gm) Sprague-Dawley rats received either single oral doses of 10 mg/kg (by gavage) or single intravenous doses of 1 mg/kg ^{14}C-SR 47436 (batch 91.01). Animals were fasted overnight before dosing to 2 hours after dosing. Urine samples were collected from each rat, separately, at 0-6 hr, 6-24 hr, then every 24 hours until 168 hours post-dose. Samples were analyzed by HPLC with UV, radioactivity and mass spectrometry detections.

Results

The results after i.v. administration were similar to those after oral administration. For both routes, SR 47436 was detected only in females and accounted for approximately 10% of the detected radioactivity. A large gender difference was observed in the qualitative metabolite profiles. The main urinary metabolites in females were three monohydroxy SR 47436 (metabolite C) isomers. In male rats, the main metabolites were four N-dealkyl monohydroxy compounds. The cumulative excretion of metabolites over 72 hours accounted for 8 and 6% of the oral dose in males and females, respectively, and for 5 and 10% of the i.v. dose in males and females, respectively. Differences between metabolite profiles would explain sex-related variability in plasma concentrations.

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2.3.4. Biliary Excretion, Profile and Identification of Biliary Metabolites Following Single Oral or Intravenous Administrations of ^{14}C-SR 47436 to Rats

Biliary metabolite profiles: Report #RS0005930623/02, Study #MET0170, Study period: March 1993 to April 1993, vol. 80.
Excretion: Report #RS0005930318/02, Study #EBI0016, Study period: December 1992 to January 1993, vol. 86.

These non GLP studies were conducted by the Department of Metabolism and Pharmacokinetics of Sanofi Recherche, Montpellier Cedex, France. Animal experimentation and samplings were same for both studies.

Two groups of four male (273-302 gm) and four female (246-272 gm) Sprague-Dawley rats (8 weeks old) received either single oral (by gavage, 5 ml/kg) doses of 10 mg/kg or single intravenous (femoral vein) doses of 1 mg/kg SR 47436 (30 µCi/kg ^{14}C-SR 47436 diluted with unlabeled SR 47436, batch 92.02). Animals were fasted overnight before treatment and fed 4 hours after administration. Biliary samples were collected from bile duct-cannulated (surgery done 24 hr before drug administration) conscious rats at 0-1, 1-3, 3-6, 6-24 and 24-48 hr postdose. The biliary samples were analyzed by HPLC with UV, radioactivity and mass spectrometry detections. Complete biliary metabolite profiles were performed on bile collected from 0-48 hours from randomly selected rats (one male and one female for each route). In other animals, biliary metabolic profiles were carried out on biliary fractions containing the maximum radioactivity, usually 1-3 hour period (see Tables 2.3.4.1 and 2.3.4.2). Animals were housed singly in metabolism cages allowing separate collection of urine and feces. Urine samples were collected at 0-6, 6-24 and 24-48 hr after dosing. Feces were collected as 24 hours fractions from 0-48 hr. Animals were sacrificed at 48 hours and carcasses were analyzed. Radioactivity was measured by liquid scintillation counting.

Results

Biliary Metabolite profiles: No major qualitative and quantitative differences were observed in biliary metabolite profiles between male and female rats following oral administration. However, quantitative differences were present between male and female rats following intravenous administration. Additionally, time-related quantitative differences were found in the relative percentage of the various detected compounds in both sets of studies. Irrespective of the sex and route of administration, parent drug was excreted in its free form in minor amounts, 0.5 to 2.9% of the administered dose (or 0.1 to 1 % of the radioactivity) over the 0-48 hour sampling period (Tables 2.3.4.1 and 2.3.4.2).

TABLE 2.3.4.1

BILIARY EXCRETION OF SR 47436 AND ITS METABOLITES FOLLOWING A SINGLE ORAL (10 MG/KG) ADMINISTRATION OF [^{14}C]-SR 47436 TO RATS

Animal #	Total rad.[1] (0-48h)	Rad.[1] in the sample period (hr)	% of the dose in bile as		Relative % in the sample[2]		
			parent drug	N_2-glucur.	parent drug ± s.d.	N_2-glucurn. ± s.d.	Other metabolites[3]
M1	79.0	31.7 (1-3)	0	26.2	0	82.8	17.2
M2	77.2	35.0 (1-3) 77.2 (0-48)	0 0.6	28.1 39.1	0 0.8	80.2 50.6	19.8 48.6
M3	79.7	Not determined due to insufficient samples					
M4	75.0	20.1 (1-3)	0.3	14.6	1.5	72.6	25.9
Mean	**77.7**	**28.9 (1-3)[4]**	**0.1 (1-3)**	**23.1 (1-3)**	**0.5 ± 0.7**	**78.5 ± 5.3**	**21.0 ± 4.5**
F5	67.9	10.5 (0-1)	0	8.8	0	84.2	15.8
F6	72.1	27.1 (1-3) 72.1 (0-48)	0.76 2.9	18.5 36.6	2.8 4.0	68.4 50.8	28.8 45.2
F7	66.2	23.7 (1-3)	0	17.4	0	73.6	26.4
F8	80.5	16.1 (1-3)	0.4	11.7	2.6	72.8	24.6
Mean	**71.6**	**19.4 (0-3)[4]**	**0.3 (0-3)**	**14.5 (0-3)**	**1.4 ± 1.6**	**74.8 ± 6.7**	**23.9 ± 5.7**

[1]: Radioactivity in bile expressed as percent of the dose administered

[2]: relative percent present in the given sample = (col 4 ÷ col 2) x 100

[3]: percent of other 13 metabolites present in the sample. The values listed here are not experimental values and are derived from columns 6 and 7

[4]: denotes bile collection period, 0-1 or 0-3 hr.

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TABLE 2.3.4.2

BILIARY EXCRETION OF SR 47436 AND ITS METABOLITES FOLLOWING A SINGLE INTRAVENOUS (1 MG/KG) ADMINISTRATION OF [^{14}C]-SR 47436 TO RATS

Animal #	Total rad.[1] (0-48h)	Rad.[1] in the sample period (hr)	% of the dose in bile as		Relative % in the sample[2]		
			parent drug	N_2-glucur.	parent drug ± s.d.	N_2-glucurn. ± s.d.	Other metabolites[3]
M9	64.3	11.5 (1-3)	0.31	1.1	2.7	9.6	87.7
M10	57.5	7.5 (1-3) 57.5 (0-48)	0.12 0.90	1.2 7.4	1.6 1.5	16.3 12.9	82.1 85.6
M11	70.8	13.7 (1-3)	0.20	1.4	1.4	10.1	88.5
M12	66.0	13.4 (1-3)	0.25	2.5	1.9	18.5	79.6
Mean	**64.7**	**11.5 (1-3)[4]**	**0.2 (1-3)**	**1.6 (1-3)**	**1.9 ± 0.5**	**13.5 ± 4.6**	
F13	57.0	7.5 (0-1)	2.6	1.7	34.8	22.6	42.6
F14	44.2	4.2 (1-3) 44.2 (0-48)	0.3 2.1	2.0 12.6	6.9 4.8	46.7 28.5	46.4 66.7
F15	48.8	3.8 (1-3)	0.8	1.7	20.9	44.6	34.5
F16	30.2	1.9 (0-1)	0.2	0.6	8.7	30.3	61.0
Mean	**45.1**	**4.4 (0-3)[4]**	**1.0 (0-3)**	**1.6 (0-3)**	**17.8 ± 12.9**	**36.1 ± 11.6**	

[1]: Radioactivity in bile expressed as percent of the dose administered

[2]: relative percent present in the given sample = (col 4 ÷ col 2) x 100

[3]: percent of other 13 metabolites present in the sample. The values listed here are not experimental values and are derived from columns 6 and 7

[4]: denotes bile collection period, 0-1 or 0-3 hr.

After oral administration fourteen metabolites were detected in the bile (Figure 2.3.4.1) of which SR 47436-N2-glucuronide was the main excreted compound in both sexes. It accounted for about 50% of the radioactivity (over 37% of the dose) excreted over 48 hr following oral and 13 to 29% (7.4 to 12.6% of the dose) following i.v. administration (Tables 2.3.4.1 and 2.3.4.2). Other quantitatively minor compounds were (see Table 2.3.4.3):

- di- and tri-hydroxy derivatives of SR 47436
- mono- and di-hydroxy derivatives of SR 49498
- SR 49498 glucuronide
- glucuronides of monohydroxy derivatives of SR 47436 and SR 49498
- SR 47436
- SR 49498
- unidentified compounds (n = 3)

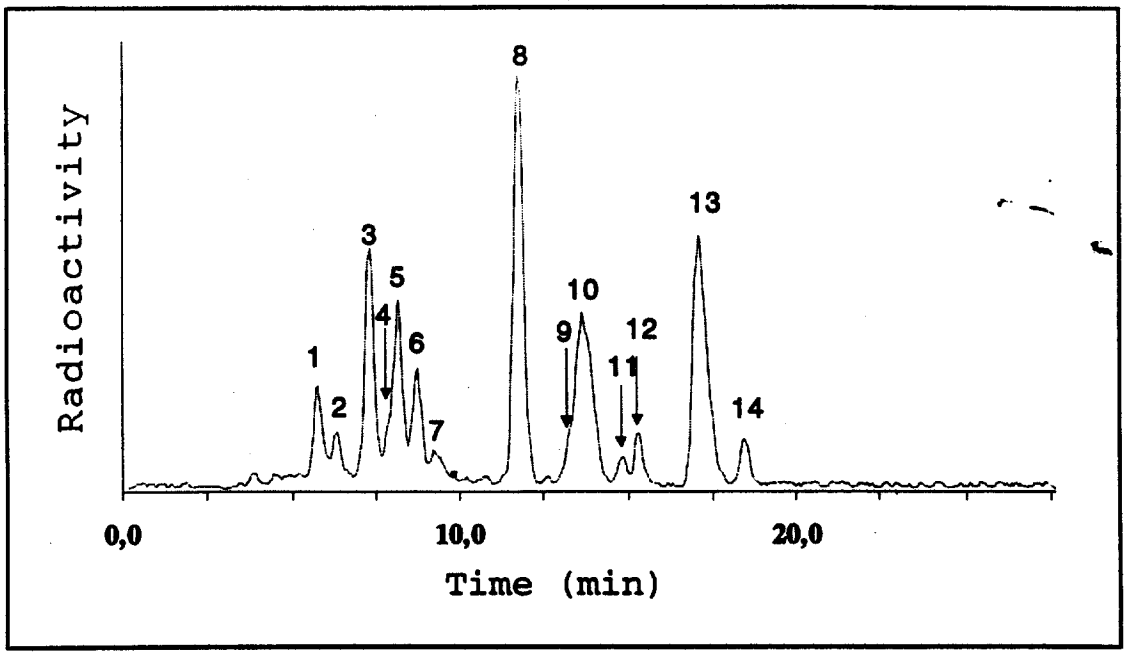


Figure 2.3.4.1.: Radiochromatogram of biliary sample following a single oral administration of ^{14}C-SR 47436 to male rat (3-6 hr sampling period). See Table 2.3.5.3 for identification of peaks.

TABLE 2.3.4.3.

CHARACTERISTICS OF THE RADIOACTIVE IDENTIFIED PEAKS (SEE FIGURE 2.3.5.1.) FOLLOWING ORAL ADMINISTRATION OF ^{14}C-SR 47436 TO MALE RAT*

This fraction (3-6h sampling period) was selected for identification of metabolites by LCMS as it contained the maximal number of metabolites (MET0170)

Peak n°	HPLC (min)	Mass (Da)	Proposed structures	Relative % in the sample	% of the dose
1	5.39	478	Dihydroxy SR 49498	5.1	1.2
2	6.15	476	Trihydroxy SR 47436	2.9	0.7
3	7.15	460	Dihydroxy SR 47436	12.2	3.0
4	7.50	393	Unknown	-	-
		460	Dihydroxy SR 47436	-	-
5	8.10	638	Hydroxy SR 49498 glucuronide	10.6 (peak 4+5)	2.6 (peak 4+5)
6	8.40	462	Hydroxy SR 49498	6.1	1.5
7	9.10	638	Hydroxy SR 49498 glucuronide	2.1	0.5
8	11.40	620	Hydroxy SR 47436 glucuronide	21.6	5.2
9	13.10	620	Hydroxy SR 47436 glucuronide	-	-
10	13.35	622	SR 49498 glucuronide	15.3 (peak 9+10)	3.7 (peak 9+10)
11	14.40	707?	Unknown	1.4	0.3
12	15.10	707?	Unknown	2.3	0.6
13	16.50	604	SR 47436 glucuronide	18.1	4.4
14	18.20	428	SR 47436	2.2	0.5

* No detailed metabolic profile data are available for female rats following either oral or intravenous administration of irbesartan, or for male rats following intravenous administration.

Excretion: In male and female rats, mean 0-48 hr biliary excretion accounted for 78 and 72%, respectively, of the oral dose and 95 and 89%, respectively, of orally administered radioactivity (Table 2.3.4.4). These biliary excretion results over a 0-48 hr sample collection period are in accordance with those observed for the fecal excretion values in the excretion balance study performed in this species after a single oral administration (see section 2.4.1). Following i.v. administration, a different pattern was observed, a slow biliary elimination relafed to the high percentage of radioactivity recovered in the carcass as compared with the oral administration (Table 2.3.4.4). These biliary excretion results are not in accordance with those observed for the fecal excretion values in the excretion balance study performed in this species after a single i.v. administration (see section 2.4.1). The high percentage of radioactivity recovered in the carcass of the intravenously dosed animals goes unexplained by the sponsor.

TABLE 2.3.4.4
MEAN CUMULATIVE URINE, FECAL AND BILIARY EXCRETION FOLLOWING A SINGLE ORAL OR I.V. ADMINISTRATION OF ^{14}C SR 47436

TIME (HR)	URINE M	URINE F	FECES M	FECES F	CARCASS M	CARCASS F	BILE M	BILE F	TOTAL RECOVERED M	TOTAL RECOVERED F
ORAL										
0-24							73.8±2.8	65.0±6.7		
0-48	3.0±2.5	1.4±1.4	6.9±5.6	0.3±0.5	7.1±4.6	15.9±3.9	77.7±2.1	71.6±6.3	94.6±2.1	89.2±3.7
I.V.										
0-24							51.6±5.7	35.9±12.1		
0-48	4.4±0.6	6.8±3.1	0.8±0.2	0.4±0.2	24.4±4.5	43.4±11.3	64.6±5.5	45.1±11.3	94.2±1.8	95.6±2.9

Values expressed as % of the administered dose, mean ± standard deviation
Total recovered = urine + feces + carcass + bile

In conclusion, for both sexes and routes of administration, the main pathway of excretion was in the bile with only a small amount of the dose excreted in the urine or feces. The amounts excreted in bile increased at about the same rate up to 24 hours after dosing. Irrespective of the sex and administration route, the unchanged drug under its free form was excreted in bile in minor amounts. SR 47436-N2-glucuronide was the main excreted compound in bile in both sexes. Several other metabolites were also identified in bile: SR 49498, hydroxy derivatives of SR 47436 and SR 49498, and their respective glucuronides.

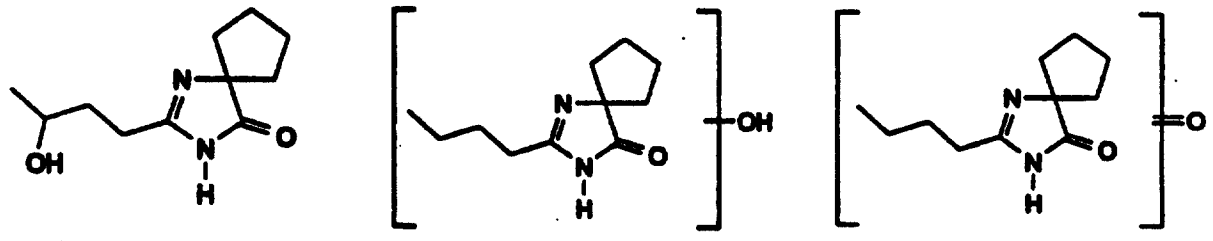
2.3.5 Profile and Identification of Urinary and Fecal Metabolites Following Single and Repeated Oral Administrations of ^{14}C-SR 47436 to Male Rats (Report #RS0005951113/02. Study #MET0241). Vol 79

This non GLP study was conducted by the Department of Metabolism and Pharmacokinetics of Sanofi Recherche, Montpellier Cedex, France from June 1995 to November 1995.

Thirtynine male Sprague-Dawley rats each received oral doses of 10 mg/kg/day ^{14}C-SR 47436 (batch 91.01) by gavage (5 ml/kg) daily for 14 days. Two suspensions were prepared: one for administrations occurring between days 1 and 7 and the second for administrations occurring between days 8 and 14. The rats were 8 weeks old and weighed 228 to 256 gm. Animals were fasted overnight before dosing to 4 hours after administration on day 1 and provided normal diet thereafter. Animals were housed singly in metabolism cages allowing separate collection of urine and feces. Urine and feces were collected from 15 animals during the 24 hour period immediately prior to dosing; from 24 animals during during the 24 hour period following dosing on day 1; from 18 animals (same animals used on day 1), during the 24 hour period follow dosing on day 7; from 6 animals (same animals used on day 7) during the 24 hour period following dosing on day 14; and from 3 animals (same animals used on day 14) during the 24 hour period beginning 144 hr after dosing on day 14. Residual radioactivity in carcass was estimated 168 hr after dosing on day 14 (the same 3 animals used for urine and feces collection 144-168 hr after dosing on day 14). Samples were analyzed by HPLC with UV, radioactivity and mass spectrometry detections.

Results

Similar quantitative and qualitative metabolic profiles were observed in both urine and feces whatever the period of observation. Thus, repeated administration did not modify the metabolite patterns. As in the previous study (see section 2.3.3), SR 47436 was not excreted in urine. Several radioactive peaks were detected in urine samples and structures have been proposed for the four main excreted compounds (Fig 2.3.5.1). All three are related to hydroxy-N-dealkylated SR 47436 derivatives, while the fourth is the 4-hydroxy (in ω-1 position)-butyl-N-dealkyl SR 47436 derivative (SR 90146). No changes in HPLC radiochromatograms were observed after hydrolysis



SR 90146(peak A) **(2 isomers) (peaks B and C)** **(peak D)**

(-OH in ω-1 position) Metabolites G Metabolite R

Figure 2.3.5.1.: Structural identification of urinary metabolites following oral administration of SR 47436 to male rats.

with ß-glucuronidase indicating that the urinary compounds were excreted in their free forms. In fecal samples, SR 47436 was detected as one of the main excreted compounds after day 1 of administration (20%), and became the main excreted metabolite after days 7 and 14 (51 and 54% of the detected radioactivity, respectively). The presence of considerable amounts of intact parent drug in the feces was not attributed to incomplete absorption of the drug from the GIT, but, rather, to the hydrolysis of the N-glucuronide metabolite in the gut. The N-glucuronide metabolite has been shown to be a major biliary excretion product (see section 2.3.4). As with urine, no glucuronides were detected after enzyme hydrolysis. Seven metabolites were identified in feces; their structures are given in Fig 2.3.5.2.

Figure 2.3.5.2.: Urinary metabolites identified following oral administration of SR 47436 to male rats

2.3.6. Profile and Identification of Plasma and Urinary Metabolites Following Single Oral Administration of ^{14}C-SR 47436 to Female Rabbits (Report #RS0005951109/01, Study #MET0239). Vol 81

This non GLP study was conducted by the Preclinical Metabolism and Pharmacokinetics department of Sanofi Recherche, Montpellier Cedex, France from January 1995 to November 1995.

Suspensions of ^{14}C-SR 47436 (batch 93.06) were administered once, orally by gavage (2 ml/kg) at a dose of 10 mg/kg to each of 9 female rabbits (hybrid New-Zealand). The animals were approximately 18 weeks old and weighed 3.24 to 3.73 kg and were fasted overnight before dosing to 4 hours after dosing.

Blood samples were collected from the jugular vein before treatment, and 1, 2, 4, 8, 24, 48 and 72 hours after treatment (n=3 animals/timepoint). Animals were used at more than one timepoint (one group sampled at 0, 2 and 8 hr; a second group sampled at 1, 4 and 24 hr; and a third group sampled at 48 and 72 hr). Urine samples were collected from a pool of 3 animals (used at 0, 2 and 8 hr time points) over the following periods: 0-8 hr, 8-24 hr and 24-48 hr. Total radioactivity, circulating and excreted compounds were analyzed by HPLC with UV, radioactivity and mass spectrometry detections.

Results

In plasma, SR 47436 was the main compound up to 8 hours, accounting for 68 to 40% of all detected compounds. This declined to about 14 and 7% at 24 and 48 hours, respectively. The major circulating metabolites were two monohydroxylated metabolites resulting from hydroxylation on the cyclopentane ring co-eluting with references BMS-M5 and BMS-M7 (metabolites C).

The parent compound was little excreted in urine, from 10% at 0-8 hr to 0.6% at 24-48 hours. The two main excreted metabolites were identified as the two cyclopentane monohydroxylated derivatives that were also identified in plasma. The other detected metabolites were the N_2-glucuronide of parent drug, SR 49498 (metabolite J) and its monohydroxy derivatives, and several other mono- and di-hydroxy derivatives resulting from ω-1 oxidation on the butyl side chain and/or on the spirocyclopentane ring, and a keto metabolite resulting from further oxidation of one of the monohydroxylated metabolites (see Figure 2.3.13). The metabolite profiles were similar for the three periods of time investigated. The sponsor asserts that the metabolites formed in rabbit fitted with those found in human.

2.3.7. Profile and Identification of Plasma Metabolites Following Single and Repeated Oral Administrations of ^{14}C-SR 47436 to Male Monkeys (Report #RS0005951116/01. Study #MET0242). Vol 81

This non GLP study was conducted by the Preclinical Metabolism and Pharmacokinetics department of Sanofi Recherche, Montpellier Cedex, France from June 1995 to November 1995.

Suspensions of ^{14}C-SR 47436 (batch 4SNP006) were administered orally by gavage (5 ml/kg) once daily for 14 days at a dose of 10 mg/kg to each of 4 male monkeys (*Macaca fascicularis*). The animals were young (precise age could not be ascertained since they were collected from the wild) and weighed 2.88 to 4.03 kg. They were fasted overnight before dosing until 4 hours after drug administration on day 1.

Blood samples were collected from each animal (from femoral vein) at 1, 2, 4, 8 and 24 hours after drug administration on days 1 and 14; before treatment on days 1, 7 and 14, and 168 hr after administration on day 14. Total radioactivity and plasma circulating compounds were analyzed by HPLC with fluorescence, UV, radioactivity and mass spectrometry detections. Metabolite profiling was carried out using pooled samples for 4 animals/sampling time.

Results

Following single and repeated oral adminstrations (10 mg/kg/day) of ^{14}C-SR 47436 to male monkeys, maximum SR 47436 concentrations in plasma were attained at 1.75 hours on both days 1 and 14. The pharmacokinetic parameters are given below. From Cmin values, steady state conditions for SR 47436 were achieved within 6 days after repeated administrations.

Pharmacokinetic parameters (mean values ± standard deviation)

Parameters	Day 1	Day 6	Day 13	Day 14
Cmax (mg/l)	0.53 (0.08)	-	-	0.84 (0.41)
Cmin (mg/l)	0.10 (0.04)	0.18 (0.08)	0.13 (0.01)	0.18 (0.04)
tmax (h)	1.75 (1.50)	-	-	1.75 (1.50)
AUC (0-24h) (mg.h/l)	5.46 (1.84)	-	-	7.52 (1.97)

Whatever the sampling time or the period of observation, SR 47436 was the main detected circulating compound and accounted for 49-79% of the radioactivity detected in plasma. The residual radioactivity was associated with several other quantitatively minor metabolites. The major one was characterized as the N2-glucuronide of SR 47436 (i.e., SR 90150 or metabolite E). Another minor metabolite was SR 49498 (metabolite J). In another related study (#RS0005930526/03), a single i.v. dose of 1 mg SR 47436/kg was administered to male monkeys. An additional minor metabolite detected in plasma in the I.V. study was the glucuronide derivative of SR 49498 (i.e., metabolite I).

2.3.8. Biliary Excretion, and Profile and Identification of Biliary Metabolites Following Single Oral or Intravenous Administrations of ^{14}C-SR 47436 to Male Monkeys

Biliary metabolite profiles: Report #RS0005930907/01, Study #MET0233, Study period: March 1993 to April 1993. Vol 82.

Excretion: Report #RS0005930624/01, Study #EBI0030, Study period: February 1993 to April 1993, vol. 87.

These non GLP studies were conducted by the department of Metabolism and Pharmacokinetics of Sanofi Recherche, Montpellier Cedex, France. Animal experimentation and samplings were done on same animals for both studies.

Male *Macaca fascicularis* monkeys (4 to 5 kg, precise age could not be ascertained since they were collected from the wild) each received a single oral dose of 10 mg ^{14}C-SR 47436 (batch 92.02 or 91.01)/kg by gavage (5 ml/kg) or a single intravenous (femoral vein) dose of 1 mg/kg (n=3/route). The animals were fasted overnight before surgery for bile-duct cannulation under anesthesia. Conscious animals were treated once, the day after surgery and then fed 4 hours after administration. Biliary samples were collected from bile duct-cannulated conscious, unrestrained animals at 0-1, 1-3, 3-6, 6-24 and 24-48 hr postdose. For each administration route, the biliary metabolic profiles were carried out on fractions containing the maximum radioactivity. However, the sponsor determined metabolite profiles for all the collection periods in one randomly selected monkey/route. In order to investigate whether or not the metabolites were excreted in a conjugated or a free form, the biliary samples were treated with ß-glucuronidase/arylsulfatase. Animals were housed singly in metabolism cages allowing separate collection of urine and feces. Urine samples were collected at 0-6, 6-24 and 24-48 hr after dosing. Feces were collected as 24 hour fractions from 0-48 hr. Biliary samples were analyzed by HPLC with UV, radioactivity and mass spectrometry detections. Total radioactivity in urine and fecal samples was determined by liquid scintillation counting.

Results

Biliary metabolite profiles: Whatever the sampling time or route of administration, one main and some minor radioactive peaks were detected. The parent compound was almost completely metabolized; it never exceeded 5% of the total detected radioactivity. The main excreted metabolite was SR 47436-N2-glucuronide (SR 90150, BMS-M8 or metabolite E), which ranged between 41.4% in the 24-48 hr fraction and 81.3% (expressed as % of the recovered radioactivity) in the 0-1 hr fraction of an orally dosed animal (n=1). Alternately, as percent of the given dose, the biliary excretion (0-48 hr) of the total radioactivity, metabolite E and parent compound were, respectively, 69%, 45% and 1.27% (Table 2.3.8.1). The metabolic patterns were qualitatively similar in animals dosed intravenously (Table 2.3.8.1).

Other quantitatively minor compounds were:

- dihydroxy derivative of parent drug
- glucuronide of dihydroxy SR 47436
- glucuronide of monohydroxy SR 49498 (2 isomers)
- glucuronide of hydroxy SR 47436 (3 isomers)
- SR 49498 glucuronide
- two unidentified compounds

TABLE 2.3.8.1
PERCENTAGE OF RADIOACTIVITY DOSE , SR 47436 AND SR 47436 N2-GLUCURONIDE EXCRETED IN BILE FRACTIONS FOLLOWING SINGLE ORAL (10 MG/KG) OR INTRAVENOUS (1 MG/KG) ADMINISTRATION OF [^{14}C] SR 47436 TO MALE MONKEYS

Bile sample	Total radioactivity (% of dose)	SR 47436 N2-glucuronide* (% of dose)	SR 47436* (% of dose)
Male PO #M1			
0 - 1 h	2.19	1.78	0.03
1 - 3 h	18.87	13.94	0.11
3 - 6 h	20.25	13.61	0.18
6 - 24 h	26.01	15.06	0.86
24 - 48 h	1.87	0.77	0.09
Total 0 - 48 h	69.19	45.16	1.27
Male IV #M5			
0 - 1 h	28.52	18.45	0.34
1 - 3 h	28.26	15.57	0.14
3 - 6 h	4.75	1.67	0.005
6 - 24 h	21.94	10.88	0.04
24 - 48 h	1.18	0.67	0.00
Total 0 - 48 h	84.65	47.24	0.53

* : values calculated as the % of the dose excreted in the considered biliary fraction multiplied by the relative percentage of SR 47436 or SR 47436-N2-glucuronide on the radiochromatogram divided by 100

Excretion: Following a single oral (10 mg/kg) or single i.v. (1 mg/kg) administration of [^{14}C] SR 47436 to bile duct-cannulated male monkeys, the radioactivity was mainly excreted through the biliary route. The largest portion was excreted during the first 24 hours of dosing. Only a small proportion of the dose was excreted in the urine (Table 2.3.8.2). The large inter-individual variability observed in the rate of excretion (high standard deviation) could be due to variabilities observed in the biliary flow of each animal and was independent of route of administration. The total 0-48 hr recovered radioactivity (bile + urine + feces + cage washes) accounted for 66 or 81% of the dose given after oral or i.v. administration, respectively. This suggests a substantial portion of the administered dose of radioactivity remained in the carcass 48 hr after dosing. However, the sponsor has not analyzed the radioactivity that remained in the carcass.

TABLE 2.3.8.2
MEAN CUMULATIVE URINE, FECAL AND BILIARY EXCRETION FOLLOWING A SINGLE ORAL OR I.V. ADMINISTRATION OF ^{14}C SR 47436 TO MONKEYS

TIME (HR)	URINE	FECES	BILE	TOTAL RECOVERED
ORAL				
0-24	12.1 ± 14.2	2.3 ± 2.7	44.3 ± 20.0	-
0-48	13.3 ± 14.5	4.8 ± 4.2	48.1 ± 18.9	66.1 ± 16.4
I.V.				
0-24	21.5 ± 20.7	0.3 ± 0.1	57.5 ± 49.5	-
0-48	22.1 ± 21.5	0.9 ± 0.9	58.2 ± 49.3	81.3 ± 27.1

Values expressed as % of the administered dose, mean ± standard deviation
Total recovered = urine + feces + bile

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2.3.9. Profile and Identification of Urinary and Fecal Metabolites Following Single and Repeated Oral Administrations of ^{14}C-SR 47436 to Male Monkeys (Report #RS0005951120/01, Study #MET0243), Vol 82

This non GLP study was conducted by the Preclinical Metabolism and Pharmacokinetics department of Sanofi Recherche, Montpellier Cedex, France from June 1995 to November 1995.

Suspensions of ^{14}C-SR 47436 (batch 4SNP006) were administered orally by gavage (5 ml/kg) once daily for 14 days at a dose of 10 mg/kg to each of 4 male monkeys (*Macaca fascicularis*). The animals were young (precise age could not be ascertained since they were collected from the wild) and weighed 2.88 to 4.03 kg. They were fasted overnight before dosing until 4 hours after administration on day 1. Urine, fecal and cage washes were collected from each animal from 0-24 hours after drug administration on days 1, 7 and 14 and from 144-168 hr after administration on day 14 (i.e., day 21). Metabolite profiling was carried out using pooled samples for the 4 animals/sampling time. Total radioactivity and excreted compounds were analyzed by HPLC with UV, radioactivity and mass spectrometry detections.

Results

In urine, similar qualitative metabolite profiles were observed for sampling days 1, 7 and 14 (data not provided for sampling day 21). SR 47436 was not the main urinary excretion product. It accounted for 2, 15 and 14% of the detected radioactivity on days 1, 7 and 14, respectively. Thirteen radioactive peaks were identified along with the unchanged parent compound. The two most prominent metabolites were SR 49498 and SR 90150, which accounted for 2.3 to 4.3% of the detected radioactivity.

In fecal samples, whatever the period of observation, free SR 47436 was detected as the main excretion product. It accounted for 66, 54.4, 60.8 and 48.4% of the detected radioactivity on days 1, 7, 14 and 21, respectively. The glucuronide of SR 47436 (SR 90150), which had been previously identified in bile (see section 2.3.8), was not identified in feces, indicating that the glucuronide derivative is hydrolyzed into SR 47436 in the GIT and then excreted in a free form, via feces. Besides parent compound, ten radioactive peaks were identified in fecal samples. Most of the compounds excreted in feces were also detected in urine. Repeated dosing for 14 days did not modfy the metabolic pattern. The proposed structures assigned to the radioactive peaks identified in urine and feces are given in Figure 2.3.9.1.

In a related study (#RS0005940128/01), a single oral (10 mg/kg) or i.v. (1 mg/kg) dose of ^{14}C SR 47436 was administered to male monkeys. The metabolite pattern in urine after oral administration was similar to that described above. However, the profile following i.v. administration was different from that observed following oral dosing, with the tetrazole N2-glucuronide of SR 47436 (metabolite E or SR 90150) as the main excreted compound. Other detected compounds in urine were the parent drug, SR 49498 (metabolite J) and its related glucuronide (relative percent of total radioactivity was not given).

Figure 2.3.9.1.: The proposed structural identification of urinary and fecal metabolites following single and repeated oral administrations (10 mg/kg) of SR 47436 to male monkeys.

2.3.10. Inter-species Comparison of SR 47436 Metabolism Using Hepatic Microsomal Fractions (Report #RS0005920224/01, Study #MIV0107[1], Report #RS0005920225/01; Study # MIV0140[2]), Vol. 83

These non GLP studies were conducted by the department of Metabolism and Pharmacokinetics of Sanofi Recherche, Montpellier Cedex, France between March 1990 and February 1992. The purpose was to evaluate hepatic metabolism and glucuronidation of SR 47436 *in vitro* using hepatic microsomal fractions prepared from various species and strains.

Hepatic microsomal fractions were prepared from the livers of Sprague-Dawley rats (3 different pools of 16, 25 or 12 males weighing 250 to 260 gm), male beagle dogs (3 males, 8.2 to 13.4 kg), *Macaca fascicularis* monkeys (two males, 2.5 and 2.8 kg, and one female, 2.3 kg), *Papio papio* baboons (four males, 4.9 to 9.6 kg), and Caucasian humans (five males and one female, 20-41 year old organ-transplant donors). The fractions were characterized by determining protein concentration and cytochrome P450 content. Biotransformation was intiated by incubating the microsomal fraction with graded concentrations (5 to 200 µM) of SR 47436 (batch 90.00 bis, dissolved in methyl alcohol or DMSO) for 30 min at 37°C. SR 47436 and metabolites were analyzed by HPLC with UV detection and liquid chromatography and mass spectrometry.

Results

The Michaelis-Menten constant (Km) of SR 47436 metabolism by cytochrome P450 monooxygeneases was evaluated in both rat and human. The "*Vmax/Km*" ratio demonstrates that rat hepatic microsomal fractions metabolized SR 47436 more rapidly than the fractions prepared from humans.

METABOLISM OF SR 47436 BY CYTOCHROME P450. ENZYME KINETICS

Species	K_M (µM)	Vmax (nmol/min/mg)	V_{max}/K_M
Rat	25.2	0.32	0.013
Human	70.6	0.39	0.006

(Km = Michaelis-Menten constant, Vmax = Maximal rate of metabolism)

The biotransformation of SR 47436 was studied in various species by incubating hepatic microsomal fractions (for 30 min) with SR 47436 in the presence or absence of NADPH, a cofactor for cytochrome P450 monooxygenases. In the absence of NADPH, SR 47436 was not metabolized or degraded. On a quantitative basis, no major difference in SR 47436 biotransformation was observed among species studied (i.e. approximately 30 to 50% of SR 47436 converted to metabolites). The kinetics of SR 47436 disappearance, as illustrated in Table 2.3.10.1, confirms the relatively slow metabolic rate of SR 47436 and the slight difference in metabolism among the five

[1] hepatic metabolism investigated in rat, dog, monkey, baboon and human

[2] glucuronidation investigated in rat, monkey and human

species (slowest in baboon monkey and dog and fastest in rat).

TABLE 2.3.10.1
KINETICS OF SR47436 DISAPPEARANCE FOLLOWING INCUBATION OF 10 μM SR47436 WITH HEPATIC MICROSOMAL FRACTIONS PREPARED FROM VARIOUS SPECIES[a]

Time (min)	Rat (R(SD)m3)	Dog (D(B)m3)	Macaca monkey (Mk(MC)m2)	Baboon monkey (Mk(B)m3)	Caucasian human (H(HL-19)m)
0	100	100	100	100	100
2	90	83	94	90	85
5	72	79	87	89	73
10	64	76	85	85	–
15	57	79	69	86	59
20	51	68	64	79	50
25	50	57	58	81	47
30	44	31	55	73	43
30	52 ± 6[b], n = 3	31 ± 1, n = 3	40 ± 7, n = 3	33 ± 2, n = 4	39 ± 17, n = 6

a: results are expressed in percentage of unchanged drug, n=1
b: percentage of SR 47436 biotransformation by hepatic microsomal fractions following a 30 min incubation with 10 μM SR 47436 (values are given as mean ± SD)

No marked qualitative differences were observed in the metabolite profiles across the species investigated. The parent drug and 7 metabolites with similar U.V. spectral properties were characterized. The main metabolites are monohydroxylated derivatives, oxidation occurring at different sites of the molecule. No dihydroxylated derivatives were detected. The N-dealkylation pathway represented only a minor metabolic process (Table 2.3.10.2). The only difference was in the relative proportion of each metabolite across species.

TABLE 2.3.10.2

IDENTIFICATION OF METABOLITES (*IN VITRO*) FOLLOWING INCUBATION OF 10 μM SR47436 WITH HEPATIC MICROSOMAL FRACTIONS PREPARED FROM VARIOUS SPECIES

Proposed Structure	Peak	Rat	Dog	Macaca Monkey	Baboon Monkey	Humans
		-	+	+	-	-
	(2)	+	++	+	+	+++
	(3)	++++	++++	+++	++	++++
	(4)	++	+++	++++	++++	+++
	(5)	+++	+++	+++	+++	++++
	(6)	+	++	++	++	+
	(7)	++	+	+	-	+
	(8)	+++++	+++++	+++++	+++++	+++++

In a related study (report #RS0005920225/01), the kinetics of formation of the SR 47436 glucuronide and Michaelis-Menten constants for glucuronide formation were investigated using microsomal fractions prepared from the livers of Sprague-Dawley rats (one pool of 17 males, 280 gm), a male *Macaca fascicularis* monkey (2.3 kg), and 13 male and a female Caucasian human (23 to 63 years old organ-transplant donors). Hepatic microsomal fractions were incubated over 30 minutes with 3 mM UDP-GA (cofactor), 0.5 mg Brij-58/mg protein (activator) and increasing SR 47436 concentrations.

Glucuronidation of SR 47436 was observed in all species investigated but with different biotransformation rates. Although test substance exhibited a higher affinity (lower Km) for UDP-glucuronosyl transferase isozyme(s) present in the rat microsomal fractions, 109.4 µM *versus* 256 and 368.6 µM for monkey and human, respectively, the rate (Vmax) of SR 47436 glucuronidation formation appears to be faster for the monkey, intermediate for human, and slowest for the rat (see below table).

Species	Km (µM)	Vmax (nmol/min/mg)	Vmax/Km
Rat	109.4	0.062	0.57
Monkey	256.0	1.404	5.48
Human	368.6	1.152	3.13

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2.3.11. Involvement of Cytochrome P4502C9 in SR 47436 Oxidation by Human Hepatic Microsomal Fractions (Report #RS0005960620/01, Study #MIV0214), Vol. 83

This non GLP study was conducted by the Preclinical Metabolism and Pharmacokinetics department of Sanofi Recherche, Montpellier Cedex, France between January 1995 and June 1995. The aim of this study was to investigate the major cytochrome P450 isoform(s) involved in SR 47436 oxidation *in vitro* using a phenotyped human hepatic microsomal bank.

Human hepatic microsomal fractions were prepared from large biopsies obtained following surgical operation in secondary liver cancer patients (Caucasian, 8 males and 4 females). The fractions were characterized by determining protein concentration, cytochrome P450 content and for their ability to perform specific enzyme reactions reported to be catalyzed by a single cytochrome P450 isoform.

Biotransformation was initiated by incubating microsomal fraction with graded concentrations (10 to 100 µM) of SR 47436 (batch 93.06 and 5ARL005, solubilized in DMSO) for 30 min at 37°C. Enzymatic reaction was initiated with the addition of NADPH, the essential cofactor for cytochrome P450 monooxygenase-dependent reactions. Interacting drugs (specific P450 isoform substrates or inhibitors, see Table 2.3.11.1) were added to the incubation mixture just before NADPH addition. At the end of incubation, enzyme reaction was stopped by the addition of 1 volume of 20% TCA-acetonitrile for 1 volume of incubation mixture. SR 47436 metabolites were quantified by HPLC with UV detection.

Additionally, the sponsor studied specific enzyme reactions of eight P450 isoforms in metabolizing SR 47436. This was investigated in microsomal fractions prepared from human ß-lymphoblastoid cell lines (purchased commercially), which are engineered to stably express human cytochrome P450 cDNA. Studies were also performed with microsomal fractions prepared from non-engineered cells (control conditions). The biotransformation procedure remained the same except for incubation time (1 hr) and SR 47436 concentration (50 µM). The specificity of cytochrome P450 isoforms to perform specific enzyme reactions are summarized in Table 2.3.11.1.

TABLE 2.3.11.1
METABOLIC CAPACITIES OF CYTOCHROME P450 ISOFORMS

CYP	Substrate	K_M (µM)	Investigated Reaction	Inhibitor	K_i (µM)

Results

In the presence of NADPH, human hepatic microsomal fractions metabolized SR 47436 to generate 4 main mono-hydroxy metabolites, « A », « B », « C » and « D ». Metabolite formation was similar irrespective of the human hepatic microsomal fraction investigated. Percentage of biotransformation varied from 30.3 to 64.6% across the subjects. Since the capacity of the different hepatic microsomal fractions to metabolize the different cytochrome P450 isoform substrates was already determined (see Table 2.3.11.1), the relationship between SR 47436 total oxidation and cy P450 isoform substrates was investigated. A positive correlation was only observed between SR 47436 total oxidation and tolbutamide 4-methylation, r^2 being equal to 0.7687. No relationship at all was observed with the biotransformation of other specific substrates (Table 2.3.11.2). Thus, the data suggest that both SR 47436 oxidation and tolbutamide 4-methylhydroxylation share a common (or closely related) isoform of cy P450.

TABLE 2.3.11.2
CORRELATION COEFFICIENTS (PEARSON VALUES) FOR IRBESARTAN TOTAL OXIDATION AND OTHER ENZYME ACTIVITY CATALYZED BY SPECIFIC *CYP* ISOFORMS

Enzyme	P450	IRB	7ER	POD	COH	DOD	TOH	AOH	NDH
P450	1	0.005	0.125	ND	0.222	0.004	0.001	0.075	0.117
IRB		1	0.023	ND	0.177	0.046	0.769	0.348	0.024
7ER			1	ND	0.001	0.002	0.055	0.148	0.333
POD				1	ND	ND	ND	ND	ND
COH					1	0.029	0.138	0.139	0.210
DOD						1	0.025	0.164	0.012
TOH							1	0.168	0.115
AOH								1	0.147
NDH									1

IRB, Irbesartan oxidation; 7ER, 7-ethoxyresorufin *O*-deethylase (*CYP*1A1); POD, phenacetin *O*-deethylase (*CYP*1A2); COH, coumarin 7-hydroxylase (*CYP*2A6); DOD, dextromethorphan *O*-demethylase (*CYP*2D6); TOH, Tolbutamide 4-methyl-hydroxylase (*CYP*2C9); AOH, aniline 4-hydroxylase (*CYP*2E1); NDH, nifedipine Dehydrogenase (*CYP*3A4). ND = Not determined

The second experiment studied the effect of different specific cy P450 isoform-substrates on SR 47436 oxidation in human hepatic microsomal fractions. Among the different substrates investigated, only two of them exhibited a potent concentration-dependent inhibitory effect on the formation of metabolites, « A », « B », « C » and « D ». At 10-fold their Km values, tolbutamide (specific metabolic probe for CYP2C9 isozyme) decreased the formation of SR 47436 metabolites by 80-100%, while nifedipine (specific metabolic probe for CYP3A4 isozyme) decreased metabolites by 50 to 85%. In order to understand the interactions between either tolbutamide and SR 47436, or nifedipine and SR 47436, enzymatic studies were performed using a larger range of concentrations (0.5 to 50-fold their Km value). In these studies, a competitive-type inhibition was observed between tolbutamide and SR 47436, while a noncompetitive-type inhibition was observed

between nifedipine and SR 47436.

A third experiment studied the effect of different specific cy P450 isoform-inhibitors on SR 47436 oxidation (see Table 2.3.11.1 for different inhibitors). Among the different inhibitors studied, both pilocarpine (inhibitor of CYP2A6) and sulfaphenazole, (inhibitor of CYP2C9) exhibited concentration-dependent inhibitory effects on the formation of SR 47436 metabolites. The inhibitory effects of pilocarpine on SR 47436 oxidation are due to its inhibition of CYP2C9 isoenzyme. The sponsor investigated the effect of an array of substrates and inhibitors on the SR 47436 metabolism and the results are summarized as follows:

- A decrease in SR 47436 metabolism was not observed in hepatic microsomal fractions lacking CYP2D6. Neither dextromethorphan (CYP2D6-substrate) nor quinidine (CYP2D6-inhibitor) decreased SR 47436 oxidation.
- Both tolbutamide and warfarin, i.e. two CYP2C9-substrates, and sulfaphenazole (CYP2C9-inhibitor), inhibited SR 47436 oxidation in a concentration-dependent manner. Moreover, tolbutamide 4- methylhydroxylation was competitively inhibited by SR 47436.
- Nifedipine (CYP3A4-substrate) powerfully inhibited the formation of the various mono-hydroxy SR 47436 metabolites. Neither verapamil nor diltiazem, two highly specific CYP3A4-substrates, nor ketoconazole and troleandomycin, two highly specific CYP3A4-inhibitors, inhibited SR 47436 biotransformation. Moreover, nifedipine oxidation was not decreased in the presence of increasing SR 47436 concentrations. It has already been reported in the literature that nifedipine inhibits CYP2C9 metabolic activity.

Finally, metabolism of SR 47436 was also investigated following a one hour incubation of 50 µM SR 47436 and 1 mM NADPH with microsomal fractions prepared from AHH-1 TK ± human ß-lymphoblastoid cell lines which have been engineered to stably express human cytochrome P450 isozymes (see Table 2.3.11.1 for 8 isozymes investigated). Among cytochrome P450 isozyme subfamilies, only two of them (CYP2C9 and CYP3A4) were able to metabolize SR 47436. CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2D6 and CYP2E1 isozymes were not able to metabolize SR 47436 to its various mono-hydroxy metabolites. CYP2C9-engineered microsomal fractions highly metabolized SR 47436 to its four mono-hydroxy metabolites (« A », « B », « C » and « D ») . CYP3A4-engineered microsomal fractions slightly metabolized SR 47436 to its mono-hydroxy metabolites, « C » and « D ».

In conclusion, CYP2C9 is the main isoform involved in the oxidation of SR 47436 to its four mono-hydroxy derivatives. This cytochrome P-450 isozyme exhibits a low inter-individual variability in humans and is not subject to a genetic polymorphism.

2.3.12. Effect of SR 47436 on Cytochrome P450 Monooxygenase Regulation Using Primary Cultures of Human Hepatocytes (Report #RS0005920117/01, Study #MIV108), Vol. 85

This non GLP study was conducted by the department of Metabolism and Pharmacokinetics of Sanofi Recherche, Montpellier Cedex, France between June and November 1990. The effect of SR 47436 on metabolic enzymes such as cytochrome P450 monooxygenase was evaluated on primary cultures of human hepatocytes.

Primary cultures of human hepatocytes were prepared from liver biopsy samples from two male (49-51 years old) and one female (31 years old) Caucasian patient undergoing hepatectomy. Cultures were incubated for 72 hr in the presence of solvent (dimethylsulfoxide); 1, 10, 25 or 50 µM SR 47436; or reference inducers (positive control), dexamethasone (P450 IIIA inducer, 50 µM) or ß-naphthoflavone (P450 IA inducer, 50 µM). After a 3-day incubation (at 37°C), hepatocytes were scraped from culture flasks and microsomal fractions were prepared. Enzyme activity indicative of cytochrome P450 isozymes (7-ethoxyresorufin *O*-deethylase, phenacetin *O*-deethylase, and nifedipine oxidase) was measured by enzymatic methods; cytochrome P450 isozymes were analyzed by Western blot methods.

Following a three day-treatment of primary cultures of human hepatocytes, SR 47436, at concentrations up to 25 µM, had no significant induction/inhibition effects on cytochrome P450 isozyme activities. At 50 µM, both 7-ethoxyresorufin *O*-deethylase (cytochrome P450 1A1) and phenacetin *O*-deethylase (cytochrome P450 1A2) were slightly increased (1.8 to 2.4-fold), but only in one of three hepatocyte cultures. SR 47436 had no effect on nifedipine oxidation (catalyzed by P450 3A gene subfamily) at all concentrations studied. Thus, the sponsor concludes that SR 47436 is neither an inducer nor an inhibitor of cytochrome P450 1A and 3A gene subfamilies in primary cultures of human hepatocytes.

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Figure 2.3.13 : Proposed metabolic pathways of SR 47436 (irbesartan); distribution of metabolites across species

[ ] position of hydroxylation, oxidation and or glucuronidation not assigned.

FIGURE 2.3.13 (CONT'D)
PROPOSED METABOLIC PATHWAYS OF SR 47436 (IRBESARTAN); DISTRIBUTION OF IRBESARTAN METABOLITES ACROSS SPECIES

Metabolite code	Pathway/ metabolite	Species	Matrix	Reference code
		Mouse	Plasma	SR 47436/BMS186295
		Rat	Plasma, urine (f), bile, feces	
		Rabbit	Plasma, urine	
		Macaque	Plasma, urine, bile, feces	
		Human	Plasma, urine, feces	
A		Rat	Bile	No reference available
B		Rat	urine, bile, feces	BMS-M1 (diol in ω-1 and in β cyclopentane ring positions)
		Rabbit	urine	
		Macaque	urine, bile, feces	
		Human	plasma, urine, feces	
C		Mouse	plasma	SR 90149 (hydroxy in α position on the butyl side chain)
		Rat	urine (f), bile, feces	SR 90148/BMS-M4 (hydroxy in ω-1 position on the butyl side chain)
		Rabbit	plasma, urine	BMS-M5 and BMS-M7 (hydroxy in the β position on the cyclopentane ring)
		Macaque	urine, bile	
		Human	plasma, urine, feces	
D		Rat	plasma, urine (f), bile	no reference available
		Macaque	bile	
E		Rat	plasma, urine (f), bile	SR 90150/BMS-M8
		Rabbit	urine	
		Macaque	plasma, urine, bile	
		Human	plasma, urine, feces	
F		Mouse	plasma,	SR 48001
		Rat	plasma (m)	
G		Mouse	plasma, urine	SR 90145 (hydroxy in α position on the butyl side chain)
		Rat	plasma (m), urine	SR 90146 (hydroxy in ω-1 position on the butyl side chain)
		Macaque	urine	

Metabolite code	Pathway/ metabolite	Species	Matrix	Reference code
H		Rat Rabbit Macaque Human	urine, bile urine urine urine, feces	BMS-M6 (keto in ω-1 position on the butyl side chain)
I		Rat Macaque	bile plasma, urine, bile	no reference available
J		Rat Rabbit Macaque Human	plasma, urine (f), bile, feces urine plasma, urine, bile, feces plasma, urine, feces	SR 49498
K		Rat Rabbit Macaque	plasma, urine (f), bile urine urine, feces	no reference available
L		Rat Macaque	bile bile	no reference available
M		Rat Macaque	plasma, urine (f), bile urine, feces	no reference available
N		Rat Human	bile (m) plasma, urine, feces	BMS-M2
O		Rat Human	bile (m) plasma, urine, feces	BMS-M3
P		Rat	bile (m)	no reference available

Metabo-lite code	Pathway/ metabolite	Species	Matrix	Reference code
Q		Rat	bile (m)	no reference available
R		Mouse Rat	urine urine	no reference available

(m) : male; (f) : female.

Biological matrices not investigated were: mouse bile and feces, rabbit bile and feces, and human bile.
J : Stability samples demonstrate the possibility of degradation of irbesartan to SR 49498 (the open ring analog). However, from comparison of amounts observed in the spiked stability samples and those observed in the collected biological samples following administration of irbesartan, the production of J as a result of systemic metabolism of irbesartan cannot be excluded.
Main metabolites characterized from "*in-vitro*" studies:

Microsomal fractions (rat, dog, macaque, baboon, human): several monohydroxy irbesartan (4 isomers) derivatives and irbesartan tetrazole N_2-glucuronide

Human hepatocytes in primary culture: irbesartan tetrazole N_2-glucuronide, monohydroxy, dihydroxy, ring opened, monohydroxy glucuronide and glucose conjugate derivatives.

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2.4. **Excretion** *Results from all studies concerning excretion are summarized in Table 2.4.5.*

2.4.1. Biliary Excretion Following Single Oral or Intravenous Administrations of ^{14}C-SR 47436 to Rats (Report #RS0005930318/02, Study #EBI0016), Vol. 86

The summary of this study is given under section 2.3.4 as a part of biliary metabolite profile.

2.4.2. Biliary Excretion Following Single Oral or Intravenous Administrations of ^{14}C-SR 47436 to Male Monkeys Report #RS0005930624/01, Study #EBI0030), Vol. 87

The summary of this study is given under section 2.3.8 as a part of biliary metabolite profile.

2.4.3. Blood Distribution, Pharmacokinetics and Urinary Excretion of Radioactivity Following Single Oral Administration of [^{14}C]-SR 47436 in Female Rabbits (Report # RS 000595103l/02, Study #LPR0066), Vol. 71

The summary of this study is given under section 2.1.3 as a part of absorption and pharmacokinetics.

2.4.4. Urinary and Fecal Excretion Following a Single Oral or Intravenous Administration of Labelled SR 47436 to Rats (Report #RS0005921007/01, Study #EBA0067), Vol. 86

This non GLP study was conducted by the department of Metabolism and Pharmacokinetics of Sanofi Recherche, Montpellier Cedex, France between July and August 1992.

Four male (266-340 gm) and four female (250-309 gm) Sprague-Dawley rats (8 weeks old) received either single oral (gavage) doses of 10 mg/kg or single intravenous doses of 1 mg/kg SR 47436 (30 µCi/kg ^{14}C-SR 47436 diluted with unlabeled SR 47436, batch 91.01). Animals were fasted overnight before treatment until 2 hours after treatment. Animals were housed singly in metabolism cages allowing separate collection of urine and feces. Urine samples were collected at 0-6, 6-24, 24-48, 48-72, 72-96, 96-120, 120-144 and 144-168 hr after dosing. Feces were collected as 24 hour fractions from 0-168 hr. Animals were sacrificed at 168 hours and carcasses were analyzed. Radioactivity was measured by liquid scintillation counting.

Results

A cumulative total of 7.86 and 6.09% of the administered dose of radioactivity appeared in urine of males and females, respectively, after oral administration of SR 47436. The cumulative total excretion in feces was 81.73 and 85.07% for males and females, respectively. A similar pattern of excretion was seen after intravenous administration of SR 47436 (Table 2.4.4.1). With both routes of administration, most of the excreted radioactivity appeared in urine and feces within 48 hr after dosing. Less than 2% of the administered dose of radioactivity remained in the carcasses 168 hr (7 days) after dosing. In summary, irrespective of the sex and the route of administration, less than 11% of the administered dose was excreted over 0-168 hr in urine, while approximately 85% was excreted over this same period in the feces. In all cases, excretion was rapid with the great majority of excreted material appearing in urine and feces within 24 hours.

TABLE 2.4.4.1
MEAN CUMULATIVE URINARY AND FECAL EXCRETION FOLLOWING A SINGLE ORAL OR I.V. ADMINISTRATION OF ^{14}C SR 47436 TO RATS

TIME (HR)	URINE M	URINE F	FECES M	FECES F	CARCASS M	CARCASS F	TOTAL RECOVERED M	TOTAL RECOVERED F
ORAL								
0-24	7.3 ± 1.4	5.1 ± 0.7	70.2 ± 8.6	56.9 ±12.0				
0-48	7.6 ± 1.3	5.7 ± 0.7	80.7 ± 1.0	79.0 ± 3.0				
0-168	7.9 ± 1.3	6.1 ± 0.8	81.7 ± 1.1	85.1 ± 2.0	1.7 ± 0.1	1.4	90.4 ± 1.8	91.5 ± 1.7
I.V.								
0-24	4.8 ± 1.8	7.4 ± 0.9	74.0 ± 5.4	53.1 ± 6.2				
0-48	5.2 ± 1.8	9.1 ± 1.0	84.1 ± 4.9	73.6 ± 7.1				
0-168	5.4 ± 1.8	10.7 ± 1.3	86.9 ± 4.8	85.4 ± 5.8	0.3 ± 0.1	1.1 ± 0.4	92.6 ± 5.0	97.2 ± 4.2

Values expressed as % of the administered dose, mean ± standard deviation, a: n = 2; b: n = 1

2.4.5. Urinary and Fecal Excretion Following a Single Oral Administration of Labelled SR 47436 to Male Monkeys (Report #RS0005921020/01, Study #EBA0068). Vol. 87

This non GLP study was conducted by the department of Metabolism and Pharmacokinetics of Sanofi Recherche, Montpellier Cedex, France between July and August 1992.

Three male (2.97-4.25 kg) *Macaca fascicularis* monkeys each received a single oral (by gavage) dose of 10 mg/kg SR 47436 (30 µCi/kg ^{14}C-SR 47436 diluted with unlabeled SR 47436, batch 91.01). Precise age of these animals is not known since they were collected from the wild. Animals were fasted overnight before treatment and then fed 2 hours after treatment. Animals were housed singly in metabolism cages allowing separate collection of urine and feces. Urine samples were collected at 0-6, 6-24, 24-48, 48-72, 72-96, 96-120, 120-144 and 144-168 hr after dosing. Feces were collected as 24 hour fractions from 0-168 hr. Radioactivity was measured by liquid scintillation counting.

Results

The excretion was rapid with a great bulk of excreted radioactivity appearing in the urine within 24 hr and in the feces within 48 hr. A cumulative total of 10.1 ± 3.3 and 81.5 ± 8.4% of the administered dose of radioactivity appeared in urine and feces, respectively, after oral administration of SR 47436. The mean overall excretion represented 91.7 ± 7.0% of the administered dose. Most of the excreted radioactivity (approximately 71 % of the dose) appeared in urine and feces within 48 hr after dosing (Table 2.4.5.1).

TABLE 2.4.5.1
MEAN CUMULATIVE URINARY AND FECAL EXCRETION FOLLOWING A SINGLE ORAL ADMINISTRATION OF ^{14}C SR 47436 TO MALE MONKEYS

TIME (HR)	URINE	FECES	TOTAL RECOVERED
0-24	7.1 ± 1.4	11.5 ± 9.3	18.6 ± 9.9
0-48	9.3 ± 3.2	62.3 ± 3.6	71.6 ± 3.3
0-168	10.1 ± 3.3	81.5 ± 8.4	91.7 ± 7.0

Mean values expressed as % of the administered dose ± standard deviation

TABLE 2.4.5
SUMMARY OF EXCRETION DATA FOR ^{14}C-SR 47436 (% OF DOSE)

Animal species	Dose (mg/kg/d)	Duration*	Route	Sex	Urine		Bile		Feces		Reference Section #
					0-24 h	0-X h	0-24 h	0-48 h	0-24 h	0-X h	
[illegible]											*2.3.1*
Rat	5	single	PO	M	4.8 ± 1.5	5.4 ± 1.7 (48 h)	44.3 ± 3.9	53.6 ± 7.0	52.9 ± 17.7	89.7 ± 7.9 (48 h)	-
				F	3.7 ± 0.2	4.7 ± 0.3 (48 h)	44.8 ± 13.1	61.5 ± 2.3	47.3 ± 30.4	92.0 ± 14.2 (48 h)	
	10	single	PO	M	7.3 ± 1.4	7.9 ± 13 (168 h)			70.2 ± 8.6	81.7 ± 1.1 (168 h)	*2.4.3*
				F	5.1 ± 0.7	6.1 ± 0.8 (168 h)			56.9 ± 12.0	85.1 ± 2.0 (168 h)	
	10[a]	single	PO	M		3.0 ± 2.5 (48 h)	73.8 ± 2.8	77.7 ± 2.1		6.9 ± 5.6 (48 h)	*2.3.4*
				F		1.4 ± 1.4 (48 h)	65.0 ± 6.7	71.6 ± 6.3		0.3 ± 0.5 (48 h)	
	10	single	PO	M			70.6 ± 2.5				-
				F			55.7 ± 3.5				
	25	single	PO	F			71.2 ± 27.1	75.0 ± 28.4			-
	30	single	PO	M	3.4 ± 1.0	3.5 ± 1.0 (168 h)			93.2 ± 5.5	99.0 ± 3.9 (168 h)	*2.2.4*
	30	single	PO	F	4.2 ± 0.7	4.6 ± 0.8 (168 h)			85.3 ± 7.1	97.4 ± 3.2 (168 h)	*2.2.4*
	10.5	single	PO	M	3.9 ± 1.1				56.1 ± 12.5		-
	10.5	7 days	PO	M	1.5 ± 0.4				84.7 ± 8.2		-
	10.5	14 days	PO	M	1.9 ± 0.4				86.5 ± 5.9		-
	1	single	IV	M	4.8 ± 1.8	5.4 ± 1.8 (168 h)			74.0 ± 5.4	86.9 ± 4.8 (168 h)	*2.4.3*
				F	7.4 ± 0.9	10.7 ± 1.3 (168 h)			53.1 ± 6.2	85.4 ± 5.8 (168 h)	
	1[a]	single	IV	M		4.4 ± 0.6 (48 h)	51.6 ± 5.7	64.6 ± 5.5		0.8 ± 0.2 (48 h)	*2.3.4*
				F		6.8 ± 3.1 (48 h)	35.9 ± 12.1	45.1 ± 11.3		0.4 ± 0.2 (48 h)	
	1	single	IV	M			30.5 ± 11.6				-
				F			17.3 ± 5.7				
[illegible]											*2.1.3*
Macaque	10	single	PO	M	7.1 ± 1.4	10.1 ± 3.3 (168 h)			11.5 ± 9.3	81.5 ± 8.4 (168 h)	*2.4.4*
	10[a]	single	PO	M	12.1 ± 14.2	13.3 ± 14.5 (48 h)	44.3 ± 20.0	48.1 ± 18.9	2.3 ± 2.7	4.8 ± 4.2 (48 h)	*2.3.8*
	10	single	PO	M	3.9 ± 1.9				6.5 ± 13.0		-
	10	7 days	PO	M	7.1 ± 3.0				84.9 ± 12.3		-
	10	14 days	PO	M	6.0 ± 3.4				77.4 ± 27.6		-
	25	single	PO	M+F			20.5 ± 0.8	22.4 ± 2.8			-
	1[a]	single	IV	M	21.5 ± 20.7	22.1 ± 21.5 (48 h)	57.5 ± 49.5	58.2 ± 49.3	0.3 ± 0.1	0.9 ± 0.9 (48 h)	*2.3.8*
	1	single	IV	M	8.9 ± 1.4	11.5 ± 2.3 (168 h)			0.1 ± 0.2	82.6 ± 8.2 (168 h)	-
[illegible]										54 ± 9 (168 h)	-
										65 ± 4 (168 h)	-

*: animals dosed once or daily for 7 or 14 days; [a]: collection of urine, bile and feces in the same animals
-: study not reviewed