CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: 21-345

PHARMACOLOGY REVIEW(S)

NDA 21, 345

Sponsor: Fonda BV
1076 HS Amesterdam, The Netherlands

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REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA ORIGINAL SUMMARY

Drug:

Trade name: Xantidar™ Injection

Generic name: Fondaparinux sodium

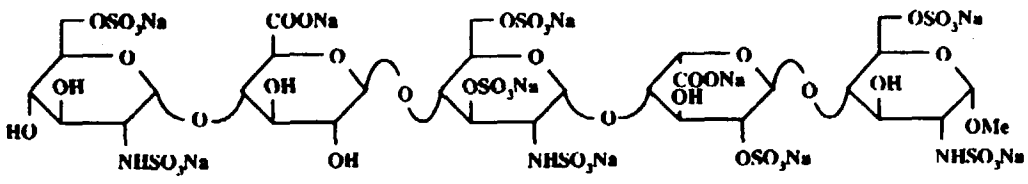
Code name: Org31540/SR90107A (Organon/Sanofi-Synthelabo Codes)

Chemical name: IUPAC Name: Methyl O-(2-deoxy-6-O-sulfo-2-sulfoamino-α-D-glucopyranosyl)-(1→4)-O-(β-D- glucopyranosyluronic acid)-(1→4)-O-(2-deoxy-3,6-di-O-sulfo-2-sulfoamino-α-D-glucopyranosyl)-(1→4)-O-(2-O-sulfo-α-L-idopyranosyluronic acid)-(1→4)-2-deoxy-6-O-sulfo-2-sulfoamino-α-D-glucopyranoside, decasodium salt.

CAS registry number: 114870-03-0.

Molecular formula/molecular weight: $C_{31}H_{43}N_3NA_{10}O_{49}S_8$/1728

Structure:



Formulation: Xantidar injection will be supplied in 10 pre-filled, —— single-use syringes affixed with an automatic needle protection system. Each pre-filled syringe contains 2.5 mg fondaparinux sodium in 0.5 ml of an isotonic solution of sodium chloride and water for injection, USP and is affixed with a 27 gauge x ½ inch needle.

Category: Antithrombotic agent.

Relevant INDs/NDAs/DMFs:

1. IND ——;

2. IND ——

Proposed Marketing Indication: Xantidar subcutaneous injection is indicated for the prevention of venous thromboembolic events in patients undergoing major orthopedic surgery of the lower limbs such as hip fracture, major knee or hip replacement surgeries.

Dose: The recommended dose of Xantidar is 2.5 mg once daily administered post-operatively by subcutaneous injection. The initial dose should be given 6 hours following surgery. The duration of treatment was not clearly indicated; however, it was assumed that the duration of treatment would not exceed the duration of clinical trial, which was 7 to 11 days.

Disclaimer: Some of the sponsor's material has been incorporated into this review.

Preclinical Studies and Testing Laboratories:

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*: Report or study number used in the IND —— /IND —— or its amendments.

**: The number appeared in boldface in the parenthesis indicates the "study code" used in this NDA 21-345.

***: New studies

1. Sanofi Recherche Paris-Gentilly, France.
2. ——————————
3. Sanofi Recherche, 371 rue du Professeuer J. Blayac, Cedex, France.
4. ——————————
5. Scientific Development Group, N.V. Organon, Oss, The Netherlands.
6. ——————
7. Sanofi Research, Malvern, PA.
8. N.V. Organon, The Netherlands.
9. ——————————

10. [redacted]

Most of the above listed studies have been reviewed previously under IND [redacted] (pharmacology reviews dated February 23, 1997; May 22, 1997; March 21, 2000; January 22, 2001; May 20, 1997; November 20, 1998) and are incorporated in the present review along with the review of the new studies.

PHARMACOLOGY:

Org31540/SR90107A is a novel sulfated pentasaccharide that is obtained through chemical synthesis. With the exception of the methoxy group at its non-reducing end, Org31540/SR90107A is analogous to the pentasaccharide sequence in heparin responsible for binding to antithrombin III (AT-III), the main endogenous regulator of blood coagulation. Pharmacology studies with Org31540/SR90107A have demonstrated AT-mediated inactivation of Factor Xa.

Primary Pharmacology

In vitro

Binding of Pentasaccharide SR90107A to Antithrombin III (Amendment #034, Report 693.4.003).

The affinity of SR 90107A for antithrombin III (ATIII) from human, rat, rabbit, and baboon was determined. The fluorescence emission spectrum of AT III is characteristic for buried trytophan residues ($\lambda_{emission}$ = 338 nm, $\lambda_{excitation}$ = 280 nm). SR90107A, as found for heparin, can increase the quantum yield of ATIII upon binding, due to a change in the hydrophobic environment of a trytophan residue. With a knowledge of the concentrations of ATIII and SR90107A and assuming a stoichiometry of interaction of 1 to 1, the dissociation constant of the ATIII-SR90107A complex can be determined using Scatchard plot analysis. The affinity of SR 90107A for AT III of different origins was as follows: rat > human > baboon > rabbit. The stoichiometry of interaction confirmed the binding of one SR 90107A (pentasaccharide) molecule to one AT III molecule.

Calculated parameters for the binding of SR90107A to ATIII of different species.

AT III Origin	K_D (nM)	Stoichiometry (mol/mol)
Human (n =4)	57.84	0.93
Baboon (n = 3)	78.57	1.00
Rabbit (n = 3)	129.87	1.02
Rat (n = 3)	47.06	0.88

Calculation of the AT III binding strength (K_d) of Org 31540 for human AT III (at pH 8.4 and 7.4) and rat AT III (at pH 7.4) from activity measurements performed at pseudo first order conditions; retrieval of raw data and justification of corrections (SDGRR 4245).

The dissociation constant, K_d, and second-order rate constant of factor Xa inactivation, k_2^+/K_z^+, were determined for the complex of Org 31540 with both human and rat AT-III. For the human AT-III/Org 31540 complex, these reaction constants were determined at both pH 7.4 and 8.4, whereas these constants were determined only at pH 7.4 for the rat AT-III/Org 31540 complex. Both reaction constants were obtained from a study of the kinetics of the factor Xa inactivation reaction at a constant concentration of AT-III and a varying concentration of Org 31540. Org 31540 binds about 10 times stronger to human AT-III at pH 7.4 (K_d = 82.1 nM) than at pH 8.4 (K_d = 754 nM). The second order rate constant of Factor Xa inactivation of this complex is 7.56 x 10^5 $M^{-1}sec^{-1}$ at pH 8.4 and 10.4 x 10^5 $M^{-1}sec^{-1}$ at pH 7.4. At pH 7.4, Org 31540 binds some three to four time stronger to human AT-III than to rat AT-III. For rat, the second order rate constant of Factor Xa inactivation of this complex is 1.97 x $10^6M^{-1}sec^{-1}$.

In vitro effects of Org 31540 on blood coagulation and platelet aggregation (Report 693.6.004, SDGRR 2913).

1. **Activated partial thromboplastin time:** Org 31540 and heparin double the activated partial thromboplastin (APTT) time, a measure of the intrinsic pathway, at concentrations of approximately 56 and 0.4 anti-Xa U/ml in human plasma, respectively. When the test was repeated with diluted activation reagent, the prolongation by Org 31540 was of the same order of magnitude as compared with undiluted reagent. In contrast, heparin displayed a stronger effect with diluted reagent. Org 31540 displayed significantly less activity than heparin with regard to prolongation of the APTT.

2. **Prothrombin time:** In human plasma, Org 31540 at concentrations ≤ 70 anti-XA U/ml did not prolong the prothrombin time, a measure of the extrinsic pathway. Heparin doubled the prothrombin time at approximately 2.5 anti-XA U/ml.

3. **Factor Xa inactivation**: Org 31540 inhibited factor Xa activity in plasma as in an AT-III buffer system when measured amidolytically using the chromogenic substrate, ——— In this system, Org 31540 was approximately 4 times more potent than heparin on a weight basis. Org 31540, at concentrations above 0.05 amidolytic anti-Xa units, dose-dependently prolonged the clotting time of plasma following addition of factor Xa and $CaCl_2$, and possessed a higher specific activity than heparin when expressed in clotting anti-Xa units. Org 31540 and heparin inhibit factor Xa activity in an amidolytic assay with a second order rate constant of 1.85×10^6 and 4.5×10^6, respectively. The dissociation constant (K_d) of Org 31540 and heparin with AT-III are 4×10^{-7} and 2×10^{-8} M, respectively. Inhibition of factor Xa generation by Org 31540 and heparin is dependent on the AT-III concentration, with Org 31540 displaying a higher dependency.

4. **Factor IIa inactivation**: Org 31540 slightly inhibited factor IIa amidolytic activity in plasma. On a weight basis, Org 31540 was approximately 1500 times less potent than heparin. Org 31540 inhibited the amidolytic activity of thrombin in an AT-III buffer system to a maximal value of 40% at a concentration of 25 to 50 μg/ml compared to 100% inhibition with heparin at 0.2 μg/ml. Org inhibited the amidolytic activity of thrombin in a heparin cofactor-II (HC-II) buffer system at concentrations 350 times higher than those of heparin. Org 31540 did not inhibit thrombin activity, as assessed by a fibrin polymerization assay, at concentrations ≤ 140 anti-Xa U/ml in the presence of either AT-III or HC-II. Org 31540 at concentrations ≥ 10 anti-Xa/ml potentiated maximal fibrin polymerization. In contrast, heparin inhibited thrombin activity with an IC_{50} of 0.25 anti-Xa U/ml in the AT-III mediated assay and an IC_{50} of 1.5 anti-Xa U/ml in the HC-II mediated assay.

5. **Inhibition of thrombin generation**: Org 31540 dose-dependently inhibited thrombin generation induced by activated factor Xa in plasma, and the inhibitory activity was increased with prolonged preincubation times. Org 31540 dose-dependently inhibited thrombin generation in human plasma using three different phospholipid sources (liposomes containing phosphatidylcholine and phosphatidylserine > Actin > Simplastin). Org 31540 and heparin dose-dependently inhibited thrombin generation over a 90 min period (T_{50} was prolonged to 30 min by 30 nM Org 31540 and 5 nM heparin). Org 31540 and heparin dose-dependently inhibited thrombin generation stimulated by factor Xa and factor V in the prothrombinase complex. In contrast to heparin, Org 31540 did not inhibit the thrombin generation induced by Factor Xa and preactivated factor V in the prothrombinase complex. Org 31540 and heparin dose-dependently inhibited thrombin generation with

fibrinogen as substrate (T_{50} was prolonged to 30 minutes by 6.5 nM Org 31540 and 1.6 nM heparin). Org 31540 dose-dependently inhibited thrombin generation initiated with activated platelets as a source of factor V and phospholipids; however, maximal inhibition achieved by Org 31540 was only 60% (IC_{50} values were 60 anti-Xa mU/ml for Org 31540 and 5 mU/ml for heparin).

6. **Inhibition of factor Xa generation**: Inhibition of factor Xa generation through direct or indirect reaction with the tenase complex (factors IXa and VIIIa, Ca^{2+}, and platelet phospholipid) was measured in the presence and absence of preactivated factor V (Va). Org 31540 and heparin dose-dependently inhibit the factor Xa generation in the absence of preactivated factor V; although, in contrast to heparin, Org 31540 does not prolong Xa generation time. The same inhibitory effects of both compounds are found in the presence of factor Va.

7. **Platelet Aggregation**: The effect of Org 31540 (0.1 to 150 µg/mL or 0.07 to 100 anti-Xa U/mL) and heparin (0.5 to 100 µg/mL or 0.08 to 16 anti-Xa U/mL) on platelet aggregation induced with ADP (10 µg/mL), collagen (2 µg/mL), or thrombin (0.15 units/mL) was compared. Org 31540 (0.01, 0.1, 1, 10, or 100 anti-Xa U/mL) did not affect ADP-induced platelet aggregation whereas heparin (0.16, 0.80, 3.2, or 16 anti-Xa U/mL) potentiated aggregation in human platelet rich plasma. Similarly, heparin (0.16-16 anti-Xa U/mL) potentiated collagen-induced platelet aggregation, whereas Org 31540 (0.01-100 anti-Xa U/mL) had no effect. The aggregation of rabbit platelets induced by thrombin was dose-dependently inhibited by Org 31540. The inhibitory activities of Org 31540 on thrombin-induced platelet aggregation was dependent on the thrombin concentration used and was independent of AT-III. The IC_{50} values for Org 31540 with or without AT-III were 2.6 and 1.8 µmole/L, respectively. The IC_{50} values for heparin with or without AT-III were 0.01 and 0.3 µmole/L, respectively. The percent inhibition of platelet aggregation induced by 0.05, 0.1, or 0.15 units/ml thrombin was 100, 64, and 20% at 2 U/mL Org 31540. The percent inhibition of platelet aggregation induced by 0.05, 0.1, or 0.15 units/ml thrombin was 100, 82, and 40% at 2.8 U/mL Org 31540.

Comparative study of the anti-factor Xa activities of heparin and SR 90107 as measured in the [redacted] assay (Report 693.4.001).

The aim of this study was to compare the effects of heparin and SR 90107A in a complex Xa assay system using the physiological substrate of factor Xa, prothrombin. Factor Xa was incubated in human plasma containing either SR 90107A (0 to 39.8 µg/ml) or heparin (0 to 41.6 µg/ml). A sample of the

incubation mixture was added to a plasma substrate supplemented with Ca^{2+} and phospholipids. Remaining Xa catalyzed the prothrombin activation of the plasma substrate and provoked its clotting. Clotting time was inversely related with the remaining factor Xa and in direct relation with the anti-factor Xa activity of heparin or SR 90107A. SR 90107A and heparin inhibited factor Xa and prolonged clotting time of the plasma-substrate in a concentration dependent way. A doubling of the control clotting time was obtained with 0.24 µg/ml SR 90107A and 5.55 µg/ml heparin. The difference in factor Xa inhibition by heparin and SR 90107A was attributed to traces of platelet factor 4 in plasma, which inhibited heparin, while having no effect on SR 90107A. High concentrations of heparin and SR-90107A did not completely suppress the coagulation of plasma-substrate, probably since not all of the factor Xa present was neutralized. This phenomenon was attributed to the fact that factor Xa inhibition was produced by the AT III-heparin or AT-III-SR 90107A complexes. At high concentrations of heparin or SR 90107A, nearly all the AT-III present is saturated by the effector. Plasma AT-III concentrations then become the limiting factor and further increases of heparin or SR 90107A have no consequence on factor Xa inhibition.

The ATIII-Mediated Inactivation of Factor IXa by Org 31540/SR90107A In Vitro (Report No. NL0018610).

Antithrombin III (ATIII)-mediated inactivation of Factor IXa by Org 31540/SR90107A in vitro was assessed. Heparin was used as a comparator. Activity of Factor IXa was measured through hydrolysis of the chromogenic substrate, ——— Final concentrations of Org 31540/SR90107A and heparin ranged from 1×10^{-8} to 1×10^{-5} M. Org 31540/SR90107A and heparin dose-dependently enhanced the AT-mediated inactivation of human factor IXa in vitro with IC_{50} values of 1×10^{-7} M and 2×10^{-7} M, respectively.

Effect of SR90107A on Thrombin Generation in Plasma (HVT0020)

This study was conducted to establish the dose-effect response of SR90107A on thrombin generation in plasma, in the intrinsic (initiated by the contact factors) as well as extrinsic pathway (triggered by thromboplastin). In this study, increasing amounts of SR90107A (0, 0.5, 1, 1.5, 3, 5, and 10 µg/ml for intrinsic pathway; 0, 0.125, 0.250, 0.5, 1, 1.5, 3, and 5 µg/ml for extrinsic pathway) was added to defibrinated human plasma and coagulation was triggered either by kaolin (intrinsic pathway) or by thromboplastin (extrinsic pathway). A plasma sample was taken every 30 minute and thrombin generation process was stopped by dilution in an appropriate buffer. The amount of thrombin generated was measured by amidolysis of chromogenic substrate ——— SR90701A inhibited thrombin generation in the extrinsic pathway (ED-50 for thrombin peak inhibition: 0.42 µg/ml) and showed a weaker effect on the intrinsic pathway (ED-50: 1.39 µg/ml).

In vivo

Effect of SR 90107 A on thromboplastin-induced thromboembolism in mice (Report 693.4.013).

The protective effect of SR 90107A evaluated in mice given thromboembolism induced with thromboplastin, and compared with heparin. SR 90107A and heparin were administered in an intravenous bolus 5 min prior to a thromboplastin challenge (1 ng IV). The percentage mortality was recorded over a 24 hr period. The effect of pentasaccharide is not considered to be related with AT-III binding because at doses above 1 mg/kg, all circulating AT-III becomes saturated. In contrast, heparin also inhibits thrombin. The protective effect of SR 90107A may be related to a weak inhibition of thrombin production.

Table 8. Effect of SR 90107A and Heparin on mortality in mice by thromboembolism induced by thromboplastin (Adapted from Sponsor's table in Report 693.4.013).

Treatment	Dose, mg/kg	Mortality	Protective Effect, %
Control		17/20	
SR 90107A	1	7/10	17
	3	5/10	41
	10	4/20	76
	15	2/10	76
Control		8/10	
Heparin	0.025	9/10	0
	0.05	11/20	31
	0.1	4/10	50
	0.15	2/10	75
	0.2	2/20	87
	0.5	0/10	100

Pharmacodynamic and Pharmacokinetic Profile of Org 31540. Effects on Thrombosis, Bleeding, and Coagulation in Experimental Models (Amendment #044; Report 2927).

The antithrombotic properties of Org 31540 were examined in several rat and rabbit models of thrombosis and compared with heparin (see table below). Both Org 31540 and heparin dose-dependently inhibited thrombus formation in the four models. When doses were expressed on an anti-Factor Xa U/kg basis, heparin was consistently more potent than Org 31540. This difference in potency was generally most pronounced when these agents were administered by the intravenous route. However, when using the subcutaneous route, this difference in potency was less pronounced, given the higher of bioavailability of Org 31540 by this route. Effects of Org 31540 and heparin on deposition of ^{125}I-fibrin and ^{51}Cr-platelets were monitored in thrombus formation with a modified AV-shunt model in rats (see table below). Org 31530 at an intravenous dose of 76 anti-Factor Xa U/kg was found to inhibit platelet and fibrin by >50%. Heparin at 80 anti-Factor Xa U/kg had no effect on platelet deposition; however, at the highest tested dose of 320 anti-Factor Xa U/kg, fibrin deposition was almost completely inhibited.

Effects of Org 31540 and heparin, administered by the intravenous and subcutaneous routes, on thrombus formation in various models of thrombosis in rats and rabbits.

Model	Compound	ED_{50} i.v. anti-Xa U/kg	µg/kg	ED_{50} s.c. anti-Xa U/kg	µg/kg
Arterio-venous shunt model rat	Org 31540	800	1,14	990	1,3
	heparin	51	0,30	497	3,1
Venous-stasis rat tissue thromboplastin	Org 31540	34	50	155	204
	heparin	16	100	69	437
Venous-stasis rat human serum	Org 31540	40	53		
	heparin	3	19		
Wessler model rabbit tissue thromboplastin	Org 31540	251	386		
	heparin	8	50		

Effects of Org 31540 and heparin administered by the intravenous route on thrombus weight and platelet and fibrin deposition.

Compound	I.V. Dose, U/kg	Thrombus wt., mg	Platelet deposition ^{51}Cr content	Fibrin deposition ^{125}I-content
Placebo	0	72.4	17.8	1.77
Org 31540	76	33.5	6.0	0.69
	240	21.2	5.4	0.53
	760	15.8	3.5	0.42
Heparin	20	69.8	17.8	1.54
	80	60.9	16.1	0.91
	320	9.9	3.1	0.07

Comparison of the antithrombotic effect of IV and SC administered Org 31540 in the rat venous stasis model (SDGRR 4235).

Earlier studies noted a discrepancy in the antithrombotic activity between IV and SC administration of Org 31540 in the rat venous stasis model with thromboplastin as inducer. Comparison between IV and SC routes of administration was complicated by differences in plasma anti-Xa activities during the thrombotic trigger. It was determined that for this model, the antithrombotic activity after IV and SC administration was similar and highly predicted by plasma anti-Xa activity just prior to the thrombotic event. Ligation of the blood vessel, in order to induce stasis, lead to retention of compound in the isolated segment, while systemic concentrations were decreased by ongoing elimination causing differences of time-dependency for IV and SC routes. It was determined that plasma Xa activity 1 hr after IV administration of Org 31540 was similar to that obtained 1.5 hr after SC administration of the same dose. In the final studies, Org 31540 was administered either 1 or 1.5 hr by IV or SC administration, respectively, prior to induction of thrombosis. Similar antithrombotic activities in this model were measured, if thrombosis was induced at a moment of similar anti-Xa levels after IV or SC administration.

Preclinical Pharmacology of the Antithrombotic Pentasaccharide Org 31540/SR90107A (Amendment #044; Report 5124).

The antithrombotic properties of Org 31540/SR90107A were examined in several animal models of thrombosis.

1. In a model of cyclic flow variation (CFV) with guinea pigs (i.e., severe stenosis of a coronary artery and damage of the subendothelium), the number of CFVs/20 min

following various intravenous treatments were as follows: placebo treatment, ——— (1.1% inhibition); 10 mg/kg aspirin, ——— (89.0% inhibition); 1 mg/kg recombinant hirudin, ——— (30.3% inhibition); 400 anti-Factor U/kg heparin, ——— (39.0% inhibition); 400 anti-Factor Xa U/kg SR90107A, ——— (22.2% inhibition); and 600 anti-Factor Xa U/kg, ——— (16.7% inhibition). Platelets play a significant role in CFV, which may explain the minimal effects of SR90107A or heparin. These two agents do not inhibit collagen-induced platelet activation.

2. In an acute occlusion model with rabbits (i.e., clamping of the carotid artery and positioning a stenotic device over the damaged part), formation of occlusive thrombi, the time to occlusion, and CF score following various intravenous treatments were as follows: with control treatment, all 8 rabbits formed an occlusive thrombus with a mean time to occlusion of 6.6 min and a mean CF score of 3.9 (full occlusion = 4); with 300 anti-Factor Xa U/kg heparin, no occlusive thrombus or CFVs were observed in six rabbits; with 600 ant-Factor Xa U/kg SR90107A, 4 of 6 rabbits developed an occlusive thrombi within 20 min and the mean CF score was 2.2; and with 600 anti-Factor Xa U/kg fraxiparine, 3 of 6 animals developed an occlusive thrombus and the CF score was 2.2. Heparin blocked the formation of occlusive thrombus suggesting that thrombin plays an essential role. Twenty min after the start of the experiment, the left carotid artery was closed and the right carotid artery was clamped and stenosed. Results were as follows: for control rabbits, the mean time to occlusion was 5.9 min and the CF score was 3.9; for heparin-treated rabbits, 3 of 6 right carotid arteries occluded within 20 min and the CF score was 1.7; for SR90107A-treated rabbits, 3 of 6 right carotid arteries occluded within 20 min and the CF score was 2.0; and for fraxiparine-treated rabbits, 5 of 6 right carotid arteries occluded and the CF score was 2.8.

3. In an acute occlusion model with rats (i.e., $FeCl_3$ treatment is used to induce an occlusive thrombi in an artery by local deendothelialization and deep wall injury exposing the smooth muscle layer), the protective effects of SR90107A, heparin, and argatroban were assessed with intravenous administration of a loading dose followed by a 60 min maintenance dose. Heparin at the high dose protected 5 out of 5 animals. SR90107A alone (75% saturation of antithrombin III) had no protective effects; however, when combined with argatroban, slight protection was observed as shown in the table below.

Table 1

Prevention of $FeCl_3$-induced occlusion of the rat carotid artery by unfractionated heparin (UFH), the pentasaccharide Org 31540 / SR 90107A (Org 31540 / SR 90107A) batches M and Q either alone or in combination with Argatroban; combinations and doses as indicated in the table. Parameters include the number out of total number of animals tested, the time to full occlusion relative to that of the untreated control after $FeCl_3$ application and the total time of patency during the 1 h evaluation period in comparison with the same control. Data represent means ± S.E.M.

treatment	dose (mg/kg load+maint.)	No. fully protected	for those not fully protected:	
			time to 100% occl. (min); [control]	total time open (min)
UFH	0.19 + 0.19	0 / 5	19 ± 2 [13 ± 1]	20 ± 2 [13 ± 1]
	0.57 + 0.57*	5 / 10	33 ± 1 [15 ± 1]	36 ± 4 [15 ± 1]
	1.90 + 1.90	5 / 5		
PS, batch M	0.38 + 0.38	0 / 3	15 ± 2 [12 ± 1]	15 ± 2 [12 ± 1]
Argatroban (A), low dose	0.18 + 0.77	1 / 6	22 ± 2 [12 ± 1]	22 ± 2 [12 ± 1]
high dose	0.34 + 2.06	0 / 6	7.2 ± 0.7**	7.2 ± 0.7**
PS-M + A, low dose		3 / 6	16 ± 1 [12 ± 1]	16 ± 1 [12 ± 1]
PS-Q + A, high dose		2 / 6	9.7 ± 1.0**	34 ± 15**

* data pooled from two separate series of experiments, performed in January and February 1997, respectively

** counting started after cessation of the Argatroban maintenance infusion which, while still running, fully protected the artery from occlusion by the $FeCl_3$

4. In a model of venous platelet thrombus formation + stenosis in the rat (i.e., a silk thread was inserted into the vena cava + partial stenosis of the vena cava, which restricted platelet involvement in the thrombus to ≤30%), thrombi weights were measured following intravenous treatment with SR90107A (6.25-50 anti-Factor Xa U/kg), heparin (20-180 anti-Factor Xa U/kg), fraxiparine (30-270 anti-Factor Xa U/kg), or clexane (30-270 anti-Factor Xa U/kg). Expressed in anti-Factor Xa units, SR90107A (ED_{50} = 19) was three times more potent than heparin (ED_{50} = 64), fraxiparine (ED_{50} = 64), or clexane (ED_{50} = 77).

5. In a model of venous platelet thrombus formation in the rat (i.e., a silk thread was inserted into the vena cava, platelet composition of thrombus was ~65%), thrombi weights were measured following intravenous treatment with SR90107A (3.6-118.4 anti-Factor Xa U/kg), heparin (40-320 anti-Factor Xa U/kg), fraxiparine (20-320 anti-Factor Xa U/kg), or clexane (40-320 anti-Factor Xa U/kg). Expressed in anti-Factor Xa units, SR90107A (ED_{50} = 52) was three times more potent than heparin (ED_{50} = 146), fraxiparine (ED_{50} = 153), or clexane (ED_{50} = 182).

6. In a model of arterial platelet thrombus formation in the rat (i.e., a silk thread was inserted into the aorta, platelet composition of thrombus was ~65%), thrombi weights were measured following intravenous treatment with SR90107A (12.1-363 anti-Factor Xa U/kg), heparin (40-320 anti-Factor Xa U/kg), fraxiparine (80-320 anti-Factor Xa U/kg), or clexane (30-1000 anti-Factor Xa U/kg). Expressed in anti-Factor Xa units, SR90107A (ED_{50} = 168) was approximately equipotent with heparin (ED_{50} = 146) and twice as potent as fraxiparine (ED_{50} = 320) or clexane (ED_{50} = 350).

7. In a model of thrombus formation on coronary stents in the rat circulation (i.e., modified arteriovenous shunt using stainless steel stents), the accumulation of [^{125}I]fibrin and [^{51}Cr]platelets was measured following intravenous treatment with SR90107A (100 and 200 anti-Factor Xa U/kg) or heparin (100, 200, and 600 anti-Factor Xa U/kg). Both heparin and SR90107A produced a dose-dependent inhibition of platelet and fibrin

deposition. The activity of heparin was more directed toward inhibition of fibrin deposition, while SR90107A displayed no preference. The potency of SR90107A to inhibit platelet accumulation was twice that observed for heparin.

Table 3

	dose (per kg)	[^{51}Cr]platelets	[^{125}I]fibrin
UFH	(100 U; 0.63 mg)	12 ± 15	51 ± 7
	(200 U; 1.3 mg)	38 ± 6; 26 ± 16	61 ± 2; 66 ± 3
	(600 U; 3.8 mg)	90 ± 0.1	90 ± 0.3
PS	(100 U; 0.14 mg)	51 ± 6	36 ± 9
	(200 U; 0.28 mg)	74 ± 2; 82 ± 6	57 ± 3; 60 ± 4

Fig. 6

8. In a model of rethrombosis, post-thrombolysis with rabbits (i.e., to test a compound for accelerated and/or improved reopening of an occluded artery while being treated with streptokinase), treatment with SR90107A (300 anti-Factor Xa U/kg IV bolus plus 150 anti-Factor Xa U/kg/hr infusion or 600 anti-Factor Xa U/kg IV bolus plus 300 anti-Factor Xa U/kg/hr infusion), heparin (160 anti-Factor Xa U/kg IV bolus plus 120 anti-Factor Xa U/kg/hr infusion or 320 anti-Factor Xa U/kg IV bolus plus 240 anti-Factor Xa U/kg/hr infusion), or fraxiparine (406 anti-Factor Xa U/kg IV bolus plus 120 anti-Factor Xa U/kg/hr infusion) was initiated immediately after the start of a 1-hr streptokinase infusion. Streptokinase alone restored blood flow for 1 of 10 rabbits. The heparin low dose had no effect during the first hr; however, blood flow was double during the next 2 hrs. The heparin high dose completely restored blood flow during the first hr. Fraxiparine produced similar results to the heparin low dose. SR90107A dose-dependently improved streptokinase-induced thrombolysis. The SR90107A low dose was similar to the heparin low dose. The SR90107A high dose produced a somewhat delayed effect with reopening of the artery at 80-110 min after the start of treatment.

9. Blood loss was examined in a subdermal bleeding model with rats that received SR90107A, heparin, or enoxaparin by the intravenous route. Both heparin and enoxaparin in a dose-dependent manner enhanced subdermal blood loss. Heparin at 120 anti-Factor Xa U/kg enhanced blood loss (8.6 times the ED_{50} stasis thrombosis). SR90107A at doses up to 60 times the ED_{50} stasis thrombosis did not enhance blood loss. In antithrombin III-supplemented rats, blood loss was increased with SR90107A, although, the difference was not statistically significant. In nephrectomized rat (e.g., renal excretion is the primary route of elimination of SR90107A), blood loss was not enhanced by SR90107A treatment despite increased plasma drug levels. SR90107A did not enhance blood loss produced by heparin (120 anti-Factor Xa U/kg) or aspirin (30 mg/kg, oral); however, it enhanced loss produced by clopidogrel (2 times 3 mg/kg, oral) and the vitamin K antagonist, ——— (4 times 15 mg/kg, oral).

Table 6

Thrombus growth inhibition and bleeding enhancement by the examined GAGs. Data represent ED_{50} and ED_{200} values with 95% confidence limits of the antithrombotic and bleeding enhancing activities. Compounds were tested in 3 doses (n=6 each in the stasis model, and n=6 each in the bleeding model).

Compound	ED_{50} Venous stasis rat anti-Xa U/kg [mg/kg]	ED_{200} Subdermal bleeding rat anti-Xa U/kg [mg/kg]	safety/efficacy ratio ED_{200}/ED_{50}
heparin, exp. 1	14 (12;16) [0.09]	130 (91;183) [0.82]	9
exp. 2		210 (160;410) [1.33]	15
enoxaparin	15 (11;21) [0.13]	340 (250;510) [3.04]	23
Org 31540 / SR 90107A	43 (37;50) [0.07]	> 2700 [>4.15]	>60

Table 7

Effect of increasing concentrations of Org 31540 / SR 90107A under conditions of increased plasma levels of ATIII and distant renal function (i.e. maintained initial plasma levels of the pentasaccharide, both unbound and bound to ATIII) towards subdermal bleeding in the rat. Data represent means and (S.E.M.)

	dose (nmol/kg)	bleeding (% placebo)	doubled ATIII (% control)	replacement (% control)
Placebo	control	100 (13)*	113 (29)(% plac.)	87 (12)(% plac.)
Org 31540 / SR 90107A	200	102 (20)	110 (10)	77 (4)
	600	130 (21)	171 (21)	104 (27)
	1000	130 (19)	207 (32)	81 (11)

*mg/kg.

APPEARS THIS WAY ON ORIGINAL

<u>Antithrombotic effect of SR 90107A in an arterio-venous shunt in the rat</u> (Report 693.4.012).

The antithrombotic effect of SR 90107A was evaluated in an arterio-venous shunt in the rat and compared with heparin. Thrombosis was induced on a silk thread placed in an extracorporeal shunt between the carotid artery and the jugular vein. Intravenous injections of 0.1, 0.3, 1, or 3 mg/kg SR 90107 A and heparin were made 5 min before induction of thrombosis. SR 90107A inhibited thrombus formation in a dose-dependent manner up to 1 mg/kg. Maximal inhibition of thrombus formation was 47% for SR 90107A. Heparin at 3 mg/kg reduced thrombus formation by 88%.

APPEARS THIS WAY ON ORIGINAL

Antithrombotic Effect of SR 90107A in the Wessler Stasis Thrombosis Model in the Rabbit (Amendment #014; Report 693.4.019).

The antithrombotic activity of SR 90107A administered by the subcutaneous route at doses of 0.051, 0.17, 0.51, and 1.02 mg/kg (doses expressed as salified form) was assessed in the Wessler stasis thrombosis model using male New Zealand rabbits. Heparin administered by the subcutaneous route at doses of 0.3, 1, 2, and 3 mg/kg was used as a reference. SR 90107A or heparin was administered two hr prior to induction of venous thrombosis (i.e., injection of thromboplastin followed by ligation of the jugular vein). Thrombi were collected after stasis for 15 min and weighted. Inhibition of thrombus formation with SR90107A doses between 0.17 and 1.02 mg/kg was essentially constant at 60-62%. In contrast, heparin produced a dose-related inhibition of thrombus formation with almost complete inhibition observed at 3 mg/kg. SR90107A at a dose of 0.17 mg/kg produced 62, 35, and 27% inhibition of thrombus formation at 2, 6, and 16 hr following treatment, respectively. Heparin at 2 mg/kg produced a 74, 64, and 13% inhibition of thrombus formation at 1, 2, and 6 hr following treatment, respectively. The duration of antithrombotic activity for SR90107A appeared to be longer than that observed for heparin.

Effects of SR90107A on Cyclic Flow Variations (CFVs) in a Stenosed and Injured Coronary in the Dog (Amendment #018; Report 693.4.020).

Effects of SR90107A on cyclic flow variations (CFVs) were assessed with an injured and stenosed coronary artery in a dog model. Using mongrel dogs, a segment of the coronary artery was isolated, moderate intimal damage was produced, and stenosis was induced with a silk thread to reduce blood flow to approximately 30% of the basal level. With intimal damage and stenosis, acute platelet thrombi, developed in the narrowed lumen, reducing blood flow to approximately zero, and then embolized to restore blood flow and produce CFVs. Following a 1-hr observation period, dogs received intravenous treatment with SR90107A at 1286 anti-Factor Xa U/kg, heparin at 500 anti-Factor Xa U/kg, or placebo (i.e., isotonic saline). After 2 hr, dogs were treated

with epinephrine by intravenous infusion at a dose of 0.2 μg/kg/min for 20 min to reinduce CFVs. SR90107A inhibited CFVs by 25 and 49% during the first and second hours, respectively. Heparin inhibited CFVs by 83-84% during both the first and second hours. Systolic arterial pressure was reduced to 75% of the basal value (141 mm Hg) in the placebo group as compared to no change in the SR90107A and heparin groups. Diastolic arterial pressure was reduced to 73.7 and 82% of basal values (99 and 106 mm Hg) in the placebo and heparin groups, respectively, as compared to no change in the SR90107A group. SR90107A treatment moderately inhibited both CFVs and decreases of systolic and diastolic blood pressures.

	CORONARY BLOOD FLOW			NUMBER OF CFVs			INHIBITION OF CFVs		REINDUCTION AFTER EPINEPHRINE 0.2 μg/kg/min
	BASAL	AFTER STENOSIS	% OF VARIATION	BASAL FREQ CFV/min	AFTER TREAT 0-1 H	AFTER TREAT 1-2 H	AFTER TREAT 0-1 H	AFTER TREAT 1-2 H	
GROUP 1 CONTROL N=7	27 ± 4	21 ± 2	-16 ± 13 %	9 ± 1	9 ± 2	8 ± 1	0 %	0 %	
GROUP 2 HEPARIN 200 UAXa/kg N=6	19 ± 1	17 ± 3*	-8 ± 12 %	9 ± 1	1 ± 1	1 ± 1	83 ± 9%	84 ± 18%	1/3
GROUP 3 SR 90107A 1250 UAXa/kg N=6	30 ± 4	21 ± 1	-27 ± 7 %	6 ± 1	5 ± 2	4 ± 2	25 ± 18%	49 ± 25%	1/3

Results are expressed in percentages of variations *versus* values before stenosis

* $p < 0.05$ | } MANN/WHITNEY 's test *versus* values before stenosis (T0).

** $p < 0.01$ |

Potentiation by SR 90107A of Recombinant Tissue-Type Plasminogen Activator-Induced Thrombolysis in the Rabbit (Report No. 693.4.017).

Methods: The effect of SR 90107A on thrombolysis induced by recombinant tissue-type plasminogen activator (rt-PA) was evaluated in a rabbit thrombolysis model. The thrombolytic effect of various treatments was evaluated by the lysis of standard-sized, preformed [^{125}I]-fibrinogen-labeled thrombi produced in the external jugular vein of New Zealand white rabbits. Thrombolysis was performed by intravenous infusion of rt-PA at a dose of 0.5 mg/kg through the contralateral marginal ear vein over a 4 hr period. SR 90107A was administered by intravenous bolus injection at 0, 0.051, 0.17, or 0.51 mg/kg with the start of the infusion. Ex vivo determinations of the plasma fibrinogen concentration, activated partial thromboplastin time (APTT), and anti-Xa activity were done at 0.5 and 4 hr after starting the infusion.

Results: Infusion of rt-PA caused 43% thrombolysis. Injection of SR 90107A enhanced the thrombolytic action of rt-PA in a dose-dependent manner; however, SR 90107A alone had no effect. The combination of 0.51 mg/kg SR 90107A with rt-PA caused 79% thrombolysis. Reduction of thrombus weight by combinations of SR 90107A with rt-PA paralleled thrombolytic effects. The plasma fibrinogen concentration was reduced from 3.3 g/L to 1.1 g/L by infusion of rt-PA; however, SR 90107A at 0.51 mg/kg had no effect and in combination with rt-PA had no further effect. Both SR 90107A and rt-PA slightly increased the APTT at 4 hr to 115-123% of the control value (16-18 sec); however, the combination was not additive. Thirty min after administration, 0.51 mg/kg SR 90107A increased circulating anti-Xa activity to 3.72 U/mL as compared with a control value of 0.012 U/mL. However, rt-PA combined with 0.51 mg/kg SR 90107A reduced the anti-Xa activity to 1.93 U/mL.

SAFETY PHARMACOLOGY

Neuropharmacological Effects

Effect of Org 31540 on locomotor activity and conditioned taste aversion in mice (Report 693.4.005, SDGRR 2987).

Methods: The effects of Org 31540 were examined in 2 different tests to detect central activity: a test for locomotor activity and a test for conditioned taste aversion in mice following subcutaneous treatment with Org 31540 (10 mg/kg). Male mice (CD-1), weighing 30 to 35 g, were used in these studies.

Results: Org 31540 had no effect on locomotor activity or conditioned-taste aversion.

Table 10. Ambulation test (Adapted from Sponsor's table in Report 693.4.005, SDGRR 2987).

Compound	Median number of movements (0 - 60 m)		Median number of movements (60 to 120 min)	
	Short	Long	Short	Long
Placebo	960	331	449	112
Org 31540	1172	326	170	102

Table 11. Conditioned taste aversion (Adapted from Sponsor's table in Report 693.4.005, SDGRR 2987).

Compound	Mean amount ± SEM (ml) of fluid drunk on test day	
	Tap Water	Sucrose solution
Placebo	0.33 ± 0.09	1.46 ± 0.27
Org 31540	0.42 ± 0.17	1.63 ± 0.26

Assessment of Effects in the Irwin Observations Test and on Body Temperature following Intravenous Administration to rats (SNX0174)

The objective of this study was to determine the potential neurobehavioral, psychotropic and neurotoxic effects SR90107A by using Irwin observational screen (spontaneous activity, ptosis, abnormal gait, spontaneous body tone, stereotypy, irregular breathing, slow breathing, body tone in response to pulling, body tone in response to gripping, and diarrhea or soft feces) after single intravenous administration to rats. In this test, rats received a single intravenous administration of SR90107A at doses of 0.3, 3 and 30 mg/kg or midazolam (reference substance at a dose of 15 mg/kg) or vehicle (physiological saline) at a dose volume of 1 ml/kg. SR90107A did not induce any neurotoxic, psychotropic or neurobehavioral effects in rats. However, SR90107A caused a slight transient increase in mean body temperature two hours after treatment (+3.2, +3.5 and +3.7% compared to pretreatment values at 0.3, 3, and 30 mg/kg, respectively). The positive control, midazolam, induced typical CNS depressant effect characterized by abnormal gait, decrease in spontaneous activity, decrease in spontaneous body tone, ptosis, slow and irregular

breathing, stereotypy etc.). Midazolam caused a decrease in body temperature one hour after treatment (-3.5% compared to pretreatment value).

Cardiovascular-Respiratory Effects

Cardiovascular and hemodynamic effects of Org 31540 in anesthetized beagle dogs (Report 693.4.006, SDGRR 2892).

Methods: This study was designed to evaluate Org 31540 for respiratory, cardiovascular, and hemodynamic effects and effects on myocardial performance in anesthetized beagle dogs. Seven animals in group 1 served as controls, while five animals in group 2 received 3.57 mg/kg (2500 anti-Xa U/kg). Dogs were anesthetized with α-choralose (120 mg/kg, and maintained under anesthesia throughout the experiment. After intratracheal intubation, spontaneous respiration and expired CO_2 were monitored. Blood samples were collected for blood gas analysis, hematology, and for anti-Xa determinations. Arterial pH, PCO_2, PO_2, $[HCO_3^-]$, base excess, O_2 content, and O_2 saturation were measured every 30 min. Left ventricular pressure, aortic blood pressure, right atrial blood pressure, cardiac output, and peripheral arterial flow, were measured throughout the experiment. For 20 minutes during the experiment, an

electrocardiogram was recorded for determination of PQ-,QRS-, and QT (actually QT(U)-) intervals. Heart rate was continuously monitored. Respiratory functions (resp. frequency, resp. flow, resp. volume, tidal volume, minute volume, and expired CO_2) were measured with flow transducer connected to a pneumotachograph.

Results:

1. **Anti-Xa activity:** Intravenous injection of 2500 anti-Xa U/kg Org 31540 resulted 5 min after injection in a mean plasma anti-Xa activity of 11.6 U/ml, which declined with time. At 250 min, it was ~3 U/ml.

2. **Cardiovascular and hemodynamic parameters:** Org 31540 produced no consistent changes in blood- and pulse pressure, heart rate, heart-minute volume, blood flows, or myocardial contractility. Changes were generally small (< 10%) and occurred in an isolated manner at 1 or 2 time points. Other changes are noted below and are considered to be minor.

a. Left ventricular heart rate was decreased at 90 and 110 min. Left ventricular peak systolic pressure decreased at 100 min. Left ventricular peak $\Delta P/\Delta T$ was decreased at 100 and 110 min.

b. The total peripheral resistance index was increased at 200 min. Cardiac output conversely decreased at 200 min.

c. Cardiac index decreased at 130 min.

d. The QT-interval was consistently higher (10 to 15%) in treatment group versus the control group through both the pretreatment and treatment periods. There was a slight lengthening (~115 vs. 95 msec, 20% increase) of the PQ-interval toward the end of the experiment.

3. **Blood gas analysis:** No relevant changes in blood gas values, pH, pCO_2, pO_2, O_2-content, base excess, and O_2-saturation were found. No effect on body temperature was found.

4. **Hematology:** No significant effects on leukocytes, erythrocytes, and platelet count were found. No changes in hemoglobin content or hematocrit were found.

5. **Urine collection:** No change in urine production was found.

6. **Electrocardiographic analysis:** No changes in electrocardiographic parameters (PQ-, QRS-, QT-, and QT-calculated intervals) were found.

Effects of SR90107A on the Action Potential of Piglet Purkinje Fibers (FIP0018)

The objective of the study was to examine the possible effects of SR90107A on the electrophysiological parameters of piglet Purkinje fibers. In this experiment, the Purkinje fibers were dissected from the right ventricle of male piglets and electrically stimulated at 1Hz (60 beats/min) by a ——— stimulator via a platinum electrode for 30 minutes. After a stabilization period of 1 hour, transmembrane action potentials were recorded by using microelectrodes. The following electrophysiological parameters were recorded: resting potential (RP), total amplitude of the action potential measured between the resting potential (RP) and the maximum value of the action potential (APA), APD_0, APD_{50}, APD_{90} (duration of the action potential at 0mv, 50% and 90% of repolarization in msec), dV/dt_{max} (maximal rate of depolarization in V/sec). The following concentrations of the test article were used: 0.01, 0.1, 1, 10, and 30 μM. SR90107A had no effects on RP, APA, dV/dt_{max} and APD_{90}. However, APD_0 was significantly decreased at 10 (-7.4%) and 30 μM concentrations (-9.3%) compared to control (238.1 msec). There was a slight but significant decrease (-4.1%) in APD_{50} at 30 μM concentration when compared to control (374.1 msec). This study is deficient in the absence of a concurrent positive control.

Gastrointestinal Effects

APPEARS THIS WAY
ON ORIGINAL

Effect of Org 31540 in some peripheral tests (Report 693.4.007, SDGRR 2898).

Methods: The aim of this study was to examine Org 31540 for possible (prohibitive) peripheral effects (i.e., body weight, body temperature, gastrointestinal function and motility, respiration, coagulation) in the rat. Org 31540 was administered subcutaneously to rats at 3.57 mg/kg (2500 U/kg) twice daily for 4 consecutive day and once on day 5. In the first experiment, weight and rectal temperature were measured; however, in a second experiment, rectal temperature was omitted. During the night after days 1 and 4, gastrointestinal function was assessed through counting the number of fecal pellets. Gastrointestinal motility was assessed by the time of appearance of steel balls in the fecal pellets. Respiration rate was measured on day 5. Autopsy was performed on day 5 and the organs were inspected for abnormalities (e.g., hemorrhages and ulcers in the gastrointestinal tract). Hematology and coagulation were assessed on day 5 (blood cell counts, prothrombin time, and APTT).

Results: In the first experiment, rectal temperature measurement caused abdominal bleeding in one rat on day 3, which resulted in death. On day 5, an abdominal hematoma was observed in one rat during autopsy. In the second experiment without rectal temperature measurement, no abdominal bleeding or hemorrhage was observed. In the second experiment, Org 31540 did not cause changes in any of the following parameters: gastrointestinal motility on day 2 or 5, fecal pellet production on day 2 or 5, body weight gain, or respiration rate. Org 31540 had no effect on blood coagulation tests, Quick Test or APTT. Blood cell counts (leukocytes, erythrocytes, platelets), hemoglobin concentration, and hematocrit were unchanged by Org 31540 treatment.

Renal Effects

The influence of Org 31540 on the hydro-electrolytic balance in rats (Report 693.4.004, SDGRR 2899).

Methods: The effects of Org 31540, 3.57 mg/kg (2500 anti-Xa U/kg) injected intravenously into Wistar rats, on urine production, and plasma and urine electrolyte and creatinine levels were examined. Thirty-two Wistar rats (16 male, weighing 220-260 g and 16 female, weighing 156-205 g) were obtained from _____ On the day of the experiment, animals were deprived of food and water from 8 am. Org 31540 2500 anti-Xa U/kg was injected intravenously into male and female Wistar rats (8 rats/sex/group) at 5 pm. Control rats (8 animals/sex/group) received the vehicle. After treatment with Org 31540 or vehicle, each animal was given 20 ml/kg distilled water orally. Urine was collected and measured 16 hr later. Urine was examined for albumin, glucose, sodium, potassium, calcium, chloride, and creatinine. A blood sample was taken for examination of hematocrit and plasma levels of sodium, potassium, chloride, and creatinine.

Results: In female rats treated with Org 31540, urine production was reduced by 25% from 6.8 ± 1.0 ml in the control to 5.1 ± 0.9 ml (-25%), and plasma creatinine levels were decreased by 12% from 41.5 ± 2.0 µmole/liter in the control to 36.5 ± 2.8 (-12%) µmole/liter. Urine albumin levels in male rats treated with Org 31540 were slightly increased (4/8, < 0.3 g/L; 3/8, 0.3 g/l, and 1/8, 1 g/L) compared with the control (8/8, < 0.3 g/L). No other statistically significant changes were noted. Org 31540 was administered to rats in a concentration of about 22 times the highest dose proposed in the clinical trial.

Effect on Hemostasis

Absence of hemorrhagic effects of SR90107A (1.7 mg/kg = 1000 anti Xa U/kg IV) in the rabbit (Report 693.4.009).

The blood loss induced by IV injection of SR 90107A at a dose of 1.7 mg/kg was measured for 10 min after incision of the rabbit ear. Blood loss and anti-Xa activity generated by SR 90107A in the rabbit. Results suggest that SR 90107A did not enhance blood loss.

Table 9. Blood loss and anti-Xa activity (Adapted from sponsor's table in Report 693.4.009).

	Control (N = 6)	SR 90107A (N = 5)
Blood loss (ml)	0.038 ± 0.01	0.026 ± 0.01
anti-Xa activity (U/ml)		1.68 ± 0.35

Neutralization by protamine sulfate of SR 90107 A induced bleeding without alteration of antithrombotic and anti-Xa activity in the rat (Report 693.4.014).

1. **Bleeding time**: SR 90107A, when administered at high doses, prolonged bleeding time. This study evaluated whether protamine sulfate was able to improve hemostasis following SR 90107A. For bleeding time determination, the tip of the tail of pentobarbital anesthetized rats was cut 5 mm from the end. Blood was blotted every 15 sec until hemostasis. SR 90107A (1, 3, 6, 10, 15 mg/kg) and heparin (0.3 to 0.6 mg/kg) were injected by IV administration just before tail transection. Protamine sulfate was administered IV as a bolus 15 min after tail transection. Bleeding time was measured for 2 hr. The IV administration of SR 90107A prolonged tail transection bleeding time in a dose-dependent manner. SR 90107A (15 mg/kg) increased bleeding about 8 fold to 4830 ± 802 sec compared with a control value of 594 ± 46 sec. Protamine sulfate, 10 and 30 mg/kg, reduced bleeding time prolongation induced by SR 90107A (15 mg/kg) to 1800 ± 51 and 1640 ± 595 sec, respectively.

2. **Anti-thrombotic effect**: In a second set of experiments, thrombus formation was induced by a combination of stasis and hypercoagulability using the rat. Human tissue factor (1 ng/kg) was injected into the dorsal vein of the penis and 10 sec later, stasis was established by tightening the two sutures. After 10 min, formed thrombi were collected, dried, and weighed. SR 90107A was injected IV at a dose of 0.16 mg/kg, 15 min before stasis. Protamine sulfate was injected 1 min before stasis. Protamine sulfate did not antagonize the antithrombotic activity of SR 90107 A observed on stasis-induced thrombosis after injection of tissue thromboplastin in the rat.

3. Anti-Factor Xa activity: In a third set of experiments, plasma anti-Xa activity was measured in rats treated with SR 90107A (15 mg/kg) alone or in combination with protamine sulfate (10 mg/kg). Protamine sulfate (10 mg/kg) decreased circulating anti-Xa activity induced by 15 mg/kg SR 90107A from 19.8 ± 1.9 to 13.6 ± 0.6 anti-Xa U/ml, a reduction of ~31%.

APPEARS THIS WAY
ON ORIGINAL

Neutralization of SR90107A-Induced Bleeding in Mice (Amendment #034; Report 693.4.018).

Blood loss, plasma anti-Factor Xa activity, and protective effects against thromboplastin challenge were assessed in male mice that received intravenous treatment with SR90107A. Heparin was used as a reference. SR90107A was administered at intravenous doses of 1, 3, 10, or 15 mg/kg (doses expressed as salified compound) and heparin was administered at intravenous doses of 0.6, 1, and 3 mg/kg. Blood loss was measured over a 20 min period starting 5 min after intravenous treatment with SR 90107A or heparin using the tail transection technique. SR 90107A and heparin both produced dose-dependent increases in blood loss. SR90107A at 10 mg/kg and heparin at 3 mg/kg both produced an approximate 12-fold increase in blood loss. Protamine sulfate at 10 mg/kg significantly reduced blood loss induced by SR90107A at 10 mg/kg (86% decrease) or heparin at 3 mg/kg (93.4% decrease). A concentrate of coagulation factors (PPSB; 30 IU/kg) also significantly reduced blood loss induced by SR90107A at 10 mg/kg (65.6% decrease). Individual coagulation factors (i.e., Factors VII, VIIa, and IX) and platelet factor 4 had no statistically significant effects on blood loss induced by SR 90107A at 10 mg/kg. The protective effect of SR 90107A or heparin against thromboplastin-induced thromboembolism in mice was determined by intravenous pretreatment at 5 min prior to thromboplastin challenge. SR90107A at 10 mg/kg produced a protective effect against thromboplastin challenge with 40% (4/10) mortality as compared to 80% (8/10) for the control. Protamine sulfate at 10 mg/kg had no antagonistic activity on the protective effect of SR 90107A at 10 mg/kg. Heparin at 0.5 mg/kg produced a protective effect against thromboplastin challenge with 20% (2/10) mortality; however, protamine sulfate at 10 mg/kg completely antagonized this protective effect leading to 100% (10/10) mortality. Anti-Factor Xa activity was measured at 5 min following intravenous treatment with SR90107A or heparin. Anti-Factor Xa activity following treatment with SR 90107A at 10 mg/kg was 16.2 U/mL, and protamine sulfate had no effect on anti-Xa activity. Anti-Factor Xa activity following treatment with heparin at 3 mg/kg was 11.7 U/mL; however, protamine sulfate at 10 mg/kg reduced anti-Factor Xa activity to 1.6 U/mL. A distinction between antithrombotic and hemorrhagic properties of Org 31540 can be observed with the use of protamine sulfate; however, this phenomenon was not observed with heparin.

Other Potential Secondary Effects

Interaction of Org 31540/SR90107A with Platelet Factor 4 (Amendment #101; Report 693.4.030).

The in vitro binding of Org 31540/SR90107A to human platelet factor 4 (PF_4) was assessed by measurement of its ability to competitively inhibit the binding the radiolabeled ^{3}H-Heparin. Unlabeled heparin at concentrations ranging from 3 to 100 µg/mL was used as a reference. PF_4 neutralizes the action of heparin and it is believed that heparin-induced thrombocytopenia it's mediated by antibodies directed against heparin-PF_4 complexes. Org 31540/SR90107A was tested at concentrations of 3, 10, 30, and 100 µg/mL. Heparin at 3, 30, and 100 µg/mL inhibited binding of ^{3}H-heparin by 79.85, 96.91 and 100.51% respectively. In contrast, Org 31540/SR90107A at 30 and 100 µg/mL produced <19% inhibition of ^{3}H-heparin binding, respectively. Results suggest that Org 31540/SR90107A possesses little or no ability to bind with human PF_4.

Comparison of cross-reactivity for induction of heparin-induced thrombocytopenia (HIT) between heparin and Org 31540/SR 90107A (SDGRR 4112).

Thrombocytopenia develops in about 5% of patients receiving heparin therapy. HIT is caused by an IgG antibody, which is formed after several days of exposure to heparin. The IgG antibody is thought to interact with heparin and a component of the platelet surface, platelet factor 4, leading to platelet activation via binding to the platelet Fcy RII receptor. This antibody is not heparin-specific as a variety of sulfated oligosaccharides are able to induce HIT, depending on their degree of sulfation. The most sensitive and specific diagnosis for HIT cross reactivity is the ^{14}C-serotonin release assay. In the present studies, platelets were radiolabeled with ^{14}C-serotonin. ^{14}C-labeled platelets were incubated with HIT sera (or Watson buffer for controls) and either Org 31540 (0.1, 1, or 10 anti-Xa U/ml) or heparin (0.1, 0.2, or 100 anti-Xa U/ml). Following a 60 min incubation, the reaction was stopped, platelets were centrifuged, and an aliquot of the supernatant was counted for ^{14}C. In the serotonin release assay, heparin, at therapeutic concentrations, caused a serotonin release of 95%. In contrast, Org 31540/SR 90107A caused a serotonin release of < 20% in the concentration range of 0.1 to 10 anti-Xa U/ml.

SR90107A does not Release Lipase Activity into the Plasma in Rats (Amendment #025; Report 693.4.021).

The ability of SR90107A to influence lipoprotein metabolism in male Sprague-Dawley rats was assessed through measurement of triglyceride lipase activity (i.e., . glycerol trioleate hydrolase activity). Heparin and nadroparin were used as reference compounds. In a single dose study, SR90107A or nadroparin were administered by the intravenous route at doses of 0.3, 1, and 3 mg/kg. Heparin was administered by the intravenous route at doses of 0.03, 0.1, 0.3, 1, and 3 mg/kg. Control animals received the vehicle, saline. In a repeat dose study, SR90107A or heparin was administered by the subcutaneous route at a dose of 1 mg/kg/day for 9 days. On day 10, SR90107A or heparin was administered by the intravenous route at a dose of 1 mg/kg. In addition, groups of rats treated with the vehicle or SR90107A by the subcutaneous route for 9 days received treatment with heparin on day 10 at an intravenous dose of 1 mg/kg. Blood for determination of lipase activity was collected at 10 min after dosing on days 1 and 10. In single dose studies, SR90107A at doses ≤3 mg/kg produced minimal effects on release of lipase activity. In contrast, heparin produced a dose-dependent release of lipase activity, which reached maximal effect at doses ≥1 mg/kg. Nadroparin at doses of 0.3 and 1 mg/kg produced releases of lipase activity, which were smaller than those observed with identical doses of heparin; however, both nadroparin and heparin at 3 mg/kg produced a similar maximal effect. In the repeat dose study, release of lipase

activity was unaffected in rats treated with SR90107A for 10 days. For rats treated with vehicle, SR90107A, or heparin for 9 days by the subcutaneous dose followed by intravenous treatment with heparin on day 10, release of lipase activity measured on day 10 was identical. Single or multiple dose treatment with SR90107A had no effect on lipid metabolism as determined by release of glycerol trioleate hydrolase activity.

The pharmacology studies examined the interaction of Org31540/SR90107A with antithrombin III (AT-III) and subsequent inactivation of factor Xa. Org31540/SR90107A induced a specific allosteric conformational change in AT at physiological pH (pH = 7.5) and ionic strength with 1:1 stoichiometry. This interaction with human AT occurred at an affinity constant of 58 nM. The Kd values obtained with AT of rat, baboon and rabbit were 47, 79, and 130 nM, respectively. Org31540/SR90107A was found to bind about 10 times stronger to human AT-III at pH 7.4 (Kd = 82.1 nM) than at pH 8.4 (Kd = 754 nM). Org31540/SR90107A was found to enhance the AT-III mediated inactivation of factor Xa. The dissociation constant (Kd) for pentasaccharide (Org31540/SR90107A) and heparin with human AT-III were $4x10^{-7}$ M and $2x10^{-8}$ M, respectively. Org31540/SR90107A slightly inhibited factor IIa amidolytic activity in plasma and was found to be approximately 1500 times less potent than heparin (IC-50 = 0.25 anti-Xa U/ml). Org31540/SR90107A also inhibited thrombin generation induced by factor Xa in the human plasma. Org31540/SR90107A did not potentiate ADP or collagen-induced platelet aggregation unlike heparin. In the ———— assay, Org31540/SR90107A and heparin inhibited factor Xa and doubled clotting time at concentrations of 0.24 and 5.55 μg/ml, respectively. Org31540/SR90107A and heparin dose-dependently enhanced the AT-mediated

inactivation of human factor IXa *in vitro* with IC-50 values of $1x10^{-7}$ M and $2x10^{-7}$ M, respectively. Org31540/SR90107A inhibited thrombin generation in the extrinsic pathway (ED-50 for thrombin peak inhibition: 0.42 μg/ml) and showed a weaker effect on the intrinsic pathway (ED-50: 1.39 μg/ml). Org31540/SR90107A, administered intravenously or subcutaneously, inhibited thrombosis induced by a combination of stasis and hypercoagulable states in a variety of models of experimental thrombosis. Reduction of thrombosis formation by Org31540/SR90107A and heparin were evaluated in a series of rat models such as arterio-venous (Org31540/SR90107A: ED-50 = 1000 μg/kg; Heparin: ED-50 = 320 μg/kg), venous stasis (Org31540/SR90107A: ED-50 = 36 μg/kg, (intravenous) IV; Heparin: ED-50 = 100 μg/kg, IV), venous stasis (Org31540/SR90107A: ED-50 = 204 μg/kg, subcutaneous (SC); Heparin: ED-50 = 437 μg/kg, SC), venous thrombosis (Org31540/SR90107A: ED-50 = 53 μg/kg; Heparin: ED-50 = 13 or 25 μg/kg), and Wessler model (Org31540/SR90107A: ED-50 = 386 μg/kg; Heparin: ED-50 = 50 μg/kg). Overall, Org31540/SR90107A may offer a number of advantages over the use of heparin for the prophylaxis of thrombosis. Org31540/SR90107A is a homogeneous preparation obtained through total chemical synthesis whereas heparin is a heterogeneous mixture of sulfated polysaccharide chains obtained through extraction from various tissues of animal origin. Org31540/SR90107A does not appear to have the liability to produce antibodies unlike heparin which, causes heparin-induced thrombocytopenia or HIT. Org31540/SR90107A showed a more selective mode of action and a higher bioavailability with subcutaneous administration when compared to heparin.

ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION:

Absorption

Rat and Rabbit

A Comparison of Plasma Radioactivity and anti-Xa Profiles Following a Single Subcutaneous Administration of [^{35}S]-SR90107A in Male Rats (Amendment #091; Report 693.5.028).

Methods: Plasma levels of SR90107A were determined in rats by radioactivity measurements or an anti-Xa bioassay following subcutaneous administration of drug. Male Wistar rats received [^{35}S]-SR90107A by the subcutaneous route at a dose of 1 mg/kg (dose expressed as non-salified form). The ^{35}S radiolabel was located in the sulphamate moiety. Blood samples were collected at 1, 4, 6, 8, and 12 hr after dosing. Nine rats were sacrificed per time point. Plasma levels of radioactivity were using a _____ The profile of plasma radioactivity was examined at 1, 4, 6, and 8 hr after dosing by HPLC analysis.

Results: Plasma $AUC_{0\text{-}12hr}$ values determined by measurement of radioactivity and anti-Xa activity were 5920 ng equiv.*hr/mL and 5970 ng*hr/mL, respectively, demonstrating close agreement. Radiochromatograms of extracted plasma samples showed one major peak corresponding to the retention time of [^{35}S]-SR90107. The peak at the 1 and 8 hr sampling times represented 95 and 71% of the applied radioactivity, respectively. A small secondary peak, probably representing free sulfate ions ([^{35}S]-SO_4^{2-}), accounted for <3% of total radioactivity at sampling times up to 4 hr; however, at 6 and 8 hr sampling times, this peak represented 9 and 12% of recovered radioactivity, respectively. Recovery of radioactivity from extracted plasma declined from 99% at 1 hr to 40% at 8 hr. The decline in recovery over time was attributed to [^{35}S]-SR90107 bound to protein (i.e., antithrombin III), which was not retained on extraction columns. At early time points, drug bound to protein that was not retained by the column would represent a negligible percentage of total radioactivity; however, at later time points as the total level of radioactivity declined, it could represent an increasingly larger proportion of total radioactivity.

Pharmacokinetic Parameters of Org 31540/SR90107A in Rats and Rabbits After Intravenous, Subcutaneous, and/or Intratracheal Administration (Report No. NL0020264).

Methods: Pharmacokinetic parameters of Org 31540/SR90107A at a dose of 100 nmole/kg (172.8 µg/kg) were assessed following intravenous, subcutaneous, or intratracheal administration to male Wistar rats and following intravenous or subcutaneous administration to male New Zealand —— rabbits. Pharmacokinetic parameters were determined through measurement of plasma levels of anti-Factor Xa activity. Org 31540 batch M and N and SR90107 batch H673d were administered by the intravenous route to rats, and blood samples were collected at time points between 0.017 and 4 hr after dosing. Org 31540 batch M was administered by the intravenous, subcutaneous, or intratracheal route to rats and blood samples were collected at 0.0167 (IV), 0.083, 0.333, 0.667, 1, 1.5, 2, 3, 4, 5, and 6 (SC and IT) hr. Org 31540 batch M or N was administered by the intravenous route to rabbits, and blood samples were collected at time points between 0.0167 and 12 hr after dosing. Org 31540 Batch M was administered by the intravenous or subcutaneous route to rabbits, and blood samples were collected at time points between 0.0167 and 6 hr after dosing.

Results: Clearance of Org 31540/SR90107A in rats, following intravenous, subcutaneous, or intratracheal administration, ranged from ———— mL/hr/kg, which is approximately equivalent to the glomerular filtration rate (181.7 mL plasma/hr/kg). The volume of distribution of Org 31540/SR90107A in rats ranged from ———— mL/kg, suggesting that drug distributed beyond the blood volume and entered tissues. Bioavailability of Org 31540/SR90107A in rats following subcutaneous

or intratracheal administration was 114.7 and 92.7%, respectively. Clearance of Org 31540/SR90107A in rabbits, following intravenous or subcutaneous administration ranged from ←——— mL/kg/hr, which is slightly less than the glomerular filtration rate (124.8 mL plasma/hr/kg). The volume of distribution of Org 31540/SR90107A in rabbits ranged from ——— mL/kg, suggesting that drug distributed beyond the blood volume and entered tissues. Bioavailability of Org 31540/SR90107A in rabbits following subcutaneous administration was 129.8%. Bioavailability values greater than 100% were apparently due to variability between samples and experimental errors that were inherent in the procedures used.

Table 2.1.1

Pharmacokinetics of three batches of Org 31540 after a single intravenous administration of 100 nmol/kg (172.8 µg/kg salt) in rats.

149.8 µg acid /kg I.V.	Org 31540 M (n=3)		Org 31540 N (n=3)		SR90107A Batch H673d (n=3)*	
	mean	± SD.	Mean	± SD.	Mean	± SD.
AUC $_{0-\infty}$ (µg.mL^{-1}.h)	0.93	0.06	1.24	0.13	1.53	0.12
AUC$_{0-Clast}$ (µg.mL^{-1}.h)	0.88	0.07	1.13	0.09	1.46	0.12
Clearance (mL/h/kg)	161.2	11.5	121.7	12.7	98.7	8.1
Distr. Vol β (mL/kg)	143.4	20.5	139.4	13.4	92.2	9.7
T1/2α (h)	0.033	0.014	0.056	0.022	0.051	0.007
T1/2β (h)	0.67	0.08	0.89	0.12	0.69	0.01
MRT $_{0-\infty}$ (h)	0.89	0.11	1.16	0.18	0.93	0.02

* = clinical ampoules of 10500 anti-Xa U.mL^{-1}, recalculated to mg. mL^{-1} by assuming an anti-Xa activity of 700 U.mg^{-1} salt = 15 mg. mL^{-1}.

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Table 2.3.1.

Pharmacokinetics of Org 31540 batch M after a single intravenous, subcutaneous or intra- tracheal administration of 200 nmol/kg (345.6 µg salt/kg) in rats.

Dose: 299.6 µg acid /kg	Org 31540 M I.V (n=3)		Org 31540 M S.C. (n=3)		Org 31540 M I.T. (n=3)	
	mean	± SD.	Mean	± SD.	Mean	± SD.
AUC $_{0-\infty}$ (µg.mL^{-1}.h)	2.07	0.22	2.38	0.17	1.92	0.18
AUC$_{0-Clast}$ (µg mL^{-1}.h)	2.00	0.20	2.34	0.16	1.57	0.15
Clearance (mL/h/kg)	146.2	16.0	126.6	9.2	157.2	13.8
Distr. Vol (mL/kg)	178.3	18.0	144.3	23.9	675.5	299.4
T1/2α (h)	0.078	0.051				
T1/2β (h)#	0.93	0.14	0.95	0.17	3.06	1.41
MRT $_{0-\infty}$ (h)#	1.24	0.21	1.92	0.09	5.21	1.56
T_{max} (h)			0.87	0.15	1.26	0.59
C_{max} (µg.mL^{-1})			0.94	0.11	0.33	0.07
% bioavailability*			114.7	8.1	92.7	8.6

* = estimated bioavailability f=1.0

= after intratracheal administration Org 31540 was slower absorbed than after subcutaneous injection which resulted in a lower C_{max} but a longer residence time or elimination half-life.

Table 3.1.1
Pharmacokinetics of two batches of Org31540/SR90107A after a single intravenous administration of 100 nmol/kg (172.8 µg/kg salt) in rabbits

dose : 149.8 µg acid / kg n=3 / batch	Org 31540 M I.V.		Org 31540 N I.V.	
	Mean	± SD	mean	± SD
AUC $_{(0-\infty)}$ (µg.mL^{-1}.h)	1.89	0.31	2.46	0.33
AUC$_{(0-Cn)}$ (µg.mL^{-1}.h)	1.80	0.28	2.24	0.35
Clearance (mL/h/kg)	81.1	12.1	61.8	7.7
Distr. Vol β (mL/kg)	176.5	21.0	208.2	30.5
T1/2α (h)	0.100	0.038	0 164	0.032
T1/2β (h)	1.62	0.12	2.64	0.50
MRT $_{(0-\infty)}$ (h)	2.19	0.08	3.43	0.66

Table 3.2.1
Pharmacokinetics of Org 31540 batch M, after a single intravenous or subcutaneous administration of 100 nmol/kg (172.8 µg/kg salt) in rabbits

Dose: 149.8 µg acid /kg	Org 31540 M I.V. (n=3)		Org 31540 M S.C (n=3)	
	mean	± SD	Mean	± SD
AUC $_{(0-\infty)}$ (µg.mL^{-1}.h)	2.22	0.12	2.88	0.46
AUC $_{(0-Cn)}$ (µg.mL^{-1}.h)	2.07	0.09	2.66	0.39
Clearance (mL/h/kg)	67.5	3.7	53.4	9.6
Distr. Vol (mL/kg)	164.6	12.5	141.9	19.2
T1/2α (h)	0.04	0.00		
T1/2β (h)	1.77	0.17	1.86	0.21
MRT $_{(0-\infty)}$ (h)	2.44	0.17	3.07	0.20
T_{max} (h)			0.84	0.20
C_{max} (µg mL^{-1})			0.78	0.09
a bioavailability			129.8	20.8

* = estimated bioavailability f= 1.0

Toxicokinetics of SR 90107A in rabbits (Report 693.5.006).

Methods: This study was designed to determine the toxicokinetic profile of SR 90107A in rabbit after a single intravenous dose. Male New Zealand rabbits (3.0 to 3.5 kg) were used in this study. SR 90107A was injected into the marginal ear vein at a dose of 100 nmole/kg or approximately 100 anti-Xa U/kg. Blood samples were collected from 2 min through 10 hr after administration.

Results: After IV bolus administration of SR 90107 A (100 nmole/kg), the plasma concentration versus time, in log-linear representation, indicated a disposition phase consisting of a distribution phase and an elimination phase. Kinetics of both phases appear to be first order. SR 90107A distributed in a volume of 63 ml/kg which corresponds to the central compartment.

Table 13. Toxicokinetics of SR 90107A in Rabbits (Adapted from Sponsor's table in Report 693.5.006).

Rabbit	$T_{\frac{1}{2}}$, elim (hr)	AUC (0 to ∞) $\mu g ml^{-1} h$	Clearance $ml h^{-1} kg^{-1}$	Vd ml/kg	MRT exp (hr)
Mean ± SD	1.76 ± 0.03	7.04 ± 1.01	24.77 ± 3.49	62.69 ± 8.66	2.31 ± 0.17

Monkey

Pharmacokinetics and animal exposure to ORG31540/SR90107A following intravenous and subcutaneous administrations of ORG31540/SR90107A in macaques or Macaca fascicularis or Cynomolgus monkeys (ABS0300)

Methods: The aim of this study was to determine the pharmacokinetic profile of ORG31540/SR90107A following single intravenous and subcutaneous dose and to assess the exposure after a repeated SC administration for 8 to 10 days. The animals were administered a single 8.8 mg/kg dose intravenously via the saphenous vein and after a wash out period of 7 days, the test compound was administered repeatedly at 10.1 mg/kg, SC, for 8 to 10 days. The blood was collected at the following time points:

- after a single IV administration at 0.083, 1, 4, 8, 12 and 24 hours post treatment
- after the first SC administration at 0.5, 1, 2, 4, 8, 12 and 24 hours post treatment
- after a repeated administration for 8/10 days, pretreatment and at 0.5, 1, 2, 4, 8, 12 and 24 hours post treatment

The ORG31540/SR90107A plasma level was determined by using a chromogenic assay.

Results: Following a single IV or SC administration, no gender difference was observed on pharmacokinetic parameters. The terminal half-life was about 5 to 6 hours. The bioavailability after SC administration was 77% and 71% in males and females, respectively [Table (5.1) 2]. The terminal half-lives and plasma concentrations at 24 hours after single and repeated administration were comparable and indicated no significant accumulation after 8/10 days treatment. The following table shows the mean (SEM) pharmacokinetic parameters after single IV or SC and repeated administration in monkeys (from vol. 9, page 30/93 of sponsor's submission).

Table (5.1) 2 - Mean (sem) pharmacokinetic parameters following a single administration

♦ *Following a single intravenous administration*

	Male (n = 3)	Female (n = 3)
C_0 (mg/L)	65.370 (0.705)	59.071 (4.306)
$AUC_{(0-24h)}$ (mg.h/L)	124.091 (6.845)	127.484 (14.828)
$AUC_{(0-inf)}$ (mg.h/L)	128.717 (8.633)	134.939 (19.001)
$t_{1/2}$ (h)	5.7 (0.5)	6.2 (1.1)
$Cl_{(tot,ref)}$ (L/h/kg)	0.0603 (0.0037)	0.0593 (0.0084)
Vd (L/kg)	0.487 (0.015)	0.511 (0.033)

♦ *Following a single subcutaneous administration*

	Male (n = 3)	Female (n = 3)
C_{max} (mg/L)	11.322 (1.105)	14.247 (1.717)
t_{max} (h)*	1.98 ——	1.05 ——
$AUC_{(0-24h)}$ (mg.h/L)	108.262 (15.611)	102.414 (13.723)
$AUC_{(0-inf)}$ (mg.h/L)	114.213 (15.748)	107.284 (14.482)
$t_{1/2}$ (h)	5.7 (0.2)	5.4 (0.3)
f (%)	77 (6)	71 (9)

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* : median value [range]

Table (5.1) 3 - Individual pharmacokinetic parameters following a repeated subcutaneous administration

Pharmacokinetic parameters	Male 101	Male 3	Female 5	Female 6
C_{max} (mg/L) t_{max} (h) $AUC_{(0-24h)}$ (mg.h/L) $AUC_{(0-inf)}$ (mg.h/L) $t_{1/2}$ (h)	[ ]			

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Pharmacokinetics of SR90107A after single 100, 250 and 500 µg/kg subcutaneous and intravenous administrations to male primates (Macaca mulatta) (ABS0118)

Methods: The purpose of this study was to assess the pharmacokinetics and absolute bioavailability (after SC) of SR 90107A after single SC and IV administration at 100, 250 and 500 µg/kg doses in macaca mulatta. The blood samples were collected for both administrations at 0, 5, 15, 30, 45 minutes and 1, 1.5, 3, 6, 12, 24, 48 and 72 hours post-treatment. The plasma drug samples were determined by measuring the anti-Xa activity using a chromogenic assay method. The assay principle was based on the *in vitro* factor Xa inhibition by the antithrombin III-ORG31540/SR90107A complex.

Results: After intravenous administration, the mean pharmacokinetic parameters were comparable across doses. The mean terminal half-life was 4.5 hours and the mean plasma clearance was 16-18 ml/h.kg. The mean distribution volume was 80 to 100 ml/kg. The AUC (0-Clast) increased dose proportionally. After SC administration, the mean pharmacokinetic parameters were also comparable across doses. The mean terminal half-life was 6 hours and the mean plasma clearance was 13 to 15 ml/h.kg. The mean distribution volume was 80 to 90 ml/kg and the mean absolute bioavailability was 100 to 140%. Cmax and AUC (0-Clast) increased dose proportionally following SC administration. The mean SR90107A pharmacokinetic parameters after IV or SC administration for each dose level are shown in the following table:

Parameter	Dose (mg/kg)					
	Intravenous (IV)			Subcutaneous (SC)		
	100	250	500	100	250	500
C0 (µg/ml)	1.94	4.75	8.70	-	-	-
Cmax (µg/ml)	-	-	-	0.13	2.68	3.97
Tmax (h)	-	-	-	0.8	1.19	3.4
Terminal half-life (h)	1.4 -	12.2	3.9*	1.7	5.7	6.0
AUC (0-Clast) (µg.h/ml)	2.23	25.60	34.50	0.90	23.36	50.38
AUC (0-α) (µg.h/ml)	1.33	29.68	30.94*	3.99	26.67	53.41
% AUC extrapolated	17.8	16.0	23	20.1	15	7
Cl (0- Clast) (ml.h/kg)	5.40	15.19	16.11	-	-	-
Cl (0-α) (ml.h/kg)	2.00	13.06	16.37*	-	-	-

Cl/F (0- Clast) (ml.h/kg)	-	-	-	1.83	14.43	12.73
Cl/F (0-α) (ml.h/kg)	-	-	-	3.14	11.78	11.61
MRT (0-α) (h)	2.2	13.4	5.1*	2.4	8.4	9.5
Vd (ml/kg)	22	136	93*	-	-	-
Vd/F (ml/kg)	-	-	-	13	95	90
F (0-Clast) (%)	-	-	-	30	99	142
F (0-α) (%)	-	-	-	40	101	139*

*: Excluding animal No. 2730.

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Toxicokinetics of SR 90107 following 10 mg/kg intravenous administration of SR 90107A (Org 31540) to male and female macaca monkeys (Report 693.5.009).

Methods: The aim of this study was to determine the toxicokinetic profile of SR 90107 following a single intravenous (10 mg/kg) administration of SR 90107A (decasodium salt) (or 8.726 mg/kg expressed as unsalified compound)to male and female macaca monkeys (macaca fascicularis). There were 4 males and 4 females. The mean body weight was 3.20 ± 0.52 kg for males and 2.82 ± 0.26 kg for females. SR 90107A was administered once intravenously in

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the saphenous vein. Blood samples were taken at the femoral vein. Animals (M1,M2,F3,F4) were sampled at 0.083, 0.5, 2, 8, and 48 hr after treatment. Animals (M5,M6,F7,F8) were sampled at 0.25, 1, 4, 24, and 48 hr. SR 90107A plasma levels were determined by an amidolytic assay of anti-Xa activity measurement.

Results: A male animal, M6, was found dead 24 hr after dosing due to a hemorrhage at the blood sampling place. Plasma concentrations were expressed as mg/L of the non-salified compound. Toxicokinetic parameters were calculated on the mean plasma concentration time curves for each sex. SR 90107A was detected and quantified up to 24 hr after dosing in one male and one female. Plasma levels of in males and females were similar. SR 90107 was characterized by a weak plasma clearance and a low volume of distribution.

Table 15. Toxicokinetics in macaca monkeys (Adapted from sponsor's table in Report 693.5.009).

Parameter	Male	Female
C_0 (mg/L)	76.86	93.55
$T_{1/2}$ (h)	2.71	2.61
AUC_{obs} (mg.h/1)	63.87	75.19
AUC (0-C_{last}) (mg.h/1)	69.43	81.90
AUC (0-Inf) (mg.h/1)	78.55	90.87
AUC_{ext} (%)	18.69	17.25
Cl (0-Inf) (1/kg.h)	0.11	0.096
Cl (0-Inf) (1/h)	0.36	0.27
V_d (1/kg)	0.44	0.36
MRT (0-Inf) (h)	3.12	2.91

Baboon

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Toxicokinetic profiles of SR90107A after single 0.27 mg/kg (175 U/kg) subcutaneous administration to baboons (693.5.002).

Methods: The aim of this study was to assess the toxicokinetic profiles of SR 90107 after a single 0.27 mg/kg (175 U/kg) subcutaneous administration in the baboon. The treatment group consisted on 3 males and 1 female of unknown age, with body weights ranging from 5.80 to 7.09 kg. Animals were fasted overnight and in the morning, 0.27 mg/kg (175 U/kg) SR 90107A (decasodium salt) was administered by the subcutaneous route. Blood samples were drawn from a femoral vein at 0 (prior to administration), 30 min, 1, 3, 6, 10, 16, 24, 32, and 48 hr after administration. The concentration of SR 90107A in plasma was determined by its ATIII-mediated anti-factor Xa effect.

Results: SR 90107A was not quantified in plasma samples 24 hr post-dose. No clinical abnormalities were observed in the animals during the course of the study. Assuming a 100% bioavailability, the SR 90107A distribution volume in the baboon was consistent with plasma or blood volume. Toxicokinetic parameters observed with SC administration in baboons were close to those observed in macaca at a similar dose level (0.25 mg/kg).

Table 14. Toxicokinetics of SR 90107A in baboons (Adapted from sponsor's table in Report 693.5.002).

Parameter	Mean ± SD
T_{max} (h)	2.50 ± 1.00
C_{max} (U/ml)	1.51 ± 0.09
AUC (0-C_{last}) (U-h/ml)	11.19 ± 1.57
AUC (0-Inf) (U-h/ml)	13.33 ± 1.17
C_{max} (mg/L)	2.32 ± 0.13
AUC (0-C_{last}) (mg.h/ml)	0.017 ± 0.002
AUC (0-Inf) (mg.h/l)	0.021 ± 0.002
AUC_{ext} (%)	16.60 ± 7.95
$T_{½\beta}$ (h)	4.37 ± 0.56
Cl/F (0-C_{last}) (ml/h.kg)	15.85 ± 2.33
Cl/F (0-Inf) (ml/h.kg)	13.23 ± 1.15
MRT (0-C_{last} (h)	4.82 ± 0.82
MRT (0-Inf) (h)	7.00 ± 0.78
V_d/F (0-Inf)	0.083 ± 0.009

Pharmacokinetic Profile of SR90107A After a Single Intravenous and Subcutaneous Administration to Male Baboons (HVV0023)

Methods: This study was conducted to assess the pharmacokinetic profile of SR90107A in male baboons (Papio ursinus) after single intravenous and subcutaneous administration at 100 nmol/kg (34.5 µg/kg, dose volume = 0.2 ml/kg, concentration = 172.8 µg/ml). Four males received a single dose of SR90107A by either IV or SC route at Day 1 or Day 15, respectively, with a 2-week wash out period. Blood samples were collected from the femoral vein at 0.5, 1, 3, 6, 10, 16, 24, 32, 48 hours after each treatment on Day 1 and 15. The concentration of the SR90107A was determined via its ATIII-mediated anti-factor Xa effect. SR90107A strongly binds to ATIII and enhances the rate of inhibition of exogenously added bovine factor Xa by ATIII-SR90107A complex. The residual factor Xa activity was measured through the rate of amidolysis of the chromogenic substrate

Results: After IV administration, the mean Cmax value was 1.8 µg/ml and Tmax was 0.5 hour and AUC (0-α) was 10.6 µg.h/ml. After SC administration, the mean Cmax value was 0.8 µg/ml and Tmax was 1.7 hour and AUC (0-α) was 8.2 µg.h/ml. The mean SR90107A pharmacokinetic parameters after IV or SC administration are shown in the following table (from vol. 9, page 18/28 and 19/28 of sponsor's submission):

Table (5.1.2) 1 - Individual and mean values of pharmacokinetic parameters after single 100 nmol/kg intravenous administration of SR90107A to baboons

Parameters	Baboon 1	Baboon 2	Baboon 3	Baboon 4	Mean	S. D.
Tmax (h)					0.5	0.0
Cmax (nmol/ml)					1.0	0.1
Cmax (µg/ml)					1.8	0.2
T1/2 ß (h)					4.7	0.9
AUC(0-Clast) (nmol.h/ml)					5.1	0.6
AUC(0-Clast) (µg.h/ml)					8.9	1.1
AUC(0-Inf) (nmol.h/ml)					6.1	0.4
AUC(0-Inf) (µg.h/ml)					10.6	0.7
Cl(0-Clast) (ml/h.kg)					19.8	2.8
Cl(0-Inf) (ml/h.kg)					18.1	2.9
Vd(0-Inf) (ml/kg)					122.2	17.0

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Table (5.1.2) 2 - Individual and mean values of pharmacokinetic parameters after single 100 nmol/kg subcutaneous administration of SR90107A to baboons.

Parameters	Baboon 1	Baboon 3	Baboon 4	Mean	S. D.
Tmax (h)				1.7	1.2
Cmax (nmol/ml)				0.5	0.1
Cmax (µg/ml)				0.8	0.1
T1/2 ß (h)				5.4	1.2
AUC(0-Clast)(nmol.h/ml)				4.4	0.7
AUC(0-Clast)(µg.h/ml)				7.7	1.3
AUC(0-Inf) (nmol.h/ml)				4.7	0.7
AUC(0-Inf) (µg.h/ml)				8.2	1.3
Cl(0-Clast) (ml/h.kg)				23.2	4.2
Cl(0-Inf) (ml/h.kg)				21.5	3.8
Vd(0-Inf) (ml/kg)				164.5	11.0

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Distribution

In vitro blood distribution of [^{35}S]-SR90107A in rat, monkey, and human (Report 693.5.011).

Methods: The objective of this study was to determine the in vitro distribution of [^{35}S]-SR 90107 in rat, monkey, and human blood. Blood was obtained from male Wistar rats, four fasted Cynomolgus monkeys, and four fasted male human volunteers. [^{35}S]-SR 90107A was combined with non-radiolabeled SR 90107A. Duplicate 2.0 ml aliquots of blood were fortified with aliquots

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of [^{35}S]-SR 90107A to give non-salified concentrations of 0.05, 0.5, 2.0, 5.0, 10.0, and 50.0 mg/l. All samples were equilibrated at 37°C for 30 min before assaying the blood for radioactivity. Samples were centrifuged at 3000 rpm for 10 min and the resultant plasma was assayed for radioactivity. The hematocrit was also determined. The stability of [^{35}S]-SR 90107A in blood was determined through spiking blood with this compound to give final concentrations of 50.0 and 2.0 mg/l non-salified. Samples were incubated at 37°C for 30 min before centrifugation to isolate plasma. Plasma samples were processed through _____ extraction units, and subsequently through NH_2 Isolute columns. Radioactivity eluted from the column was quantitated. Portions of the extract were analyzed by _____. Mobile phases were (A) 0.001% DMSO in water, pH 3, and (B) 0.5 M NaCl, pH 3. A gradient program was used to elute [^{35}S]-SR 90107A and the eluate was monitored with a radiochemical detector.

Results: [^{35}S]-SR 90107A was found almost exclusively in plasma in rat (96%), monkey (96%), and man (101%). A very low fraction was associated with blood cells in all species. The distribution of SR 90107 was independent of concentration and no species differences were observed. [^{35}S]-SR 90107A was found to be radiochemically stable in human blood over 30 min at 37°C (purity > 95%). Measured plasma concentrations should be a true reflection of circulating drug levels.

The in vitro protein binding of [^{35}S]-SR 90107A in rat, monkey, and human plasma (Report 693.5.010).

Methods: The objective of this study was to determine the *in vitro* protein binding of [^{35}S]-SR 90107A in rat, monkey, and human plasma. Blood was obtained from male Wistar rats, four fasted Cynomolgus monkeys, and four fasted male human volunteers. [^{35}S]-SR 90107A was combined with non-radiolabeled SR 90107A. The degree of protein binding was determined at concentrations of 0.05, 0.5, 2.0, 5.0, 10.0, and 50.0 mg/L. All samples were equilibrated at 37°C for 30 min before aliquots were taken for ultrafiltration. Plasma samples were subjected to ultrafiltration using _____ ultrafiltration devices with a _____. The stability of [^{35}S]-SR 90107A in plasma was assessed in a similar manner as that described above. Ultrafiltrate samples were also assayed for [^{35}S]-SR 90107 using HPLC.

Results: Saturable plasma protein binding was observed for SR 90107A over the concentration range examined. At the lowest drug concentration (0.05 mg/l), [^{35}S]-SR 90107A was highly protein bound in rat (89.6%), monkey (87.7%), and man (92.5%). This level of protein binding remained approximately constant as

the drug concentration increased to 2.0 mg/L. However, as drug concentrations were further increased, the fraction bound to plasma decreased sharply such that at 10 mg/L, the binding had fallen to 71.2% in rat, 72.4% in monkey, and 75.3% in man. Thereafter, the extent of protein binding declined more slowly, and at the highest concentration (50 mg/L), the binding was 59.8% in rat, 67.1% in monkey, and 70.0% in man. There were no species differences in plasma protein binding. At clinically relevant concentrations (1 to 2 mg/L), plasma protein binding for SR 90107 was approximately 90%.

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Tissue Distribution of Radioactivity Following a Single (0.4 mg/kg expressed as decasodium salt) Subcutaneous Administration of [^{35}S]-SR90107A (Org31540) to the Male Wistar Rat (DIS0216)

Methods: Seven animals (1 animal/time) were treated with a single 0.4 mg/kg of [^{35}S]-SR90107A (0.28 Ci/mmol) at a dose volume of 1 ml/kg. Blood, plasma and tissue samples were collected at 0.25, 0.5, 1, 3, 6, 24 and 48 hours after treatment. The radioactivity in the samples was measured by [redacted]

Results: In most tissues, maximum radioactivity concentration was observed at 0.25 hour after treatment. Maximal radioactivity was observed in bladder content (urine), in tissues involved in the metabolism and excretion (blood, lung, kidney and liver). The tissue concentrations to plasma concentrations ratios are shown in the table 5.1 (5) of the sponsor's submission (from Vol. 10, page 33/155). Lower levels of radioactivity were observed in other tissues as shown in the following table (from Vol. 10, page 29/155 and 30/155 of sponsor's submission). Levels of radioactivity decreased greatly from 24 to 48 hours after treatment. The terminals half lives were different in different tissues (blood, esophagus, lung, myocardium, skin, adrenals, brown fat, gut wall, lymph node, pancreas, and salivary gland: 1 to 2 hours; thymus, hypophysis, plasma, colon, testes, spleen, spinal cord, thyroid, seminal vesicle: 2 to 10 hours; Harderian gland, liver, eye, bone marrow, and kidneys: 10 to 20 hours; cartilage: 41 hours) indicating different rate of disappearance of radioactivity from different tissues. Overall, no retention or accumulation of the test compound was observed and no target organ was observed.

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Table (5.1) 5 - Tissue concentration to plasma concentration ratio after a single subcutaneous administration of [^{35}S] SR90107A (ORG 31540) to the male Wistar rat.

TISSUES	SAMPLING TIMES (in hours)					
	0.25	0.5	1	3	6	24
Adrenals	0.226	0.177	0.172	0.207	0.354	ND
Adrenals cort.	0.103	0.098	0.134	0.175	0.308	ND
Adrenals med.	0.339	0.295	0.244	0.284	0.462	ND
Bladder content	5.637	3.060	ND	18.002	99.785	20.333
Bladder wall	0.131	ND	ND	0.480	ND	ND
Blood (*)	0.701	0.702	0.723	0.576	0.728	ND
Bone marrow	0.136	0.075	0.100	0.246	0.615	4.667
Brain	0.013	0.009	0.011	0.019	0.062	1.000
Brown fat	0.240	0.192	0.194	0.251	0.462	ND
Cartilage	ND	ND	ND	ND	1.667	33.333
Colon	ND	0.121	0.129	0.232	1.031	ND
Seminal vesicula	0.046	ND	0.009	0.035	0.138	1.000
Eye	0.005	ND	0.014	0.013	0.092	1.000
Gastric content	0.003	ND	0.686	0.025	0.154	ND
Gastric wall	0.176	0.070	0.059	0.150	ND	ND
Gut content	0.018	ND	0.046	0.017	6.462	18.667
Gut wall	0.167	0.177	0.163	0.292	0.462	ND
Harder's gland	0.105	0.121	0.119	0.117	0.477	4.333
Hypophysis	0.092	0.087	0.110	0.134	0.615	ND
Kidneys	0.673	0.490	0.541	0.685	4.400	58.667
Kidney cort.	0.404	0.390	0.477	0.902	6.000	86.000
Kidney med.	1.484	0.463	0.597	0.461	1.800	10.333
Liver	0.331	0.358	0.298	0.392	0.800	2.000
Lung	0.490	0.492	0.404	0.399	0.662	ND
Lymph nodes	0.330	0.306	0.237	0.322	ND	ND
Muscle	0.065	0.048	0.050	0.075	0.185	1.667
Myocardium	0.271	0.218	0.197	0.211	0.354	ND
Oesophagus	0.053	0.143	0.053	0.033	0.077	ND
Pancreas	0.082	0.075	0.100	0.123	0.338	ND
Plasma	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**
Salivary gland	0.126	0.132	0.160	0.132	0.569	ND
Skin	0.172	0.197	0.295	0.259	0.569	ND
Spinal cord	0.009	0.009	0.011	0.029	0.138	0.667
Spleen	0.076	0.064	0.086	0.152	0.569	3.000
Testis	0.027	0.027	0.085	0.180	0.523	1.667
Thymus	0.061	0.050	0.060	0.073	0.277	ND
Thyroid gland	0.156	0.111	0.127	0.186	0.677	4.333
White fat	0.006	0.042	0.014	0.121	0.354	1.000

ND = Not determined

Table (5.1) 1 - Tissue concentrations estimated from [redacted] after a single subcutaneous administration of [^{35}S] SR90107A (ORG 31540) to the male Wistar rat (expressed as mg Eq. decasodium salt/kg of tissue) (adrenals to hypophysis)

TISSUES	SAMPLING TIMES (in hours)						
	0.25 (1)	0.5 (2)	1 (3)	3 (4)	6 (5)	24 (6)	48 (7)
Adrenals	0.453	0.282	0.278	0.123	0.027	ND	ND
Adrenals cort.	0.208	0.157	0.215	0.104	0.022	ND	ND
Adrenals med.	0.680	0.470	0.393	0.170	0.034	ND	ND
Bladder cont	11.312	4.878	ND	10.749	7.433	0.069	0.013
Bladder wall	0.264	ND	ND	0.287	ND	ND	ND
Blood	1.406	1.119	1.165	0.344	0.055	ND	ND
Bone marrow	0.273	0.120	0.160	0.146	0.046	0.016	0.010
Brain	0.025	0.015 (*)	0.017	0.011	0.005 (*)	0.005	ND
Brown fat	0.481	0.306	0.313	0.150	0.034	ND	ND
Cartilage	ND	ND	ND	ND	0.125	0.115	0.063
Colon	NO	0.194	0.208	0.138	0.077	ND	ND
Seminal vesicula	0.091	UDL	0.015	0.021	0.010	0.003	ND
Eye	0.010 (*)	UDL	0.023	0.008	0.007	0.003	ND
Gastric content	0.007 (*)	UDL	0.105	0.015	0.011	ND	ND
Gastric wall	0.354	0.112	0.095	0.089	ND	ND	ND
Gut content	0.036	UDL	0.074	0.010	0.482	0.065	0.012
Gut wall	0.336	0.282	0.262	0.175	0.035	ND	ND
Harder's gland	0.211	0.193	0.192	0.069	0.035	0.015	ND
Hypophysus	0.184	0.139	0.176	0.080	0.046	ND	ND

(1) = LOD = 0.0050 mg Eq./kg - LOQ = mg Eq./kg
(2) = LOD = 0.0048 mg Eq./kg - LOQ = mg Eq./kg
(3) = LOD = 0.0006 mg Eq./kg - LOQ = mg Eq./kg
(4) = LOD = 0.0002 mg Eq./kg - LOQ = mg Eq./kg
(5) = LOD = 0.0016 mg Eq./kg - LOQ = mg Eq./kg
(6) = LOD = 0.0008 mg Eq./kg - LOQ = mg Eq./kg
(7) = LOD = 0.0002 mg Eq./kg - LOQ = mg Eq./kg
(*) = Under Quantification Limit
ND = Not Determined
NO = Not Observed
UDL = Under Detection Limit

Table (5.1) 2 - Tissue concentrations estimated from ——————————— ; after a single subcutaneous administration of [^{35}S] SR90107A (ORG 31540) to the male Wistar rat (expressed as mg Eq. decasodium salt/kg of tissue) (kidney to white fats)

TISSUES	SAMPLING TIMES (in hours)						
	0.25 (1)	0.5 (2)	1 (3)	3 (4)	6 (5)	24 (6)	48 (7)
Kidneys	1.350	0.782	0.872	0.409	0.328	0.201	0.081
Kidney cort.	0.811	0.623	0.768	0.538	0.447	0.296	0.122
Kidney med.	2.978	0.739	0.963	0.275	0.134	0.035	0.014
Liver (8)	0.663	0.571	0.480	0.234	0.060	0.007	0.004
Lung	0.983	0.784	0.651	0.238	0.050	ND	ND
Lymph nodes	0.661	0.488	0.382	0.192	ND	ND	ND
Muscle	0.129	0.077	0.081	0.045	0.014	0.006	ND
Myocardium	0.544	0.347	0.318	0.126	0.026	ND	ND
Oesophagus	0.106	0.288	0.086	0.020	0.006	ND	ND
Pancreas	0.164	0.119	0.160	0.074	0.025	ND	ND
Plasma (8)	2.007	1.595	1.611	0.597	0.075	0.003	UDL
Salivary gland	0.253	0.211	0.258	0.079	0.043	ND	ND
Skin	0.346	0.315	0.475	0.155	0.042	ND	ND
Spinal cord	0.018	0.014 (*)	0.017	0.017	0.010	0.002 (*)	ND
Spleen	0.152	0.102	0.138	0.091	0.042	0.010	ND
Testis	0.055	0.043	0.138	0.107	0.039	0.006	ND
Thymus	0.122	0.079	0.096	0.044	0.021	ND	ND
Thyroid gland	0.313	0.178	0.205	0.111	0.051	0.015	ND
White fat	0.012 (*)	0.066	0.022	0.072	0.026	0.003	ND

(1) = LOD = 0.0050 mg Eq./kg - LOQ = mg Eq./kg
(2) = LOD = 0.0048 mg Eq./kg - LOQ = mg Eq./kg
(3) = LOD = 0.0006 mg Eq./kg - LOQ = mg Eq./kg
(4) = LOD = 0.0002 mg Eq./kg - LOQ = mg Eq./kg
(5) = LOD = 0.0016 mg Eq./kg - LOQ = mg Eq./kg
(6) = LOD = 0.0008 mg Eq./kg - LOQ = mg Eq./kg
(7) = LOD = 0.0002 mg Eq./kg - LOQ = mg Eq./kg
(8) = Values obtained using LSC
(*) = Under Quantification Limit
ND = Not Determined
NO = Not Observed
UDL = Under Detection Limit

Animal Treatment and Determination of Radioactivity in Plasma and in Milk of Lactating Rats Following Single Intravenous Treatment with [^{35}S]-Org 31540/SR90107A (Amendments #037 and #054; Report 5040).

Methods: Radioactivity concentrations in maternal plasma and milk were determined following intravenous treatment of lactating female Sprague-Dawley rats with [^{35}S]-Org 31540/SR90107A at a dose of 10 mg/kg. Five dams, each with a litter, were used in this study. Lactating dams were treated with radiolabeled drug approximately 10 days after pup delivery. At least 1 hr prior to milk collection, litters were removed from their mothers. Milk and blood samples for determination of milk and plasma radioactivity concentrations, respectively, were collected at 0.5, 1, 3, 6, and 25 hr after dosing using 5 dams per sampling time. After the 1, 3, and 6 hr collection times, litters were returned to their mothers for feeding. Dams received intraperitoneal treatment with 3 I.U. oxytocin at 15 min prior to each time point to facilitate milk collection except at the 1-hr time