CENTER FOR DRUG EVALUATION AND RESEARCH

Application Number 20-718

PHARMACOLOGY REVIEW(S)

NDA 20-718 Review # 1

Reviewer: Indra Antonipillai, Ph.D.
Pharmacologist, HFD-180

MAR - 3 1997

Sponsor and Address: COR Therapeutics, Inc.
South San Francisco, CA

Date of Review: February 25, 1997

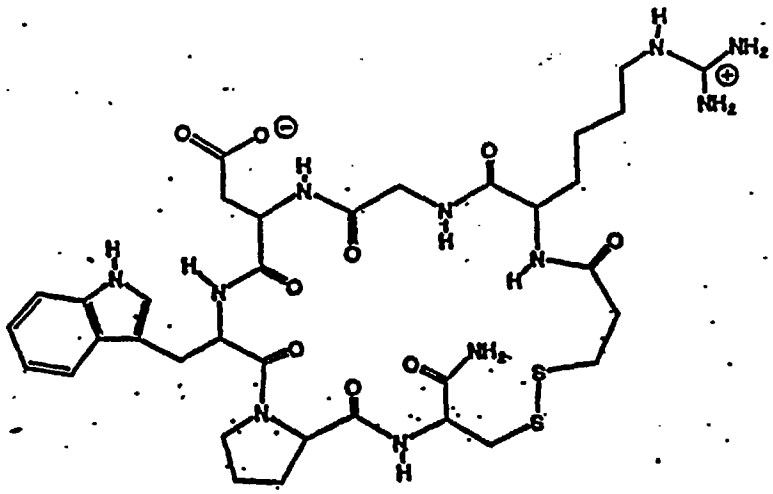
Date of Submission: April 1, 1996

Date of HFD-180 Receipt: April 2, 1996

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
Original Summary

DRUG: INTEGRILIN/Intrifiban/C-68-22/SCH 60936 Injection.

Cyclo(S-S)mercaptopropionyl-L-homoarginine-glycyl-(L)aspartyl-(L)tryptophanyl-(L)Propyl-(L)Cysteinamide



MW 832.1 Molecular Structure $C_{35}HS_2N_{11}O_9S_2$

Formulation: Bolus 10 ml injection vial contains 2.0 mg/ml of the drug, and the continuous 100 ml infusion vial contains 0.75 mg/ml. Both vials also contain 25 mM citric acid buffer, pH 5.25.

Category: Antithrombotic/anti-aggregating agent/inhibitor of platelet glycoprotein (GP) IIb-IIIa.

PROPOSED MARKETING INDICATION:

It is indicated as an adjunct to aspirin and heparin in patients undergoing percutaneous transluminal coronary angioplasty (PTCA), for the prevention of acute cardiac ischemic complications (such as death, myocardial infarction, need for urgent intervention) related to abrupt closure of the treated coronary vessel.

DOSE:

The recommended dose of integrilin is a 135 μg/kg bolus injection, followed by a 0.5 μg/kg/min infusion for a 20-24 hour period.

RELATED INDs/NDAs:

PRECLINICAL STUDIES AND TESTING LABORATORIES:

Type of Study	Study/Report #	Drug Batch #	Testing Lab.	Review Page #
Pharmacology				5 - 15
Absorption:				
Rat	P-6202/RT1-64C-5483	36201-21-11/931201		16 - 17
Monkey	P-6203	36201-23-5		17 - 18
Distribution:				
Male rats	P-6205/RT1-64C-5483	36201-21-11/931201		19 - 21
Pregnant rats	P-6204	36201-23-5		19 - 21
Metabolism:				
Rat	P-6202	36201-21-11		21
Monkey	P-6203	36201-23-5		21
Excretion:				
Rat	P-6202/RT1-64C-5483	36201-21-11/931201		21 - 22
Monkey	P-6203	36201-23-5		21 - 22
Acute Toxicity: i.v.				
Rat	PH406-COR-001-90/ PH406-COR-002-90	A0001A A0001A		23 - 24
Rabbit	PH458-COR-001-90/ PH458-COR-002-90	A0001A A0001A		23 - 24
Monkey	BIO 52543	A0001A		23 - 24
Subacute Toxicity: i.v.				
Rat				
14-Day	BIO 52485	A0001A		25 - 26
28-Day + 14-Day recovery	BIO 54291	D0017A		26 - 28
3-Day Toxicokinetic Study	P-6166	E0023A & E0028A		28 - 29
Monkey				
14-Day	BIO 52484	A0001A		29 - 31
28-Day + 14-Day recovery	BIO 54292	D0017A		31 - 34
3-Day Toxicokinetic Study	P-6165	E0023A & E0028A		34

Reproductive Toxicity:			
Segment I. Fertility and Reproductive Performance Rats, i.v.	BIO 95628	D0015A, E0019A	35 - 39
Segment II. Teratology Rats, i.v.	BIO 95635	D0015A	40 - 43
Rabbits, i.v.	BIO 95636	E0019A	44 - 47
Segment III. Perinatal/Postnatal Rats, i.v.	BIO 95629	D0014A, E0019A	47 - 53
Special Toxicity Studies:			
1. Antigenicity in guinea pigs	PH742-COR-001-90	A0001A	54
2. Acute Irritation studies in rabbits	PH422-COR-001-90	A0001A	55
3. Delayed type hypersensitivity	PH711-COR-001-90	A0001A	54
4. Test of production of hemolysis	PH725-COR-001-90	A0001A	55
5. Test for production of plasma protein flocculation	PH726-COR-001-90	A0001A	55
6. 14-Day i.v. toxicity of degraded Integrilin in monkeys	BIO 54516	E0028A	56 - 58
Mutagenicity:			
1. Ames Test	HWA 16265-0-422 CHV 17110-0-422	E0019A E0028A	59 - 61
2. Mouse lymphoma cell (TK locus) forward gene mutation test	HWA 16265-0-431	E0019A	61 - 62
3. Human lymphocyte chromosome aberration test	CHV 17110-0-449	E0028A	62 - 63
4. Mouse micronucleus test	HWA 16265-0-455	E0019A	64 - 65

Some in vivo secondary pharmacology studies, acute toxicity studies, 14-day toxicities in the albino rats and cynomolgus monkeys, antigenicity in guinea pigs, delayed type hypersensitivity, acute irritation study in rabbits, and tests for production of hemolysis and plasma protein flocculation were submitted Previous IND reviews for these studies, are incorporated in appropriate places.

PHARMACOLOGY:

Integrilin is a cyclic heptapeptide containing 6 amino acids. Integrilin is a specific inhibitor of platelet GP IIb/IIIa. In acute cardiovascular syndromes (such as unstable angina, abrupt closure due to post-PTCA, or in myocardial infarction, reocclusion following thrombolysis), platelet-mediated arterial thrombosis is a troubling complication, and aspirin and heparin have limited roles. Therefore, antiplatelet agents, such as integrilin, which has a short half life, may be useful in these syndromes. Primary pharmacology studies have been carried out to determine if integrilin is a specific inhibitor of von Willebrand factor, and fibrinogen binding, to GP IIb/IIIa receptor. In vivo studies, in a baboon and a dog model of thrombosis, have been carried out to see if integrilin inhibits platelet aggregation, and is anti-thrombotic. Pharmacodynamic studies of the drug have been carried out in healthy baboons with both, aspirin and heparin, to determine its interaction with these two drugs.

Primary Pharmacology:

In Vitro Studies

1a. Effects of Integrilin on glycoprotein (GP) IIb/IIIa receptor binding and platelet aggregation:

This study examined the effects of the drug, on the binding of integrins (the adhesive proteins) to GP-IIb/IIIa receptor, as well as on platelet aggregation.

Cell adhesion (using M21 melanoma cell adhesion to vitronectin) and platelet aggregation were measured. Integrilin inhibited fibrinogen binding to GP IIb-IIIa in a dose related manner, with an IC_{50} of 100-120 nM, Table 1. It was a more potent inhibitor of von Willebrand factor binding to GP IIb/IIIa, with an IC_{50} of <5 nM. Integrilin was a poor inhibitor of vitronectin binding to purified vitronectin receptor $\alpha_v\beta_3$, with an IC_{50} of 5 μM. Integrilin inhibited vitronectin-mediated cell adhesion at an IC_{50} of 5-10 μM, and ADP (20 μM)-induced human platelet aggregation at 100-120 nM respectively. Integrilin completely inhibited the platelet aggregation induced by ADP, collagen (5 μg/ml), thrombin receptor activating peptide (TRAP, 10 μM), and U46619 (thromboxane A_2 analog, 1 μM) at a concentration of 1.0 μM.

These studies indicate that integrilin specifically inhibits von Willebrand factor and fibrinogen binding to GP IIb/IIIa receptor at IC_{50} values of <5 and 100-120 nM respectively, and inhibits ADP-induced platelet aggregation at 100-120 nM.

Table 1

Integrilin inhibition of fibrinogen binding to GP IIb/IIIa IC_{50} values	Integrilin inhibition of von Willebrand factor binding to GP IIb/IIIa IC_{50} values	Integrilin inhibition of vitronectin mediated cell adhesion IC_{50} values	Integrilin inhibition of ADP-induced platelet aggregation IC_{50} values
100-120 nM	< 5 nM	5-10 μM	100-120 nM

1b. Effects of Integrilin on Ex-vivo Platelet Aggregation Induced by ADP, in Platelet-rich Plasma Samples of Human and Different Animal Species:

The IC_{50} values in 5 animal species along with IC_{50} values in humans, are shown in Table 2. Platelets of human, monkeys, and baboon were more sensitive to integrilin's action, followed by the dog, rabbit and rat, suggesting that differences in homology of the GP IIb/IIIa receptor exist, in different species.

Table 2

Inhibition of ADP-induced platelet aggregation	IC_{50} values
Rat	9.41 ± 0.80 μM
Rabbit	4.96 ± 1.92 μM
Monkey	120 ± 29 nM (range 102-154 nM)
Dog	1.9 μM
Baboon	390 nM
Human	50-240 nM

2. Potent and specific integrin antagonists, which are highly specific for GP IIb/IIIa, can be designed:

This published paper (J.Biol.Chem. 286: 1066, 1993) describes, how a potent and selective peptide can be designed, which acts as an antagonist of GP IIb/IIIa. A member of the snake venum-derived peptide family, containing Arg-Gly-Asp (RGD) is among the most potent antagonist of adhesive proteins to platelet GP IIb/IIIa. However, if Arg in RGD is substituted with Lys (K), thereby making it KGD, it now makes these peptides very specific, for inhibition of GP IIb/IIIa. One such peptide is barbourin (a novel member of the disintegrin family of peptides). For example, a KGDX anolog (cyclo(S,S)-CKGDWPC-NH2) which contains the most hydrophobic aromatic side chains, was a more potent inhibitor of GP IIb/IIIa and gave the IC_{50} values of 5.9 μM, in inhibiting ADP-induced platelet aggregation, vs another anolog, from the same family, with less hydrophobic aromatic side chains, (cyclo(S,S)CKGDW(sar)c-NH2), with the IC_{50} values of 92 μM. Furthermore, formation of disulfide bridges, changes in the conformational sequence, optimization of secondary hydrophobic binding sites, and derivatization of the lysyl side chain are important to make these peptides optimal, which now have both, the high selectivity and affinity for GP IIb/IIIa.

In Vivo Studies

1. Studies in Baboons

1a. Effects of Integrilin on Thrombosis, in the 'in Vivo' Baboon Model:

Baboons and humans have similar coagulation, proteins-induced platelet aggregation systems, bleeding times and platelet morphology. In this study, an established primate model of vascular graft thrombosis was used (Arteriosclerosis 5: 595, 1985) in which insertion of Dacron vascular grafts, as extension segments into chronic femoral arteriovenous silastic shunts, induces thrombus formation. Following infusion of ^{111}In-oxine labeled platelets into baboons, thrombus formation onto the dacron graft can be measured with scintillation camera. In platelet rich plasma (PRP) of baboons, ADP (20 μM)-induced platelet aggregation was inhibited, at IC_{50} of 390 nM integrilin. Infusion of integrilin (3.5 μg/kg/min) into a peripheral vein (n=3 animal) caused a 50% inhibition, in ex vivo platelet aggregation within 20 minutes, whereas 5 or 10 μg/kg/min (n=1) completely inhibited ex vivo platelet aggregation after ~25 minutes. Bleeding times (measured at ~30 minutes) are shown in Table 3, integrilin caused a dose-related increase in bleeding times. The platelet aggregation was normalized, within 15-30 min, and the bleeding times after 30 minutes of termination of the drug.

Table 3. Bleeding times in baboons

Infusion Dose (μg/kg/min)	Bleeding times (minutes)
Controls (n=5)	~ 4
5 (n=5)	7 ± 1.4
10 (n=5)	12 ± 1.5

Table 4 shows the thrombus formation, which was inhibited by 15% and 54% by 5 and 10 μg/kg/min doses of integrilin resp. Greater inhibition (44% and 80% vs 15% and 54% respectively) was achieved, when grafts were placed 15 minutes after integrilin infusions, because the drug had already reached the steady state. The thrombus formation is normalized after stopping the integrilin infusion.

Table 4. Effects of integrilin on platelet deposition (thrombus formation).

Dose (μg/kg/min)	Incidence of Thrombus Formation, (grafts were placed 15 minutes before integrilin infusion)	Incidence of Thrombus Formation, (grafts were placed 15 minutes after integrilin infusion)
5 (n=5)	15%	44%
10 (n=5)	54%	80%

No effects on behavior, general appearance, changes in spontaneous bleeding or on blood pressure were seen in animals (n=13) with the clinical-grade of integrilin (5-10 μg/kg/min). However 2 baboons treated with the laboratory grade of the drug (10 μg/kg/min) showed transient thrombocytopenia (no data were given). To confirm this, in a separate study, 2 groups of baboons received 20 or 30 μg/kg/min of the drug, to see if there was a maximal effect on thrombocytopenia. The maximum response was achieved at 20 μg/kg/min (with 40-80% reduction in platelet count at 30 minutes). The 30 μg/kg/min dose did not further alter the platelet count. Within 2 hours the platelet count returned to normal. Therefore, the mechanisms involved in reductions in thrombus size are not clear, if it was a direct effect on platelet thrombus formation, or an indirect one due to a mild thrombocytopenia.

In conclusion, integrilin at doses of 5-10 μg/kg/min has a good anti-thrombotic effect in baboons without reducing the peripheral circulating platelet count. However, higher doses (20-30 μg/kg/min) cause a 40-80% decrease in platelet count. Platelet aggregation was normalized within 15-30 min, and the bleeding times after 30 minutes of termination of the drug.

1b. Studies of integrilin with aspirin and heparin in baboons:

Since both heparin and aspirin are clinically used in coronary artery syndromes, the interaction of these two drugs with integrilin were evaluated. A model of Dacron grafts in baboons was used again. Thrombus formation is measured in this model, by quantitating the amount of platelets deposited onto the dacron graft, using a scintillation camera. Healthy, male baboons received oral aspirin (35 mg/kg, a dose which abolishes the platelet thromboxane A2 formation) for 1-2 hours. Heparin (40 or 80 units/kg) bolus iv + a continuous heparin infusion (40 or 80 units/kg/hr) was given for an hour. Integrilin (5, 10, or 20 μg/kg/min) was administered by a continuous infusion for 75 minutes. Bleeding times (BT), activated partial thromboplastin time (aPTT), and the thrombus formation were measured. Table 5 (reproduced from volume 26, reference 5, page 5) shows, that heparin or aspirin alone did not alter bleeding times (4.4 vs 5.0 ± 1.5 and 5.5 ± 0.8 min respectively). Integrilin, in combination with heparin + aspirin, significantly increased the bleeding times to 21.7 ± 3.5 and 26.9 ± 1.9 min, with 40 and 80 unit/kg of heparin resp. Integrilin (5 μg/kg/min)-mediated bleeding time was slightly increased by 80 unit/kg of heparin (7.3 ± 1.3 vs 9.6 ± 1.9 min), and was significantly increased by aspirin (14.0 ± 2.3 min).

The basal aPTT was unchanged by aspirin alone (31 ± 2 vs 33 ± 2 sec). This was significantly increased by 80 units of heparin alone (226 ± 21 sec), and decreased by combination of integrilin (5 μg/kg/min) + aspirin + heparin (63 ± 3 and 171 ± 37 sec respectively with 40 and 80 units of heparin).

Table 5. Hemostatic effects of integrilin, heparin and aspirin.

Integrelin (µg/kg-min)	Heparin (unit)	Aspirin (mg)	BleedingTime (min)	aPTT (sec)	N
0			4.4 ± 0.2	31 ± 2	8
5			7.3 ± 1.3	31 ± 2	5
10			11.7 ± 1.2	34 ± 2	5
20			>30	33 ± 2	4
	80		5.0 ± 1.5	226 ± 21	5
2	80		8.8 ± 1.2	–	3
5	80		9.6 ± 1.9	201 ± 32	4
		35	5.5 ± 0.8	33 ± 2	5
5		35	14.0 ± 2.3	–	5
5	40	35	21.7 ± 3.5	63 ± 3	4
5	80	35	26.9 ± 1.9	171 ± 37	5

Integrilin alone, at 5 µg/kg/min significantly decreased the thrombus formation, ($p < 0.01$). Heparin (80 units/kg) alone also decreased the thrombus formation ($p > 0.1$). The combination of two, although further decreased the thrombus formation, the effect was not statistically significant from the integrilin alone ($p > 0.2$). No changes in thrombus formation (or accumulation of platelets on the graft) were seen with aspirin alone, or in combination of all 3 (aspirin + integrilin + heparin).

In conclusion, 5 µg/kg/min of integrilin, slightly increases bleeding times, but has significant antithrombotic effects. Heparin (80 units/kg) slightly increases bleeding times of integrilin, but potentiates its antithrombotic action. In contrast, aspirin has no effect on its antithrombotic action but significantly increases the bleeding time of integrilin. The combination of all 3 agents may have additive effects on increasing the bleeding times, as shown in Table 3.

2. Studies in Dog

2a. Effects of integrilin on a canine model of thrombosis:

An established dog model was used to form a platelet-rich thrombus in one of the major artery (J. Lab. Clin. Med. 103:, 204, 1984), which induces cyclic blood flow reductions (CFR). A laboratory grade developmental batch of integrilin was used in

total of 14 mongrel dogs (1 to 5/group). In platelet rich plasma (PRP) of dogs, ADP and epinephrine-induced platelet aggregation was inhibited by integrilin with an IC_{50} of 1.9 μM. Integrilin at 1 (n=2), and 4 μg/kg/min (n=4) caused a 50%, and 100% inhibition resp, in ex vivo platelet aggregation in PRP. At a continuous infusion rate of 1-4 μg/kg/min, inhibition of platelet aggregation reached a steady state by 30 min, at this dose a >50% inhibition in platelet aggregation was noted for 120-150 min, even after termination of the drug. Up to 4 μg/kg/min of the drug did not affect the bleeding times in dogs. Complete inhibition of the thrombus formation was observed within 20 minutes, after infusion of ≥ 1.0 μg/kg/min. No effects of the drug on behavior, general appearance, changes in spontaneous or excessive bleeding, platelet count, hematocrit, or any effects on blood pressure, heart rate, cardiac, output, systemic or pulmonary vascular resistance were seen. These studies suggest that, integrilin has a significant anti-thrombotic effect in this dog model.

2b. Effects of Integrilin in a closed-chest canine model of coronary thrombolysis:

A chronic model of thrombolysis in the coronary artery, induced by electrical injury to epithelium was used (J. Clin. Invest. 77: 496, 1986). Two hours after coronary occlusion, t-PA (10 μg/kg/min by continuous infusion) was given. Integrilin (2, 5, or 10 μg/kg/min) or vehicle was delivered by continuous iv infusion, 30 minutes before t-PA and continued for 2-hours after reperfusion. Integrilin, dose-dependently produced inhibition of reocclusion (4/6, 3/6, 0/5 vs 6/6), following coronary thrombolysis with t-PA. The platelet aggregation was inhibited at doses of 2, 5 and 10 μg/kg/min (18 ± 12, 5 ± 4, 1 ± 1 vs 78 ± 2) and bleeding times were increased at 5 and 10 μg/kg/min (6.3 ± 3, 8.1 ± 2.1 vs 1.2 ± 0.5). These studies show that integrilin increases the t-PA induced thrombolysis and decreases reocclusion.

2c. Effects of Integrilin and Thrombin Inhibitor, Hirudin on Thrombolysis in a canine model of Intra coronary Thrombosis:

This study examined if the combination of platelet inhibitor and a thrombin inhibitor (hirudin) would improve thrombolysis in the occluded coronary artery. A thrombus was produced by stimulation of an electrode, implanted in the coronary artery of dogs. Animals were given t-PA (1 mg/kg) for 20 minutes to lyse the clot, along with following agents for 90 minutes: group 1 received saline, group 2 integrilin (5 μg/kg/min), group 3 integrilin (2.5 μg/kg/min) + recombinant hirudin (10 μg/kg/min), group 4 hirudin (20 μg/kg/min). Either agent given with t-PA enhanced the rate of lysis of the occlusive thrombus, but failed

to change the reocclusion rate (57% and 63% resp. vs saline 83%). However, low dose combination of both completely restored the coronary blood flow and significantly decreased the reocclusion rate (25%). Therefore, both of these drugs given with t-PA may be effective in preventing thrombosis.

2d. Effects of Integrilin on Platelet Aggregation during Cardiopulmonary Bypass (CPB):

CPB patients, after surgery have postoperative bleeding, due to thrombocytopenia and impaired platelet function (due to the platelet adhesion to foreign surfaces, such as oxygenator, bypass tubing). This study evaluated the effects of integrilin on platelet adhesion, after CPB, using a dog model, to examine the beneficial effects of the drug. Mongrel dogs were subjected to 2.5 hours of hypothermic CPB, and were then observed for 6-hours. Dogs (n=6) received either integrilin (90 μg/kg/min, iv bolus + 2 μg/kg/min continuous infusion during CPB) or vehicle. At 2-hours, controls had significantly lower platelet counts (35.2 ± 4.6%, % of pre-CPB values) compared to integrilin group (68.2 ± 4.9%). After 6 hours, the integrilin treated animals had reduced blood loss (248 ± 30 ml vs 447 ± 97 ml in the control), and platelet function was better preserved in the integrilin group (expressed as % of pre-CPB aggregation induced by 10 μmol/L ADP 20.8 ± 3.0% vs 43.9 ± 10.4% in the control group). There were no changes with respect to $Pa0_2$, lung weight, histology or myeloperoxidase activity between two groups. Thus, integrilin reduces post-surgery bleeding after CPB.

Secondary Pharmacology:

In vitro Studies

1. Effects of Integrilin on Cardiac Metabolism in the Guinea Pig Heart:

Integrilin was infused at 0.1, 1.0 and 10 μM conc, in the isolated guinea pig heart, and following parameters were examined: left ventricular developed pressure (LVDP, control 63-75, treated 55-62 mm Hg, at 0.1-10 μM), its first derivative dP/dT (881-1201 vs treated 822-1022 mm Hg), coronary perfusion pressure (CPP, 46-58 vs 39-47 mm Hg), and tissue conc. of creatinine phosphate (CP, 57 vs 53-57), ATP (19 vs 19-20), ADP (2.2 vs 2.7-3.2), and AMP (0.35 vs 0.27-0.36) were examined. None of these parameters were significantly different from the vehicle control. Thus, the drug has no effect on myocardial contractility or cardiac metabolism.

2. Effects of Integrilin on Isolated Hog Coronary Arteries:

This study examined the direct effects of integrilin on contraction and relaxation-parameters in the isolated hog coronary arteries with and without endothelium. The strips were

placed in Kreb's solution. The baseline tension of the strips was not altered by 0.1-10 μg/ml conc of integrilin. Similarly 0.1 to 3 μg/ml had no effects, but 3-10 μg/ml had minimal effects on relaxation of $PGF_{2\alpha}$-induced contractions. These studies suggest, that integrilin has no contractile activity, and very minimal or no relaxation activity in this model.

3. Effects of Integrilin on Human Vascular Smooth Muscle Cell (VSMC) Proliferation in the Presence or Absence of Platelet Derived Growth Factor (PDGF):

The primary cultures of human VSMC (without growth factors) were incubated in the presence of PDGF BB (5 to 160 ng/ml), to induce cell proliferation. Integrilin (7, 20, 60, and 65 μM) was added to see if it had an effect on basal or PDGF-induced mitogenesis. Integrilin alone (65 μM), or in the combination with PDGF, did not alter cell proliferation.

In Vivo Studies

1. Intrinsic Cardiovascular Effects of Integrilin in Conscious Rats:

This study examined the cardiovascular activity of integrilin, after a single infusion dose of 50 μg/kg/min for 45 min or the vehicle (0.9% aqueous saline), into conscious rats. Blood pressure, heart rate, and cardiac electrical activity were monitored up to 4 hours. Integrilin did not affect the PR, QRS, QT, and RR intervals, or blood pressures and heart rates, suggesting that integrilin has no effect on intrinsic cardiovascular activity.

2. Acute Effects of Integrilin on Kidney Function in Dogs and Rats:

Anesthetized dogs or rats were given iv infusion of either integrilin (50 μg/kg/min) for 45 min or saline. Urine flow, renal plasma flow, and glomerular filtration rates were evaluated during and post-infusion times, to examine the potential effects of the drug on kidney. These parameters exhibited a similar time-dependent decrease in both drug and saline treated animals (for example, urine flow rate in control dogs decreased from 0.27 to -0.07 and -0.12, vs in treated dogs, from 0.50 to -0.11 and -0.18 resp), suggesting that the integrilin does not have any adverse effects in the kidney in either species.

Following 'in vivo' studies were submitted to and were reviewed on 10/4/1990. These are reproduced below.

Effects on CNS

For each of the following tests, three mice were given single i.v. bolus injection of 300 mcg/kg of C68-22.

1. Reflux Inhibition/Muscle Relaxation/Motor Activity/Catalepsia Maintenance: C68-22 had no effect.

2. Anti-Metrazol Seizure Activity: C68-22 had no effect on metrazol induced seizures and death.

3. Anti-electric Shock Assay: C68-22 was ineffective in protecting against mild electric shock effects.

4. Behavioral Depression: Slight behavioral depression was seen in treated mice.

5. Tetrabenzene Antagonism Test: Treated mice were slightly protected from tetrabenzene ptosis. Indicating a slight dopamine antagonistic activity.

Anti-Inflammatory Activity

1. Carageenin-Induced Paw Edema: A single i.v. dose of C68-22 (300 mcg/kg) given to rats had no effect on carrageenin induced edema (positive control was not included).

2. Arachidonic Acid-Induced Ear Inflammation: A dose of 300 mcg/kg into mice ear, had no effect on arachidonic acid induced inflammation in the ear.

3. Rat Gait and Anit-Pyretic Test in Rats: A single dose of 300 mcg/kg into rats paw had no effect on the pyretic and gait response induced by injection of 35% suspension of brewers yeast into the rats paw.

Gastrointestinal Evaluations

1. Rat Antisecretory Test: C68-22 (300 mcg/kg) administered by intravenously to rats had no significant effect on volume and pH of the gastric fluid.

2. Rat Anti-ulcer Assay: C68-22 (300 mcg/kg, i.v.) had no effect on aspirin induced ulcer in the rats.

Cardiovascular System Assays

1. Antihypertensive Action in SH Rats: C68-22 (300 mcg/kg, i.v.) did not have any significant antihypertensive effect and had no effect on heart rate in SH rats.

2. Chloroform-Induced Arrhythmia: C68-22 (300 mcg/kg, i.v.) had no effect on chloroform induced arrhythmia.

Following 'in vivo' studies were submitted to IND 35,465, amendment dated January 24,1991, and were reviewed on 2/13/1991. These are reproduced below.

1. Effects of C68-22 on Cardiac and Hemodynamic Function in the Dogs: Mongrel dogs were given incremental infusions of C68-22 at 5, 10, 20, 30, 50, and 100 mcg/kg/min for 30 minutes each with a 30 minute rest period for anesthesia at the end of the 10 and 30 mcg/kg/min doses. A dose of up to 30 mcg/kg/min of C68-22 did not result in any significant changes in right arterial pressure, pulmonary capillary wedge filling pressures, pulmonary artery pressure and systemic blood pressure. However at 50 and 100 mcg/kg/min a significant decrease in mean arterial pressure and decline in peripheral vascular resistance were noted (data presented graphically). A dose as high as 100 mcg/kg/min had no significant effect on cardiac conduction, and did not cause any cardiac ischemia or ventricular or atrial dysrhythmias.

2. Effects of C68-22 on Renal Function in Dogs: Anesthetized female beagle dogs (n=4) were given i.v. infusion of C68-22 at 5, 25, and 50 mcg/kg/min for 45 minutes with a 15 minutes washout between each dose levels.

A dose dependent decrease in creatinine clearance was seen in treated animals (control = 25 ml/min, low dose = 20 ml/min, mid dose = 16 ml/min and high dose = 12 ml/min). At the end of 25 and 50 mcg/kg/min infusions the mean arterial blood pressures were reduced by 18% and 26% and heart rates by 10% and 16% respectively. No significant changes in serum and urine electrolyte levels were seen. Sponsor has also submitted an addendum to this report, in which decreases in creatine clearance, filtrate fraction and blood pressure were seen in vehicle treated animals and the extent of changes were similar to that found in drug treated animals. Thus C68-22 had no biologically significant adverse effect(s) on renal function of the dog.

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION (ADME)

Pharmacokinetics:

The pharmacokinetics of integrilin have been studied in adult male Albino rats (276-306 g), and in adult male Cynomolgus monkeys (males 3.0-3.6 kg) after a single iv bolus injection.

Rats

Pharmacokinetics, Metabolism And Excretion of ^{14}C-Integrilin (SCH 60936) Following A Single Intravenous Bolus Dose To Male Albino Rats: (Report # P-6202, Study # 950442).

Methods: In albino rats (Sprague-Dawley, Crl:CD(SD)BR, 4/time point), integrilin (^{14}C-SCH 60936) was given as a single iv dose at the conc of 2 mg/ml/kg (batch No. 36201-21-11) to groups 1-3. Controls (group 4) received the vehicle (placebo) to determine the background levels of radioactivity. Rats in group 1 were sacrificed at 2, 5, 10, 15, 30, 45, 60, 90, 120, 180, 240, and 360 minutes after the drug administration, and blood was collected. Urine was collected from the group 2 animals at 0-4, 4-8, 8-24, 24-48, 48-72, 72-96, 96-120, 120-144, and 144-168 hrs after treatment, and feces at 0-24, 24-48, 48-72, 72-96, 96-120, 120-144, and 144-168 hrs. Group 3 rats were sacrificed at 5, 30, and 90 min after dosing, and metabolites were characterized in the blood. The metabolic patterns in urine and feces were determined

The results of metabolism and excretion studies are presented under those sections.

Results: In male Albino rats, the pharmacokinetics variables are shown in Table 6. The highest mean plasma concentration (7.10 μg/ml) and radioactivity (10.6 μg equiv/g) were seen at 2 min. By 45 min, the conc of the drug was below the detection level. The half life of integrilin was 7.8 min (0.13 hr). The proportion of the drug to total radioactivity in plasma decreased over time and by 30 min only 15% of the drug was found in plasma. The total radioactivity in whole blood vs plasma was consistently lower (at 5 and 90 min, these were 6.46 and 0.49 equivalents/g resp in plasma, vs 3.76 and 0.28 μg equivalents/g resp in blood), which suggests uneven distribution of the drug between cellular components of blood and plasma, whereas hematocrit conc did not change significantly with time. After reaching peak conc, plasma levels declined rapidly, 56% of the administered dose was recovered in urine within first 4 hours.

Table 6. Pharmacokinetics parameters of integrilin in Albino rats, after a single iv injection.

Parameter	PK of Integrilin
Cmax (μg/ml)	7.10
Tmax, (min)	2.00
$AUC_{0-73\ h}$, (μg.hr/ml)	0.895
t½, (hr)	0.131

Pharmacokinetics, Metabolism And Distribution of Integrilin (C68-22) Administration Intravenously To Sprague Dawley Rats: (Report # RT1-64C-5483).

Methods: In Sprague-Dawley male rats, 2 and 20 mg/kg (lot # 931201) of ^{14}C-integrilin were given, as a single iv dose. Single blood samples, urine and feces, as well as tissues were collected at sacrifice. Radiolabeled trapping solutions from the breath traps were also collected. Radioactivity in plasma, various tissues, and urine/feces/cage rinses was determined

The results of metabolism and distribution studies are presented under those sections.

Results: In Sprague-Dawley rats, monophasic decay as a function of time, was observed, after a single iv dose, with a plasma half life of 11-12 min, and there was a linear clearance of the drug with the dose. At 24 hours, >90% of the radioactivity was excreted.

Monkeys

Pharmacokinetics, Metabolism And Excretion of ^{14}C-Integrilin (SCH 60936), Following A Single Intravenous Bolus Dose To Male Cynomolgus Monkeys: (Report # P-6203, Study # 95344)

Methods: In Cynomolgus monkeys (4 males, assigned to 2 study groups), integrilin (^{14}C-SCH 60936) was given at doses of 2 mg/ml/kg (batch # 36201-23-5), by a single iv injection. Group 2 animals were used exclusively for metabolism studies. Blood was collected at baseline and at 5, 10, 30, and 60 minutes, and at 2, 4, 6, 12, 24, and 48 hr after the drug administration. Blood samples from the group 2 were collected at 10, 60 min and 6 hours after treatment. Urine and feces were collected at various intervals, up to 504 hrs after dosing. The metabolic patterns in urine and feces were determined

Concentration of the drug was measured

The results of metabolism and excretion studies are presented under those sections.

Results: In Cynomolgus monkeys, the highest mean plasma concentration (6.0 μg/ml) and radioactivity (6.5 μg equiv/g) were seen at 5 min, after a single iv dose, Table 7. By 4 hours, the conc of the drug was below the detection level. The half life of integrilin was 17.58 min (0.291 hr). The proportion of the drug to total radioactivity in plasma decreased over time. The total radioactivity in whole blood vs plasma was consistently lower, as was seen in the albino rat, suggesting uneven distribution of the drug between cellular components of blood and plasma, whereas hematocrit conc did not change significantly with time. After reaching peak conc, plasma levels declined rapidly.

Table 7. Pharmacokinetics parameters of integrilin in Cynomolgus monkeys, after a single iv injection.

Parameter	PK of Integrilin
Cmax (μg/ml)	6.03
Tmax (min)	5.33
$AUC_{0-48\ h}$ (μg·hr/ml)	1.81
t½ (hr)	0.291

In humans: The half life of the drug in healthy subjects is 0.6-1.7 hours. However patients, undergoing coronary angioplasty have reduced plasma clearance (100-150 vs 200-300 ml/kg/hour), and increased half life (2-3 hours). The patients with renal impairment, and healthy post-menopausal women have half life of 2.5 hrs and 1.8 hrs resp. No gender differences were noted. There was a rise in the drug concentration, in proportion to dose, in the range of 0.5-1.5 μg/kg/min.

These studies suggest that half life of the drug is similar in species, it was 8 min in rats, and 17 min in monkeys. In humans, it was 2.2 hours, after 18-24 hours of iv infusion. Continuous infusion of the drug led to steady state increases in the drug conc in plasma, and no sex differences were noted between species.

Distribution:

Tissue Distribution Of Radioactivity in Male Sprague Dawley Rats, and in Pregnant Female Rats, After A Single Intravenous Bolus Dose Of ^{14}C-Integrilin: (Report # P-6205, and # P-6204)

Methods: Distribution of ^{14}C-integrilin (SCH 60936, 2 mg/kg) was examined, after a single bolus iv dose of the drug (lot # 36201-21-11 and 36201-23-5) or placebo to male Sprague Dawley rats (3 rat/group), and to healthy pregnant rats, at 19 days of gestation (n=3/time period). Rats were sacrificed at 0.1, 0.25, 1, 2, 6, 24, and 48 hours, after the injections, and the radioactivity in plasma, various tissues, and urine/feces/cage rinses was determined The distribution of radioactivity was also determined
Distribution was also examined in male rats after 2 and 20 mg/kg doses as indicated above (study #RT1-64C-5483).

Results: The radioactivity was widely and rapidly distributed throughout the body. In male rats, the peak tissue levels were attained within 0.1 hour, and decreased rapidly with time (except in liver, GI tract, and testes). By 48 hours, only trace amounts (<1%) were seen in tissues and carcass. Only organs that had higher conc than the circulating drug level, were bladder, small intestine contents, and kidney (2.1, 2.2, and 2.8 fold resp compared to plasma, at 0.25 hour, shown as distribution ratio of tissue to plasma), Table 8 (reproduced from volume 47, reference 8, page 23), suggesting that the drug was eliminated at early time points by biliary excretion, but the major route of elimination was via the renal system. The tissues in the central nervous system had very little conc of the drug, indicating that it does not cross the blood-brain barrier. In male rats, 2 and 20 mg/kg doses showed comparable tissue distribution profiles and were dose related. Similar patterns of distribution were found in maternal tissues, the peak tissue levels were attained within 0.25 hour in the kidney and bladder, suggesting rapid clearance of the drug. Bladder, small intestine contents, and kidney had 3.5, 2.9, and 2.3 fold higher conc, compared to plasma, at 0.25 hour. However fetal tissue uptake was slower, and maximal tissue conc were seen at 6 hours after dosing. In male rats, visual inspection of autoradiography indicated, the presence of radioactivity in the sclera and cornea of the eye from 0-2 hour, which diminished by 6-hour and reappeared at 6 hours (report # P-6203) in the outer lens of the eye, and this was even detectable at 48 hours.

Table 8. Distribution ratio of tissue to plasma in male rats after a single iv bolus dose of ^{14}C-integrilin.

	Mean						
	0.1 hr[a]	0.25 hr[a]	1 hr[a]	2 hr[a]	6 hr[a]	24 hr[a]	48 hr[a]
Plasma	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Blood	0.640	0.620	0.621	0.686	0.843	1.035	1.259
Liver	0.790	1.350	1.682	2.956	5.177	5.335	4.759
Bladder	3.419	2.131	2.116	1.955	2.025	1.976	2.429
Kidney	2.968	2.803	2.798	3.714	5.540	5.090	5.151
Submaxillary gland	0.225	0.211	0.250	0.452	1.438	1.293	1.492
Stomach	0.349	0.357	0.300	0.466	0.874	1.216	1.612
Stomach contents	0.056	0.306	0.265	0.213	2.033	1.581	0.225
Small intestine	0.169	0.385	1.288	4.367	0.870	1.463	1.577
Small intestine contents	0.400	2.230	22.294	63.891	5.001	3.373	1.447
Cecum	0.245	0.230	0.303	1.285	2.642	2.233	2.801
Cecum contents	0.032	0.039	0.052	5.812	94.807	15.026	2.540
Large intestine	0.242	0.239	0.270	0.778	2.558	1.835	1.995
Large intestine contents	0.048	0.051	0.055	0.439	72.882	16.379	5.016
Testes	0.087	0.167	0.174	0.377	0.654	0.777	0.882
Seminal vesicle	0.327	0.191	0.353	0.279	0.859	1.077	1.985
Epididymis	0.266	0.344	0.447	0.534	0.935	1.128	1.336
Prostate	1.324	0.571	0.706	0.821	1.521	2.529	3.833
Heart	0.217	0.210	0.247	0.458	1.199	1.250	1.379
Spleen	0.162	0.215	0.229	0.856	1.029	1.540	2.035
Abdominal artery	1.091	1.090	0.916	1.217	1.501	1.494	1.870
Mesenteric lymph nodes	0.260	0.256	0.306	0.598	0.876	1.220	1.616
Thymus	0.096	0.110	0.146	0.291	0.917	1.418	2.028
Pancreas	0.193	0.171	0.321	0.774	2.430	2.020	1.818
Thyroid	0.254	0.295	0.565	0.540	1.613	1.680	2.219
Adrenal gland	0.202	0.217	0.399	0.962	2.485	3.692	3.776
Hypophysis	0.222	0.232	0.324	0.505	1.445	1.904	0.585
Lung	0.462	0.604	0.778	1.119	1.212	1.288	1.626
Trachea	0.689	0.673	0.594	0.845	1.531	1.591	1.605
Eyeball	0.104	0.133	0.177	0.294	0.967	1.438	1.164
Harderian gland	0.160	0.140	0.166	0.295	1.560	4.508	4.654
Abdominal fat	0.091	0.096	0.129	0.175	0.133	0.538	1.083
Brown fat	0.270	0.273	0.321	0.390	1.035	2.060	1.933
Skin (from back)	0.491	0.699	0.557	0.531	0.821	1.112	1.707
Skeletal muscle	0.084	0.075	0.111	0.402	1.255	1.788	1.688
Bone (femoral)	0.080	0.102	0.097	0.289	0.642	0.969	1.311
Bone marrow (femoral)	0.153	0.151	0.193	0.412	1.512	2.513	2.508
Spinal cord	0.045	0.042	0.064	0.205	0.884	0.959	0.920
Cerebrum	0.014	0.016	0.041	0.145	0.594	0.632	0.647
Cerebellum	0.016	0.016	0.044	0.174	0.692	0.728	0.708
Medulla	0.018	0.020	0.047	0.188	0.745	0.805	0.827
Carcass	0.281	0.517	0.405	0.655	1.397	1.670	1.993

a Time after administration.

Protein binding effects of integrilin were examined in rat, rabbit, monkey and humans, using pooled platelet poor plasma, and 0.05, 0.5, 2, and 15 μg ^{14}C-integrilin (the steady state plasma conc). No dose-related effects on %-binding were noted in any species. The mean binding was 12.3% in rat, 14% in rabbit, 18.4% in monkey, and 24% in human plasma. Previous study in human plasma had similarly shown 30-40% of integrilin was bound to plasma proteins.

Metabolism

The metabolism of ^{14}C-integrilin was studied in rats and monkeys as indicated above (report # P-6202, and P-6203 resp). The metabolic patterns in urine and feces were determined The drug was extensively metabolized to deaminated product, as well as to 3 more polar metabolites in plasma and urine of male rats. In rat plasma, at early time period (5 min after dosing), the radioactivity consisted exclusively of unchanged integrilin, however, by 30 min total conc of integrilin was 40% and of deaminated integrilin was 30%. Additionally, 3 more polar metabolites, each representing 10% of the total radioactivity were also observed. In 24-hr rat urine, 60% of radioactivity consisted of integrilin + deaminated product, along with more polar metabolites (8-25%). In monkey plasma, at early time period (10 min after dosing), the radioactivity consisted exclusively of unchanged integrilin, however, by 60 min, total conc of integrilin decreased to 31% and total radioactivity of deaminated integrilin was 11%. Additionally, 2 more polar metabolites, representing 27% and 31% of the total radioactivity, were also observed and by 6 hrs, no radioactive peaks were detected. In male monkeys 24-hour urines showed the presence of unchanged drug, deaminated product and at least 5 more polar metabolites (5-31%).

Excretion

The excretion of ^{14}C-integrilin was studied in rats and monkeys as indicated above (report # P-6202, RT1-64C-5483, and P-6203 resp). In male albino rats, after receiving 2 mg/kg of ^{14}C-integrilin, 56% of the administered dose was recovered in urine within first 4 hours, and 5% was recovered in the feces, at 24 hours. At the end of 7 days < 1% of the dose was found in carcass. The drug was not fully recovered, due to its expulsion in CO_2. In Sprague-Dawley male rats, after 2 and 20 mg/kg (single iv dose) of ^{14}C-integrilin, at 24 hours, >90% of the radioactivity was excreted, 70-76% was seen in urine, 5-10% in feces, and 10% as CO_2. In monkeys after 2 mg/kg of ^{14}C-integrilin, 25-51% of the administered dose was recovered in urine within first 24 hours, and 6% in the feces. The radioactivity was not fully recovered again, due to its expulsion in CO_2.

The enterohepatic circulation (recipient, n=5) and biliary excretion (donor, n=4) of integrilin was studied in 2 groups of bile duct cannulated male Sprague Dawley rats. The donors received a single iv dose of 2 mg/kg of ^{14}C-SCH 60936 (integrilin), the recipient rats were given intraduodenally pooled 0-24 hour bile obtained from donor rats. Bile was obtained at intervals of 0-2, 2-4, 4-6, 6-8, 8-24, and 48 hours, and urine and feces, up to 48 hours, in both groups of animals. The drug was extensively metabolized into a number of metabolites, including the deaminated product. Within 4 hours, 21% of the radioactivity was excreted in bile, and 76% in urine, and 14% of the drug injected, underwent enterohepatic circulation, Table 9 (reproduced from volume 48, reference 10, page 6). The radioactivity in bile from donor rats was primarily the deaminated integrilin, which subsequently undergoes enterohepatic circulation. In the urine of the recipient rats, there was a further metabolism of the deaminated integrilin to more polar metabolites. In conclusion, integrilin given iv to donor rats, undergoes rapid metabolism primarily to deaminated product, which is then excreted into the bile (~21%), or eliminated by filtration via the kidney (~76%). Thus, majority of the drug derived radioactivity excreted into the bile is resorbed, and most of the resorbed radioactivity is excreted by the kidney.

In humans, there is an evidence of extra-renal clearance of integrilin, as the estimated clearance of the drug consistently exceeded normal glomerular filtration rate, and urinary recovery was below 100% of the given dose. In humans, 50% of the administered dose is excreted in urine as integrilin, and as deaminated integrilin, within 16 hours in urine.

These studies indicate that pharmacokinetics of integrilin are similar in rats, monkeys and humans.

Table 9. Total excretion of radioactivity in donor and recipient rats.

Group	Mean Percent of Dose (%CV)					
	Bile	Urine and Cage Wash	Feces	GI Contents	Carcass	Total
Donor	20.5 (51)	76.2 (13)	0.15 (93)	0.10 (29)	1.30 (58)	98.3 (3)
Recipient	0.89 (15)	38.6 (25)	15.1 (105)	8.35 (131)	8.50 (35)	71.4 (12)

TOXICOLOGY:

ACUTE TOXICITY

Following studies were submitted to amendment dated January 24, 1991, and were reviewed on 2/13/1991. These are reproduced below.

Methods: The acute toxicity of C68-22 after a single 90 min i.v (infusion) was studied in rats, rabbits and monkeys. Control animals received i.v. infusion of vehicle (placebo for C68-22, lot # A0002A). In rats and monkeys the maximum volume of injection for vehicle or the drug was 0.25 ml/kg/min., and the volume of injection in rabbits were not mentioned. For administration of lower level of doses the volume of injections were reduced accordingly. All animals were observed for the toxic signs and mortality at frequent intervals on the day of treatment and daily thereafter for 14 days. At the end of observation period, all animals were sacrificed and submitted to complete necropsy.

Results: No mortality occurred during the study period. In rats, red urine (hemoglobinuria ?) was seen during 0-2 hours after drug administration in 1/5 males and 2/5 female rats at high dose (500 mcg/kg/min for 90 min), red urine was also seen in 3/5 female rats of the control group. At necropsy, mottled kidneys (control: 2/5 males & 0/5 females; 10 mcg/kg/min: 4/5 males & 1/5 females; 50 mcg/kg/ min: 0/4 males & 1/6 females; and 500 mcg/kg/min: 0/5 males & 0/5 females) and mottled lungs (control: 0/5 males and 0/5 females and 500 mcg/kg/min: 5/5 males and 5/5 females) were observed.

In rabbits, no mortality was recorded, dose dependent thrombocytopenia was seen in female rabbits only (control: $381 \times 10^3/mm^3$, 10 mcg/kg/min: $338 \times 10^3/mm^3$, 50 mcg/kg/min.: $263 \times 10^3/mm^3$ and 500 mcg/kg/min.: $125 \times 10^3/mm^3$). However, 24 hr after the drug administration, the platelet counts recovered to some extent. Transient clinical signs such as loss of righting reflex, dyspnea, decreased muscle tone, decreased activity, ptosis, dark red urine, gel like fecal discharge, nasal discharge and abnormal stance were seen in 2/3 females at high dose levels. These signs disappeared at 24 hr after the termination of the infusion. Only at high dose, mottled lungs, pale kidneys, multiple cysts on the ovaries and tan area on left lateral liver lobe (4 cm x 2 cm) were seen at necropsies.

In monkeys, no mortality was recorded, and only petechial hemorrhages at the femoral and/or abdominal regions in 2/3 monkeys were evident at 500 mcg/kg/min, which lasted 1-3 days after the drug administration.

In the above mentioned acute toxicity studies in rats, rabbits and monkeys, sponsor did not use sufficiently high doses to elicit toxicities and mortalities. The contract labs which conducted these tests, mentioned that "500 mcg/kg/min is the maximum concentration that could be obtained with the test material as furnished."

Acute I.V. (Infusion) Toxicity Study of C68-22 in Rats, Rabbits and Monkeys

Species (Strain)	No./Sex/Group	Dose (mcg/kg/min for 90 min	LD_{50}	Highest Non-lethal Dose (mcg/kg/min)
Rats (Sprague-Dawley)	10	0, 10, 50[1], 500	ND	500
Rats (Sprague-Dawley)	5	0, 0.11, 0.55, 5.55	ND	5.55
Rabbits (Albino New Zealand White)	3	0, 10, 50, 500	ND	500
Rabbits (Albino New Zealand White)	3	0, 0.11, 0.55, 5.55	ND	5.55
Monkeys (Cynomolgus)	3[2] (males)	0, 500	ND	500

1 = This group consists of 4 male and 6 female rats.
2 = Only 1 male monkey was used in the control group.

APPEARS THIS WAY
ON ORIGINAL

Following studies were submitted to amendment dated January 24, 1991, and were reviewed on 2/13/1991. These are reproduced below.

APPEARS THIS WAY
ON ORIGINAL

SUBACUTE TOXICITY:

14-Day Continuous I.V. Toxicity Study of C68-22 in the Albino Rats
(Study # 52485)

Testing Laboratories:

Study Started: June 27, 1990

Study Completed: January 4, 1991

GLP Requirements: A statement of compliance with the GLP regulations and quality assurance unit was included.

Animals: Male (268-370 g) and female (201-261 g) Sprague-Dawley (albino) rats (11-13 weeks old)

Drug Batch No.: A0001A (2 mg/ml)

Methods: Groups of 10 male and 10 female rats were given continuous i.v. infusion (via indwelling catheter implanted in the right femoral vein) of C68-22 at 0 (placebo for C68-22, lot # A0002A), 2, 10 and 50 mcg/kg/min for 14 consecutive days. The volume of injection were fixed at 1.5 ml/kg/hour. For treating the animals at high dose (50 mcg/kg/min), undiluted drug (stock: 2 mg/kg) was used, while drug was diluted in 0.9% saline to appropriate concentrations before administration of the drug to low and mid dose group animals. Two additional groups (5/sex/group) were included in the study, one received vehicle and the other received high dose (50 mcg/kg/min) for the same length of time, and they were observed for 14 additional days after the termination of the treatment (recovery study group). All animals were observed daily for clinical signs and mortalities, body weights, food consumptions were recorded weekly. Ophthalmoscopic examinations were performed on all animals during the pretreatment period, at the end of the treatment period and at the end of the recovery period. Blood samples were collected from the orbital sinus following an overnight fasting, once during pretreatment and prior to necropsy for hematological and blood chemistry tests. Urinalysis were also done prior to necropsy. At the end of experiment all animals were sacrificed except the animals in the recovery groups, which were sacrificed at the end of 14-day recovery period. All animals were subjected to complete necropsy and histological examinations.

Results:

1. Observed Effects: No treatment related clinical signs were seen.

2. Mortality: There were two deaths (one in control group and one in mid-dose group). The control group female (#1663) was in moribund condition on day 11 and died during blood sampling while the mid-dose group female (#3562) was found dead on day 5.

3. Body Weight/Food Consumption/Water Consumption: Increased/decreased body weight gains were seen in treated animals. These effects were not dose related and considered not to be treatment related. No significant effects on food consumption were seen during the study period.

4. Hematology/Coagulation/Bone Marrow: No treatment related effects were seen.

5. Blood Chemistry/Urinalysis: No treatment related effects were seen.

6. Vital Signs/Physical Examination/Ophthalmic Examination: No treatment related effects were seen.-

7. Organ Weights: No treatment related effects were seen.

8. Gross Pathology: No treatment related effects were seen.

9. Histopathology: No treatment related effects were seen except thrombosis, proliferation of intima, and local inflammation and edema were present at the infusion sites in control as well as high-dose treated animals. Infusion sites of low and mid dose groups were not examined.

Thus the highest dose (50 mcg/kg/min for 14 days) did not produce any toxicity, and this is the maximum dose which can be delivered (1.5 ml/kg/hr) to rats from the drug supplied from the sponsor (stock concentration = 2.0 mg/ml). Sponsor did not mention any limitation on the solubility of the drug, therefore they could have used sufficient higher concentrations of the drug to elicit toxicity.

A 28-day continuous Intravenous Toxicity Study of Integrilin in the Albino Rats, with the 14-Day Recovery Period (Study # BIO 54291)

Testing Laboratories:

Study Started: February 7, 1995

Study Completed: August 24, 1995

GLP Requirement: A statement of compliance with the GLP regulations and quality assurance unit was included.

Animals: Male (218-298 g, 8-9 weeks old) and female (214-249 g, 11-12 weeks old)) Sprague-Dawley albino rats.

Drug Batch No.: D0017A (0.5 mg/ml), E0021A (2.0 mg/ml, a batch used clinically).

Methods: 4 Groups (10-15/group) of male and female rats were given continuous intravenous infusions of integrilin (by an indwelling catheter, placed in the right femoral vein) at 1, 5, and 50 μg/kg/min (or 0, 1.44, 7.20, and 72 mg/kg/day) for 28 consecutive days. 1.5 ml/kg/h of the drug was infused via an implanted catheter in the right femoral vein. A control and a high dose group consisted of 15 rats/group, the other groups had 10 animals/group. Control group received, 25 mM citrate solution, pH 5.25 (placebo). Additionally, 3 males and 3 females from the high dose group were used for toxicokinetic studies. Integrilin was available in stock solutions of 0.5 mg/ml, and 2.0 mg/ml. For 1.44 and 7.20 mg/kg/ day treated groups, 0.5 mg/ ml stock solution of integrilin was diluted with placebo, to give ratios of infusion solution of 0.04 mg/ml and 0.2 mg/ml resp. For 72 mg/kg/day, integrilin was employed, as furnished, i.e. 2 mg/ml stock solution. Dose selection was based on a previous 14-day iv toxicity study in rats, where 50 μg/kg/min produced no toxic effects in rats. The dosing solutions from the first and the last day were analyzed. All animals were sacrificed at the end of the study except the extra 5 animals in the control and a high dose group, which were kept for additional 14 days for recovery periods. Mortality and clinical signs were observed twice daily. Body weights were noted twice weekly prior to start of the study, and once weekly during the treatment/recovery periods. Food consumptions were observed weekly. Ophthalmoscopic examinations were performed on all animals prior to treatment, and before the end of the treatment and recovery periods. For hematology and clinical chemistry tests, blood was collected from the abdominal aorta of all animals prior to sacrifice, after overnight fasting. Urinalysis were done prior to treatment and before sacrifice. Bone marrow smears were prepared from each sacrificed rat, which were just saved and not examined. For toxicokinetic studies, blood (for measurement of drug conc) was collected from 3 additional groups of high dosed rats, at baseline and on days 7, 14, 21, and 28 days (time of collection was not given), however, there was loss of activity in these samples following thawing, therefore, these were not analyzed, and would be reported in future. At the end of the 28-day study, all animals were sacrificed, except the two groups which were kept for 14-day recovery periods. Gross pathology and complete necropsy with histopathological examinations were carried out on all animals.

Results:

1. Observed Effects: Incidental minor clinical signs such as scab, area of skin red or blue, fur thin cover, or mass at the femoral inguinal area were observed.

2. Mortality: One high dosed male died on the 23rd day of the study, gross pathology indicated a firm well defined mass at the injection site, microscopy indicated a severe inflammation, which may have been due to infection.

3. Body Weight/Food Consumption: The initial and final (28 day) mean body weight of control male rats was 276.1 g and 408.8 g, and of female rats was 232.7 g and 274.7 g resp. The initial and final (29 day) mean food consumption of control male rats was 28.7 g/animal/day and 26.3 g/animal/day, and of female rats was 22.8 g/animal/day and 20.6 g/animal/day resp. In treated rats, both body weights and food consumptions were unaffected.

4. Hematology/Coagulation/Bone Marrow: No significant drug related effects were noted.

5. Blood Chemistry/Urinalysis: No treatment related changes in blood chemistry or urinalysis were observed.

6. Organ Weights: No treatment related effects were noted.

7. Gross Pathology: No treatment related effects were noted.

8. Histopathology: No treatment related effects were noted.

These studies indicate that doses of integrilin, up to 50 μg/kg/min, did not produce any toxicity in rats, when given for 28 continuous days. The sponsor could have used higher amounts to determine the possible organs of toxicity. However, 50 μg/kg/min (72 mg/kg/day) of integrilin is a 'no effect dose' in rats.

A 3-Day Toxicokinetic Study of Integrilin (SCH 60936), Administered by Continuous Intravenous Infusion in Sprague-Dawley Rats: (Study # P-6166).

Methods: In sprague-Dawley rats (30/sex/dose), integrilin (lot # E0023A and E0028A) was given by a continuous iv infusion (1.5 ml/kg/hr) for 3-days, at doses of 1.44, 7.2, and 72 mg/kg/day (these doses were similar to those used in 28-day toxicity studies in rats). Blood was collected at baseline (0 time), 30 min, 2, 24, 48, 60, and 72 hours, and again on day 4, at 10, 30, 60, and 90 min after the infusion. Plasma concentration of the drug were measured '

Result: In Sprague-Dawley rats, following a continuous 3-day iv infusion of the drug, the plasma conc increased rapidly, and a steady state was reached by 30 min, the values were not different between males and females, these are shown in Table 10. The values at 1.44 mg/kg/day were too low to measure, but values from

mid and high doses, decreased one hour after ending the infusion, the dose-related increase was seen in steady state plasma conc (C_{ss}) and area under the curve (AUC, from zero to ~73 hrs). Apparent half life of the drug was ~30 min, as it was determined from the high dosed rats.

Table 10. Toxicokinetic parameters of integrilin in Sprague Dawley rats, after a continuous 3-day infusion.

Parameter	Dose (mg Integrilin™/kg/day)					
	7.20			72.0		
	Male	Female	Combined	Male	Female	Combined
Css (ng/ml)	279	154	216	2104	2438	2271
AUC_{0-73h} (μg·hr/ml)	21.4	11.3	16.4	175.6	153.2	164.4

Following studies were submitted to IND 35,465 amendment dated January 24, 1991, and were reviewed on 2/13/1991. These are reproduced below.

14-Day Continuous I.V. Toxicity Study of C68-22 in the Cynomolgus Monkey (Study # 52484)

Testing Laboratories:

Study Started: July 31, 1990

Study Completed: January 17, 1991

GLP Requirements: A statement of compliance with the GLP regulations and quality assurance unit was included.

Animals: Young adult male (3.3-5.7 kg) and female (2.4-2.6 kg) cynomolgus monkey

Drug Batch No.: A0001A (2 mg/ml)

Methods: Groups of 5 monkeys (4 males and 1 female for control, low dose and mid dose groups respectively and 3 males and 2 females for high dose group) were given continuous i.v. infusion (via indwelling catheter implanted in the right femoral vein) of C68-22 at 0 (placebo for C68-22, lot # A0002A), 1, 5 and 50 mcg/kg/min for 14 consecutive days. The

volume of injection were fixed at 1.5 ml/kg/hour. For treating the animals at high dose (50 mcg/kg/min), undiluted drug (stock: 2 mg/kg) was used, while drug was diluted in 0.9% saline to appropriate concentrations before administration of the drug to low and mid dose group animals. All animals were observed daily for clinical signs and mortalities, body weights were recorded weekly and food consumptions were recorded daily (by visual estimation). Ophthalmoscopic examinations were performed on all animals during the pretreatment period, and during the last week of treatment. ECG's and blood pressure were monitored once pretreatment and weekly thereafter. Blood samples were collected following an overnight fasting, once during pretreatment, approximately 3 hours after treatment on day 1, and on day 3, 8 and 14 day of the study for hematological and clinical chemistry tests. Urinalysis were also performed, once during pretreatment period, on day 8 of the study and just before final sacrifice. At the end of experiment all animals were sacrificed and were subjected to complete necropsy and histological examinations.

Results:

1. Observed Effects: Clinical signs were noted only in high-dose group animals such as decreased activity, excessive bleeding from nose and/or mouth (4/5), contusions (3/5), facial petechial hemorrhages and/or swelling (nose/limb) (2/5).

2. Mortality: Three out of 5 animals of high dose group died or killed in moribund condition during the study period (1 male and 2 females).

3. Body Weight/Food Consumption/Water Consumption: No significant effect on body weights were seen in the treated animals except one female (#4522) of high dose group which was sacrificed on day 8 due to poor health lost its weight by 21%. No significant effects on food consumptions were evident except 3 high-dose animals which died/killed during the study period ate significantly less food.

4. Hematology/Coagulation/Bone Marrow: At the end of 8 days of treatment, all surviving high-dose monkeys (2 males and 2 females) had severe anemia. Additionally, the two monkeys (# 4511 & # 4522) which were killed on day 8 and 10 of the study also had increased mean cell volume (MCV), reticulocytosis, erythoblostosis, polychromasia, anisocytosis and hypochromasia. These findings suggest regenerative anemia in these animals.

Effects on Hematological Parameters

Parameters	Day 8		Day 14	
	Control	High Dose	Control	High Dose*
RBC $10^6/mm^3$	5.9±0.59	2.92±1.61	5.8±0.61	3.7±0.36
Hemoglobin (g/dl)	11.0±0.61	6.0±3.27	10.7±0.67	8.0±1.70
Hematocrit (Hct %)	35.9±2.38	19.5±9.92	35.3±2.80	26.1±3.75
MCV (uM^3)	61.1±3.84	68.9±6.84	61.1±3.44	69.9±3.39
Reticulocytes %	--	13.7±13.06	--	5.3±1.56

* = mean of only 2 animals.

No significant effects on hematological parameters were evident at low and mid dose.

5. Blood Chemistry/Urinalysis: At the end of 8 days of treatment, there was a dose dependent decrease in ALT activities (control = 110 U/L, low dose = 69.0 U/L and high dose = 53.8 U/L), along with decreases of 22%, 21% and 25% in totaled protein, albumin and globulin at high dose respectively. At the end of treatment period, at which point only 2/5 animals remained in the study had significant decreases in BUN (22%), calcium (10%), ALT activities (54%), total protein (9.3%), albumin (12%), and globulin (4.7%), and increase in alkaline phosphatase activity (131%). Apart from a 34-59% decrease in ALT activities, there were no significant effects on clinical chemistry parameters at low and mid dose levels. Urinalysis were not remarkable.

6. Vital Signs/Physical Examination/Ophthalmic Examination /ECG/Blood Pressure: No treatment related effects were seen.

7. Organ Weights: The relative thyroid weights were decreased by 12%, 12% and 38% at low, mid and high dose respectively.

9. Gross Pathology: Discoloration of intestinal contents were seen in the treated animals (control = 0/5, low dose = 0/5, mid dose = 1/5 and high dose = 3/5). Additionally clot was seen in 3/5 animal of high dose group.

10. Histopathology: At high dose, focal hemorrhages were seen in the heart (2/5), lung (1/5), thymus (2/5) and subcutaneous muscle (1/1). Additionally, focal hemorrhages were also seen in kidney (1/5) and skeletal muscle (1/5) at mid dose level. No abnormalities were seen in animals treated with low dose.

Thus focal hemorrhages and excessive bleeding were seen at mid and high dose, and the "no effect dose" is 1 mcg/kg/min and 5mcg/kg/min is well tolerated dose.

A 28-day continuous Intravenous Toxicity Study of Integrilin in the Cynomolgus Monkeys, with the 14-Day Recovery Period (Study # BIO 54292)

Testing Laboratories:

Study Started: February 9, 1995

Study Completed: August 25, 1995

GLP Requirement: A statement of compliance with the GLP regulations and quality assurance unit was included.

Animals: Young adult male (2.6-4.8 Kg) and female (2.5-3.9 kg) cynomolgus monkeys.

Drug Batch No.: D0017A (0.5 mg/ml).

Methods: 4 Groups of male and female monkeys were given continuous intravenous infusions of integrilin (by an indwelling catheter, placed in the right femoral vein) at 1, 5, and 12.5 µg/kg/min (or 0, 1.44, 7.20, and 18 mg/kg/day) for 28 consecutive days. 1.5 ml/kg/h of the drug was infused via an implanted catheter in the right femoral vein. A control and a high dose group consisted of 5 monkeys/group, the other groups had 3 animals/group. Control group received, 25 mM citrate solution, pH 5.25 (placebo). Integrilin was available in stock solution of 0.5 mg/ml. For 1.44 and 7.20 mg/kg/day treated groups, 0.5 mg/ml stock solution of integrilin was diluted with placebo, to give ratios of infusion solution of 0.04 mg/ml and 0.2 mg/ml resp. For 18 mg/kg/day, integrilin was used as supplied, i.e. 0.5 mg/ml stock solution. Dose selection was based on a previous 14-day iv toxicity study in monkeys, where sponsor indicates, that 5 µg/kg/min produced no toxic effects in monkeys. The dosing solutions from the first and the last day were analyzed. All animals were sacrificed at the end of the study except the extra 2 animals in the control and a high dose group, which were kept for additional 14-days for recovery periods. Mortality and clinical signs were observed twice daily. Body weights were noted twice weekly at baseline, and once weekly during the treatment/recovery periods, food consumptions/appetence were observed weekly. Ophthalmoscopic examinations were performed on all animals prior to treatment, and before the end of the treatment and recovery periods. ECGs were obtained at baseline and on days 28, and 42. For hematology and clinical chemistry tests, blood was collected from the cephalic or saphenous vein of all animals, on days -1, 7, 14, 21, 28 and 42. Urinalysis was done prior to treatment and before sacrifice. Bone marrow smears were prepared from each sacrificed animal, but were not examined, they were not requested by the sponsor. Blood (~2 ml) for measurement of the drug conc was collected from all 4 groups of monkeys, on day 2 (~24 hrs post start of infusion), 7, 14, 21, and 28, however due to marked variability in values, due to poor sample collection, these were not analyzed, instead a separate repeat dose toxicokinetic study was initiated. At the end of the 28-day study, all animals were sacrificed, except the two groups which were kept for 14-day recovery periods. Gross pathology as well as complete necropsy with histopathological examinations were carried out on all animals.

Results:

1. Observed Effects: No drug related clinical signs were observed.

2. Mortality: No drug related mortalities were observed.

3. Body Weight/Food Consumption/Appetence: The initial and final (29 day) mean body weight of control male monkeys was 3.5 kg and 3.9 kg, and of female monkeys was 3.12 kg and 3.40 kg resp. In treated monkeys, body weights and food consumptions/appetence were all unaffected.

4. Hematology/Coagulation/Bone Marrow: No significant effects were noted, except in one male monkey, at 12.5 μg/kg/min, which showed decreased hematocrit (at 12.5 μg/kg/min 22.1%, vs control 36-41.1%), hemoglobin (at 12.5 μg/kg/min 6.8 g/dl, vs control 11.1-12.8 g/dl), RBC count (at 12.5 μg/kg/min 3.9 vs control 5.8-6.5 10^6/mm), MCV (at 12.5 μg/kg/min 57 UM^3, vs control 60 - 65.3 UM^3), and MCH (at 12.5 μg/kg/min 17.5 pg, vs control 18.7-20.3 pg), on day 28. These parameters were also altered on day 21. Mean platelet counts in males, on day 28, in controls vs at 1, 5, and 12.5 μg/kg/min were, 537±70, 438±79, 326±27, and 334±70 X $10^3/mm^3$ resp. These were significantly decreased at 5 and 12.5 μg/kg/min doses vs controls. No changes in female platelet counts were observed.

5. Blood Chemistry/Urinalysis: No treatment related changes in blood chemistry or urinalysis were observed.

6. Cardiovascular: The group mean heart rate (from ECG recordings) of high-dosed males were significantly increased compared to controls (217.0±14.0 vs 260.6±10.5 beats/min), but it was not considered to be significant by the cardiologist, as these are not uncommon in monkeys.

7. Organ Weights: No drug related effects were noted.

8. Gross Pathology: No drug related effects were noted.

9. Histopathology: None, except in one high dosed male monkey (which had shown changes in hematological parameters), had moderate hemorrhage in skeletal muscle area, along the vena cava. Sponsor suggests that it may have been due to an experimental procedure.

These studies indicate that doses of integrilin, up to 5 μg/kg/min, did not produce any toxicity in monkeys, when given for 28 continuous days. At 12.5 μg/kg/min, one male monkey had hemorrhage in the skeletal muscle, which may have been due to the pharmacological effect of the drug on platelet aggregation. 5 μg/kg/min (7.2 mg/kg/day) of integrilin is a 'no effect dose' in monkeys.

A 3-Day Toxicokinetic Study of Integrilin (SCH 60936), Administered by Continuous Intravenous Infusion in Cynomolgus Monkeys: (Report # P-6165, Study # 395372).

Methods: Integrilin (Lot # E0023A and E0028A) was given by a continuous iv infusion (1.5 ml/kg/hr, 2 monkeys/sex/dose) for 3-days to monkeys, at doses of 1.44, 7.2, and 18 mg/kg/day (these doses were similar to those used in 28-day toxicity studies in monkeys) and blood was collected at baseline (0 time), 4, 8, 24, 36, 48, 60, and 72 hours, and again on day 4, at 20, and 60 min, and 2, 4, and 8 hours after the infusion. The concentration of the drug was measured.

Results: Following a continuous iv infusion of a drug, the plasma conc increased rapidly, and a steady state was reached by 8 hour, the values were not different between males and females, and the values declined two hours after ending the infusion, Table 11. In one male monkey, the values at 1.44 mg/kg/day, were undetectable, sponsor indicates that this monkey may not have been treated with the drug. The dose-related increase was seen in mean plasma conc (C_{ss}) and $AUC_{0-73\ h}$. Apparent half life of the drug was ~1.2 to 1.5 hours, the accurate half life could not be calculated due to sparsity of samples and rapid decline.

Table 11. Toxicokinetic parameters of integrilin in Cynomolgus monkeys, after a continuous 3-day infusion.

	Dose (mg Integrilin™/kg/day)								
	1.44			7.20			18.0		
Parameter[a]	Male[b]	Female	Combined	Male	Female	Combined	Male	Female	Combined
Css (ng/ml)	99.4	91.0	93.8	528	503	516	909	1180	1050
$AUC_{0-73\ h}$ (μg·hr/ml)	7.09	6.25	6.5	37.2	32.3	34.7	65.7	83.7	74.7

a Mean pharmacokinetics parameter (n = 2/sex)
b n = 1

REPRODUCTIVE TOXICITY STUDIES

Segment I. Effects of Integrilin (Continuous IV Infusion) on Male and Female Fertility Studies in Rats
(Study # BIO 95628)

Testing Laboratories:

Study Started: March 22, 1994

Study Completed: August 24, 1995

GLP Requirement: A statement of compliance with the GLP regulations and quality assurance unit was included.

Animals: Male and female Sprague Dawley (Crl:CD(SD)BR) rats, (males 309-416 g; females 209-284 g weight, 12 to 13 weeks of age),

Drug Batch No.: D0015A, E0019A.

Methods: Four groups of 25 male and 25 female rats were given continuous iv infusion of integrilin (by an indwelling catheter, placed in the right femoral vein), at doses of 0, 1, 5, and 50 μg/kg/min (or 0, 1.44, 7.2, and 72 mg/kg body weight/day). The drug was administered, at an infusion rate of 1.5 ml/kg/h, for a period of 28 days and 14 days respectively, before the matings. The dose selection was based on the availability of the highest dose of 2.0 mg/ml stock solution in a vehicle. Five males, from each treated group and controls, were randomly chosen for the toxicokinetic studies. Males were treated during the matings, until their necropsy. Females were treated for 14 days prior to mating, throughout the mating, and during days 0 to 7 of gestation. Mated females were sacrificed on the 20th day PC clinical sign(s) were recorded prior to drug treatment, and daily during treatment, till day 20 PC. Body weights were recorded weekly in all animals and in mated females on days 0, 3, 7, 9, 12, 15, 18, and 20 PC. Food consumptions were recorded weekly before matings, and on days 0-3, 3-7, 7-9, 9-12, 12-15, 15-18, and 18-20 of gestation. Estrous cycles were assessed in each rat for 10 days prior to matings, by examining the vaginal lavages. The drug treated males and females were mated with untreated partners. The vaginal lavages in females were also checked daily during matings for the presence of sperms. The females were necropsied, the corpora lutea were counted, uterus were weighed, the number of implants, the number of dead or resorbed fetuses (F1), the number of live fetuses were examined. All fetuses were individually weighed, and externally examined. Fetuses sex was