

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: 020807

PHARMACOLOGY REVIEW(S)

NDA 20-807

REVIEW # 1

Reviewer: Tanveer Ahmad, Ph.D.
Pharmacologist, HFD-180

MAY 9 1997

Sponsor and Address: Behringwerke AG
Marburg, FRG
U.S. Agent: ClinTrial Research Inc., NC 27709

Date of Review: May 8, 1997

Date of Submission: Initial Submission: December 31, 1996
Amendment BZ: March 24, 1997
Amendment BP: March 26, 1997

Date of HFD-180 Receipt: Initial Submission: January 2, 1997
Amendment BZ: March 24, 1997
Amendment BP: March 27, 1997

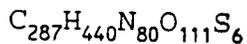
REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
(Original Summary)

Drug: Lepirudin (i.v.)

Chemical Name: r-Hirudin [leu¹,Thr²]-63-desulfohirudin

-Company Code: HBW 023 or I 88 0239

1	5	10	15
Leu Thr Tyr Thr Asp Cys Thr Glu Ser Gly Gln Asn Leu Cys Leu			
16	20	25	30
Cys Glu Gly Ser Asn Val Cys Gly Gln Gly Asn Lys Cys Ile Leu			
31	35	40	45
Gly Ser Asp Gly Glu Lys Asn Gln Cys Val Thr Gly Glu Gly Thr			
46	50	55	60
Pro Lys Pro Gln Ser His Asn Asp Gly Asp Phe Glu Glu Ile Pro			
61	65		
Glu Glu Tyr Leu Gln			



M.W. = 6979.5 daltons

Formulation: Each vial contains 50 mg lepirudin. It is to be reconstituted in water for injection or isotonic saline.

Category: Anticoagulant (Thrombin inhibitor).

Proposed Marketing Indication: Lepirudin is indicated for anticoagulation in adult patients with heparin-associated thrombocytopenia (HAT) type II and thromboembolic disease.

Dose: 0.4 mg/kg i.v. bolus followed by 0.15 mg/kg/hr i.v. infusion for 2 - 10 days or longer if clinically needed.

BEST POSSIBLE COPY

BEST POSSIBLE COPY

PRECLINICAL STUDIES AND TESTING LABORATORIES				
Type of Study	Study #	Drug Lot #	Testing Laboratories	Review Page #
Pharmacology				
Absorption: Rat, Rabbit, Dog, Pig & Monkey				4
Distribution: Rat				25
Metabolism: Rat & Monkey				40
Excretion: Rat, dog & Monkey				46
				51
Acute Toxicity				
<u>Mouse</u>				
I.V.	134-11 134.2-11 134.4-11	Ch.-B U001 Ch.-B A016 Ch.-B:616/114/011	BG BG BG	55
S.C.	134-11.1	Ch.-B C003	BG	55
<u>Rat</u>				
I.V.	134-12 134.2-12 134.2-12.1 134.4-12	Ch.-B U001 W015 A016 Ch.-B: 616/114/011	BG BG BG BG	55
S.C.	134-12.1 134.4-12.1	Ch.-B C003 Ch.-B: 616/114/01	BG	55
<u>Monkey</u>				
I.V.	134.4-17.1	Ch.-B: 616/114/011	BG	55
Subacute/Subchronic Toxicity:				
<u>Rat</u>				
3-Days (i.v. bolus + i.v. infusion)	11588TSR	Ch.-B: 616/114/011		57
1-Month (i.v.)	88.1094	U001		60
13-Week (i.v.)	134.4-52	115011		62
1-Month (s.c.)	134.4-42	118011	BG	64
3-Month (s.c.)	90.0391 (89.0648)	C003		66
<u>Monkey</u>				
3-Days (i.v. bolus + i.v. infusion)	11589TSP	616114011		69
15-Day (i.v. bolus + i.v. infusion)	13326TSP	115011	BG	72
1-Month (i.v.)	2549-100	U002		75
13-Week (i.v.)	12507-TCF	115011		78
3-Month (s.c.)	90.0762 (89-0539)	C003/009	PR	86

Reproductive Toxicity Studies:				
Fertility & Reproductive Performance (Segment I)				
Rat (i.v.)	12505RSR	115011		91
Teratology (Segment II)				
Rat (i.v.)	9583RSR	20		93
Rabbit (i.v.)	9584RSL	20/19		98
Perinatal/Postnatal (Segment II/III)				
Rat (i.v.)	12506RSR	115011		102
Mutagenicity:				
Ames Test	88.1571 (88.1077) 95.0266 (95.0051)	U001 116001	PR RR	104
In Vitro UDS Test in an A549 Mammalian Cell Line	88.2085	U001	PR	105
In Vitro Chromosomal Aberration Test in V79 Chinese Hamster Cells	89.1239 (90.0325) 95.0266	C004 116001	PR PR	106
HGPRT Forward Gene Mutation Assay in V79 Cells	95.0050	116001	PR	107
Mouse Micronucleus Test	95.0250	116001	PR	108
Special Toxicity Studies:				
Local Tolerance Studies in Rabbit After I.V., I.A., P.V. and S.C. Dose	134-24, 134.2-24, 134.24.1, 134.24.2, 134.2-24.3, 134.2-24.1, 134.2-24.2, 134.4-24, 134.4-24.1, 134.4-24.2, 134.4-24.3	#13, #Ch.-BU001, #Ch.-BU003, #C005, 114011	BG	109
Antigenicity Studies:				
Guinea Pigs	134-03, 134-03.2, 134.2-03, 134-03.1, 134.4-09	U001, A016 118011	BG	113
Rabbits	134-04	Ch.-B.24	BG	116
Monkeys	134.4-07.1	118011	BG	118
Dermal Sensitization Test	95.0151	118011	PR	124
Allergic Reaction to Yeast-Derived Contaminating Proteins From r-hirudin	P-248	--	BG	125
Antigenicity of the Yeast Protein T3329 in Sheep	9725	--	PR	126

BG = Behringwerke AG, Germany

PR = Pharma Research, Hoechst, Frankfurt, Germany

Most of the above mentioned studies were submitted under and were reviewed (dates of reviews: September 25, 1995 and December 27, 1996). These reviews are incorporated below. Additional studies included in this NDA are reviewed. These are: (1) miscellaneous pharmacology studies, (2) 1-month s.c. and 13-week i.v. toxicity studies in rats, (3) Segment I. fertility and general reproductive performance study in rats, (4) Segment II/III perinatal/postnatal study in rats and (5) genotoxicity studies: V79/HGPRT forward gene mutation assay and mouse micronucleus test, and special toxicity studies: local tolerance studies in rabbits, dermal sensitization test and antigenicity studies in guinea pigs.

PHARMACOLOGY:

**APPEARS THIS WAY
ON ORIGINAL**

Primary Pharmacology:

HBW 023 (r-Hirudin) is the recombinant Hirudin isoform [Leu1,Thr2]-63-desulfohirudin from yeast. It differs biochemically from natural hirudin isoforms in that the first and second N-terminal amino acids are leucine and threonine instead of either valine, valine or isoleucine and threonine. Finally, r-Hirudin differs from natural hirudin in that it is not sulfated on Tyr-63. R-Hirudin, like natural hirudin non-covalently binds to and inactivates the enzymatic activity of thrombin (namely the cleavage of fibrinogen to fibrin), with an inhibition constant (Ki) of 123 fM. Since the cleavage of fibrinogen to fibrin is the final step in clot formation, common to both intrinsic and extrinsic pathways of coagulation, r-Hirudin can cause prolongation of all of the tests for hemostasis [i.e. TT (thrombin time), PT (prothrombin time), APTT (activated partial thromboplastin time), PTT (partial thromboplastin time), TPT (thromboplastin time) and bleeding time.

**APPEARS THIS WAY
ON ORIGINAL**

In Vitro Effects:**In Vitro Effects of r-Hirudin in Blood from Dogs and Human:**

In in vitro studies the effects of r-Hirudin (0.0001 mg/ml to 1.0 mg/ml) hematological parameters, coagulation, and fibrinolysis were examined using citrated blood samples from human and dog. At doses \geq 0.001 mg/ml, r-Hirudin produced extreme prolongation of thrombin times, marked prolongation of prothrombin times; marked reductions in Quick values, marked increases in times of thromboelastography (TEG; TEG r = reaction time until start of clot, TEG k = clot forming time) and marked reductions in thrombus stability (no coagulation; TEG maximum amplitude = clot stability). However, r-hirudin had no adverse effects on blood cell numbers, (leukocytes, erythrocytes, or platelets), hematocrit, hemoglobin, mean packed cell volume, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), or collagen-induced platelet aggregation.

In Vitro Evaluation of the Potency of r-Hirudin as Compared with Other Anticoagulants.

In vitro studies evaluated the inhibitory potency of r-Hirudin (Hirudin-Hoe) (effects on TT, APTT, and PT) using normal and antithrombin III deficient human plasma and compared its potency to antithrombotic agents such as antithrombin III, heparin, LMW-heparin, synthetic thrombin inhibitors, and thromboplastin inhibitor. Tables 1 (A and B; succeeding page), show the concentrations of r-hirudin needed to produced a doubling of coagulation times for TT, APTT, and PT as compared to other anticoagulants.

APPEARS THIS WAY
ON ORIGINAL

Briefly, the data in Tables 1 (A and B) show that Hirudin Hoe (HBW 023) and the other two hirudin analogs behaved similarly in all three tests (TT, APTT, and PT). In addition, comparisons made on a μM basis showed that hirudin appeared to be the most active antithrombotic agent tested, with the exception of the APTT test, where heparin at concentrations $> 0.1 \mu\text{M}$ was more potent than Hirudin. Finally, Table 1 shows that the concentrations of hirudin needed to double the coagulation times in AT-III-depleted plasma was essentially the same as those needed in standard human plasma, indicating that unlike heparin, hirudin does not require the presence of a cofactor.

Table 1 Antithrombotic Concentrations Leading to a Doubling of the Coagulation Times.

APPEARS THIS WAY
ON ORIGINAL

A: STANDARD HUMAN PLASMA

Antithrombotic	TT		APTT		PT	
	$\mu\text{g/ml}$	μMol	$\mu\text{g/ml}$	μMol	$\mu\text{g/ml}$	μMol
Hirudin Hoe (r-Hirudin, HBW 023)	0.29	0.04	1.16	0.17	6.06	0.88
Val-Val-Hir	0.23	0.03	1.02	0.15	8.29	1.20
Ile-Thr-Hir	0.29	0.04	1.10	0.16	9.40	1.36
Heparin	0.92	0.06	1.99	0.13	26.04	1.74
LMW-Heparin	2.43	0.49	5.48	1.10	494	98.86
AT-III	1122	17	10844	166	19117	294
AT-III-Heparin	4.76	0.07	10.81	0.17	128.67	1.98
TPI	41545	1298	148	4.61	422	14.06
Foy	64.63	155	188	452	832	1992
MD-805	0.08	0.15	1.08	2.06	1.65	3.14
STI	1.15	2.45	28.71	60.83	48.72	103

B: AT-III-DEFICIENT PLASMA

Antithrombotic	TT		APTT		PT	
	µg/ml	µMol	µg/ml	µMol	µg/ml	µMol
Hirudin Hoe (r-Hirudin, HBW 023)	0.23	0.03	0.88	0.13	5.16	0.75
Val-Val-Hir	0.23	0.03	1.34	0.19	6.97	1.01
Ile-Thr-Hir	0.36	0.05	1.06	0.15	7.74	1.12
Heparin	3.39	0.23	18.11	1.21	819	54.63
LMW-Heparin	299	59.89	46.36	9.27	1919	384
AT-III	1403	21.59	12474	192	14785	227
AT-III-Heparin	11.53	0.18	17.16	0.26	127	1.95
SIT	1.11	2.35	32.76	69.41	40.16	85.08

Val-Val-Hir and Ile-Thr-Hir = Hirudin analogs; LMW-Heparin = Low Molecular Weight Heparin; AT-III = Antithrombin III; TPI = Thromboplastin Inhibitor; MD 805, Foy, and STI BW = synthetic thrombin inhibitors;

**APPEARS THIS WAY
ON ORIGINAL**

Other in vitro studies compared the inhibitory effects of heparin versus r-hirudin (at equivalent antithrombin units) on the activity of thrombin bound to fibrin polystyrene beads and on thrombolysis-induced coagulation activation. In the studies using thrombin bound to fibrin polystyrene beads, r-hirudin produced a dose-dependent inhibition of the activity of thrombin (up to approximately 70 %), whereas equivalent doses of heparin produced less than 25% inhibition of bound thrombin activity. In studies using standardized blood clots suspended in normal plasma, hirudin abolished both the activation of coagulation induced by clots both before and after thrombolysis with r-tissue plasminogen activating factor as well as the formation of soluble fibrin. In comparison, heparin, at equivalent thrombolytic concentrations was less potent in inhibiting thrombin activity and still resulted in the formation of fibrinopeptide A and soluble fibrin at doses up to 10 U/ml. Thus, comparisons made at equivalent anti thrombin units, suggested r-Hirudin may be more suitable than heparin for preventing re-thrombosis which occurs after thrombolytic therapy.

Ex Vivo Effects on Coagulation Parameters and/or Bleeding Times:

Rats: In rats the ex vivo effects of r-hirudin on blood coagulation and/or bleeding times were investigated following intravenous (i.v.) subcutaneous (s.c.), oral (p.o.) and nasal administration.

In an i.v. study in rats (n=5/group), r-hirudin produced pronounced dose-dependent prolongation of TT, with a threshold dose of 0.0625 mg/kg (lasting for 30 min) and maximal effects (i.e. prolongation > 300 sec) observed at doses \geq 0.125 mg/kg (lasting up to 3 hours at the 10 mg/kg dose). In comparison, only slight dose-dependent prolongation of PTT, was observed over a dose range of 0.0625 to 10 mg/kg. Maximal increases in PTT up to 38.3 sec (relative to a control value of 16.5 sec) lasted for up to 2 hours after dosing at the 10 mg/kg dose.

In two other i.v. studies in anesthetized rats, the effects of r-hirudin on mesenteric arterial or venous bleeding times were evaluated. In the arterial study, i.v. infusion of r-hirudin at a dose rate of 10 and 20 and μ g/kg/min for 1 h had no effects on mesenteric arterial bleeding times. At doses of 40 and 80 μ g/kg/min, r-hirudin produced significant prolongation of bleeding times (46 and 114%, relative to control times of 142 sec, respectively).

In the venous bleeding time study, following a single i.v. bolus dose of 3 mg/kg, r-hirudin produced increased mean bleeding times (up to 224, 140, 108, 121, and 154 sec measured at 15, 30, 60, 120, and 240 min after dosing, respectively). In comparison, bleeding times for control animals ranged from 108 to 116 sec. The nature of the moderate prolongation in bleeding time at the 240 min time point was not apparent.

In a s.c. study in rats (n=5/group), r-hirudin produced pronounced dose-dependent prolongation of TT, with a threshold dose of 0.0625 mg/kg and maximal effects (i.e. prolongation > 300 sec) observed at doses \geq 0.25 mg/kg and lasting from 2 hr at the 0.065 mg/kg dose up to 5 hours at the 10 mg/kg dose. In comparison, only moderate dose-dependent prolongation of PTT, was observed over a dose range of 0.0625 to 10 mg/kg. Maximal increases in PTT up to 41.1 sec (relative to a control value of 16.5 sec) lasted for up to 4 hours after dosing at the 10 mg/kg dose. TT and PTT prolongation following s.c. dosing was increased by a factor of 2-4 relative to that reported following i.v. dosing in rats.

In an oral study in rats (n=5/group), administration of r-hirudin by gavage at a dose of 10 mg/kg had no effects on either TT or PTT.

Finally, ex vivo studies in rats showed that nasally administered r-hirudin at a dose of 1 mg/kg in rats, produced a pronounced increase in TT

which lasted for up to 1-hour, but only slight increases in PTT at 15 and 30 min after dosing. Increases in TT were correlated with r-hirudin plasma concentrations of 153 and 118 ng/ml at 15 and 30 min after nasal administration in rats.

Rabbits: In rabbits, single i.v. bolus injections of r-hirudin at doses of 0.1 and 1.0 mg/kg produced small prolongations of bleeding times at both doses (up to 62 and 60 sec, relative to control bleeding times of 31 and 38 sec) at 30 and 10 min after administration, respectively. At the said doses r-hirudin also produced dose-dependent prolonged coagulation times (from control values of 14.7 min up to 74.08 min and ≥ 120 min at 10 min after dosing for the 0.1 and 1.0 mg/kg doses, respectively). Finally, the thromboelastography analysis showed that i.v. doses of r-hirudin produced a clear dose-dependent retardation of thrombus formation (i.e. prolonged TEG r-time 46 and 156% and prolonged TEG r+k-time 52 and 143% at 10 min after dosing at the 0.1 and 1.0 mg/kg doses, respectively), but had no effects on stability of thrombus formation (i.e. no effect on TEG maximal amplitude). Effects on coagulation parameters were prolonged for 2-4 hours, whereas effects on bleeding time, which returned to normal by 60 min post dosing.

Dogs: Ex vivo studies in dogs (n =3/dose level) investigated the effects of r-hirudin on coagulation parameters [TT, PTT, thromboplastin time (TPT), and thromboelastography, (TEG; TEG r = reaction time until start of clot, TEG k = clot forming time, TEG maximum amplitude = clot stability) following administration as a single i.v. bolus dose or as a single subcutaneous dose.

In the i.v. bolus dose study in dogs (n=3/dose), r-hirudin produced dose-dependent prolongation of TT (from control values the 0.031 and 0.5 mg/kg doses, respectively). Effects on TT lasted from 30 min up to 2 hours after dosing at the 0.031 and 0.5 mg/kg doses, respectively. In contrast, i.v. doses up to 0.5 mg/kg produced only slight increases in PTT (27%) and TEG (from TEG r and TEG k times of 10.3 5.7 min, up to 17.0 and 8.0 min, respectively) and had no effects on TPT.

In the s.c. dose study in dogs (n=3/dose), r-hirudin (0.125 and 0.5 mg/kg) produced a pronounced dose-dependent prolongation of TT

Effects on TT lasted from 4 hours up to 6 hours after dosing at the 0.031 and 0.5 mg/kg doses, respectively (markedly longer than the 2 hour response period observed following i.v. bolus dosing at the 0.5 mg/kg dose in dogs). PTT, TEG and TPT were not changed at the s.c. doses tested.

Monkeys: In ex vivo studies in rhesus monkeys (n=2 or 3 monkeys/dose), the effects of r-hirudin, administered as single i.v. injections, at doses of 0.031, 0.062, 0.125, 1.0, and 10.0 mg/kg, on blood coagulation parameters [TT, PTT, thromboplastin time (TPT), and thromboelastography, (TEG)] and bleeding times were investigated. At the said doses, r-hirudin produced dose-dependent increase in TT (from control values of 20.6-26 min up to 75 min at the 0.031 mg/kg dose and > 300 min at doses \geq 0.125 mg/kg, respectively). TT prolongation lasted up to 30 min at the 0.031 mg/kg dose and up to 8 hours at the 10 mg/kg dose. Less pronounced prolongation of PTT was observed and only slight increases in TPT were observed at the 0.5 and 10 mg/kg doses. Thromboelastography results showed increased TEG r (reaction time until start of clot) at doses \geq 0.5 mg/kg and increased TEG k (clot forming time) only at the 10 mg/kg high dose. However, at the 0.5 and 10 mg/kg i.v. doses, r-hirudin had no effects on bleeding times in monkeys.

In another ex vivo study in monkeys, the effects of r-hirudin on blood coagulation parameters were assessed following single subcutaneous administration at doses of 0.1 and 0.5 mg/kg. At the said doses, R-hirudin produced distinct and dose-dependent prolongation of thrombin time (up to >300 sec) and moderate prolongation of PTT (increase relative to mean control values of 30.4 sec), but only slight effects on TPT (increase relative to mean control values of 10.9 sec), respectively. Finally, the 0.1 and the 0.5 mg/kg s.c. doses produced slight effects on effects on thromboelastography parameters (TEG r increased TEG k increased from mean of 2.5 min up to 4 min), but had no effect on TEG maximum voltage (i.e. thrombus stability).

APPEARS THIS WAY
ON ORIGINAL

Interactions of r-hirudin, _____, and prothrombin on bleeding time in rats

Urethane-anesthetized rats were administered a single bolus injection of r-hirudin into the tail vein. One minute later, either _____, prothrombin, or a _____ was intravenously administered via the tail vein. The abdomen was opened, small veins were punctured, and bleeding times were recorded.

As shown in the following table, intravenously administered r-hirudin increased mean and median bleeding times in rats. The factor VIII product _____ produced a treatment-related inhibition of r-hirudin-induced increases in mean and median bleeding times. Prothrombin produced a dose-related inhibition of r-hirudin-induced increases in mean and median bleeding times. At the doses studied, the inhibitory effect of the _____ on r-hirudin-induced increases in bleeding times was not additive. Thus, either _____ or prothrombin might be an effective antidote for inadvertent r-hirudin overdosing.

Interactions of r-hirudin, _____ and prothrombin on mean
and median bleeding times in rats

Group No.	Treatment	Mean bleeding time (min)	Median bleeding time (min)
1	r-Hirudin (30 mg/kg, i.v.) + isotonic saline	652	645
2	r-Hirudin (30 mg/kg, i.v.) + FII/VIII Buffer	729	751
3	r-Hirudin (30 mg/kg, i.v.) + Beriate HS [®] (2.0 U)	493	544
4	r-Hirudin (30 mg/kg, i.v.) + Beriate HS [®] (250.0 U)	498	394
5	r-Hirudin (30 mg/kg, i.v.) + Prothrombin (1.0 U)	617	675
6	r-Hirudin (30 mg/kg, i.v.) + Prothrombin (5.0 U)	347	271
7	r-Hirudin (30 mg/kg, i.v.) + Prothrombin (25.0 U)	235	231
8	r-Hirudin (30 mg/kg, i.v.) + Prothrombin (250.0 U)	299	191
9	r-Hirudin (30 mg/kg, i.v.) + Beriate HS [®] (250.0 U) + Prothrombin (250.0 U)	239	219

APPEARS THIS WAY
ON ORIGINAL

In Vivo Effects of r-Hirudin in Various Models of Thrombosis:

Wire Coil Thrombus Model in Rats:

In vivo studies in rats examined the effects of r-hirudin on early stage thrombus formation induced by insertion of a stainless steel wire coil into the vena cava. Studies in this model showed that r-hirudin (injected intravenously, shortly after insertion of the wire) produced a dose-dependent inhibition of venous thrombus formation, with an ED₅₀ dose of 0.17 mg/kg (corresponding to 2022 anti thrombin units/kg).

In this same model heparin also produced a dose dependent inhibition of thrombus size, with an ED₅₀ of 163 IU and a duration of action of 1 hr. A separate examination of the time course for the antithrombotic effects r-hirudin (0.25 mg/kg, i.v.) in this model showed that the antithrombotic effects of r-hirudin were time dependent with a duration of action of approximately 60 min. This latter study also showed that r-hirudin at the 0.25 mg/kg i.v. dose resulted in increased mesenteric arterial bleeding times which only lasted for only 20-30 min. Other studies on the duration of r-hirudin's anti-thrombotic effects in the wire coil rat thrombosis model showed that at i.v. bolus doses of 0.3, 1.0, and 3.0 mg/kg, r-hirudin (administered 5 min after thrombus induction) resulted in inhibitory effects which lasted up to 4 hours at the 0.3 mg/kg dose and up to 48 hours at the 1.0 and 3.0 mg/kg doses.

In still other studies using the rat wire coil thrombus model, r-hirudin, administered subcutaneously at 30 min prior to insertion of the wire, also inhibited thrombus formation with an ED₅₀ of 0.17 mg/kg and complete inhibition observed in 5 of 8 rats tested at a s.c. dose of 3 mg/kg. Finally, studies on the duration of action of a 1 mg/kg s.c. dose of r-hirudin indicated that antithrombotic effects were evident following dosing up to 6, but not 8 hours prior to thrombus induction.

Laser-Induced Thrombus Model in Rats: In another rat thrombus model, the effects of r-hirudin on the induction of thrombus formation via 1/30 sec single laser shots in rat mesenteric arterioles or venules were assessed. The number of laser shots needed to induce thrombus formation was used to gauge the effects on thrombus formation. In this latter model, subcutaneous administration of r-hirudin (10 and 30 mg/kg), at 1 hour prior to thrombus induction, produced significant inhibition of the laser-induced thrombus formation (increase in the number of laser shots needed), whereas lower s.c. doses (0.5, 1.0, or 3.0 mg/kg) had no significant effects. In addition, r-hirudin produced significant inhibition of the laser-induced thrombus formation following 60 min of i.v. infusion at doses of 40 and 80 µg/kg/min (48 and 42% increase in the number of laser shots needed), with no significant inhibitory effects observed at lower doses (10 and 20 µg/kg/min). For comparison, R-hirudin's said antithrombotic effects were analogous to those seen with clinically relevant concentrations of Heparin-Na (660 and 3300 IU/kg s.c. or i.v. infusion of 25 and 50 IU/kg/min for 60 min).

APPEARS THIS WAY
ON ORIGINAL

Effects of r-Hirudin and Heparin on Induced Pulmonary Platelet Aggregation in Rats: The antiplatelet aggregatory effects of r-hirudin (1 and 5 mg/kg, s.c.) and that of standard heparin (25 IU/kg, i.v.) were evaluated in a rat model of pulmonary platelet aggregation, wherein aggregation of [⁵¹Cr]-labelled platelets occurs in the thoracic cavity subsequent to thrombin injection (50 IU/kg, i.v.). In this model s.c. injection of r-hirudin at the 1 and 5 mg/kg doses (1 hour prior to thrombin injection) resulted in dose-dependent inhibition of thrombin-induced pulmonary platelet aggregation (30 and 64%, respectively). In comparison, the 25 IU/kg heparin dose also produced a 42% inhibition of platelet aggregation induced by thrombin.

APPEARS THIS WAY
ON ORIGINAL

Stenosis-Induced Thrombosis Model in Rabbits: In this platelet-dependent model, thrombosis was induced in the jugular vein and carotid artery by damaging the endothelium and constricting the vessel. Intravenous bolus injection of r-hirudin (10 min prior to thrombus induction) at doses 0.6, 1.2, and 2.0 mg/kg produced a dose dependent inhibition of thrombosis formation in both the jugular veins and carotid arteries. The 0.6 mg/kg dose of r-hirudin resulted in a 50 and 38% reduction in the incidence of thrombosis in arteries and veins, respectively, whereas the 2.0 mg/kg dose completely inhibited arterial thrombus formation and resulted in 75% inhibition of venous thrombus formation. ED₅₀ values for inhibition of the incidence of thrombus formation were 0.7 and 0.97 mg/kg in arteries and veins, respectively. In comparison, doses of Heparin-Na required to produced significant inhibition of thrombus formation were 100 IU/kg in veins and 300 IU/kg in arteries.

In this same model, r-hirudin's effects on carotid arterial thrombosis formation was evaluated following i.v. bolus injection (femoral vein) at doses ranging from administered 1 min prior to induction of thrombus formation. In this, like the previous study, r-hirudin produced a dose-dependent inhibition of thrombus formation (ED50 dose = 0.03 mg/kg) compared to saline treated control rabbits.

Finally, using this rabbit model, both the potency and the duration of r-hirudin's antithrombotic effects in both jugular veins and carotid arteries were assessed following subcutaneous injection. For the potency portion of the study, r-hirudin was injected subcutaneously at dose levels of 3, 5, and 10 mg/kg at 30 min prior to induction of thrombosis (vascular damage and stenosis). For the duration of effect portion of the study, r-hirudin, at a dose level of 10 mg/kg, was injected subcutaneously at 3, 4, 6, and 8 hours prior to thrombus induction. In the potency portion of the study, r-hirudin produced a dose dependent inhibition of thrombus formation in both arteries and veins up to 88% at the 10 mg/kg dose (ED₅₀ values for inhibition of the thrombosis incidence were = 3.9 mg/kg in carotid arteries and 4.8 mg/kg in jugular veins). Plasma

levels of r-hirudin measured at 30 min following s.c. injection at doses of 3, 5, and 10 mg/kg were 1.05 ± 0.15 , 1.73 ± 0.14 , 3.29 ± 0.20 $\mu\text{g/ml}$. In the duration portion of the study, r-Hirudin (10 mg/kg, s.c.) significantly inhibited the incidence of thrombus formation when injected up to 6 hours prior to thrombus induction in arteries and up to 4 hours in veins. Plasma levels of r-hirudin at 2, 4, 6, and 8 hours following s.c. injection of the 10 mg/kg dose averaged 6.2 ± 0.4 , 2.5 ± 0.1 , 1.7 ± 0.4 , and 0.3 ± 0.06 , $\mu\text{g/ml}$. The aforementioned plasma levels were all ≥ 0.3 $\mu\text{g/ml}$, which prolongs thrombin clotting time to > 300 sec, pooled rabbit plasma.

APPEARS THIS WAY
ON ORIGINAL

Thrombin-Induced Thrombosis Model in Rabbits: In this rabbit model, thrombosis was induced by injection of 2 IU of thrombin in to the blood column of clamped segments of rabbit jugular vein. The effects of r-hirudin on thrombosis formation and/or thrombolysis was evaluated following i.v. injection at a dose of 0.1 mg/kg administered 15 or 60 min prior to thrombosis formation (thrombin injection) or at i.v. doses of 0.1 and 1.0 mg/kg administered at 15 min after thrombosis formation. R-hirudin (0.1 mg/kg, i.v.) completely inhibited thrombus formation when administered at 15 and 60 min prior to thrombin injection. When administered 15 min after thrombus induction, r-hirudin (0.1 mg/kg, i.v.) resulted in thrombolysis in 2 of the 5 animals tested after 136 and 234 min, whereas the 1.0 mg/kg i.v. dose produced thrombolysis in 4 of 5 animals tested, after 105 to 143 min. In comparison, fibrinolytics such as tPA and urokinase normally lead to thrombolysis after approximately 15 min. Thus, while r-hirudin is effective in preventing thrombus formation, it has only weak thrombolytic activity in this model.

Comparison of r-Hirudin and Standard Heparin in Preventing Thrombus Formation in a Wessler Rabbit Model.

The in vivo antithrombotic effects of r-Hirudin (12.5 to 400 $\mu\text{g/kg}$) given as an i.v. bolus at 5 min prior to induction of thrombosis were compared with that of standard heparin (90 $\mu\text{g/kg}$) using the Wessler rabbit model of thrombosis, wherein clot formation in ligated sections of Jugular veins is induced by i.v. injection of human serum into the marginal ear. R-hirudin produced a dose-dependent inhibition of clot formation in the right jugular vein (ID_{50} values of 200 $\mu\text{g/kg}$). Plasma IC_{50} concentrations for inhibition of clot formation were 0.917 $\mu\text{g/ml}$ in the right jugular vein and 6.032 $\mu\text{g/ml}$ in the left jugular vein. R-Hirudin doses which were equivalent to the ID_{50} dose of 90 $\mu\text{g/kg}$ for standard heparin in the Wessler rabbit model, were 110 $\mu\text{g/kg}$, 150 $\mu\text{g/kg}$, and 170 $\mu\text{g/kg}$, based on thrombus size, thrombus weights, and protein dosage of the clots as end points, respectively. Finally, the plasma concentrations of r-Hirudin which inhibited clot formation to a degree equivalent to that induced by a 90 $\mu\text{g/kg}$ dose of standard heparin were 0.261 $\mu\text{g/ml}$ using clot score (0-4, 0 being no clot and 4 being occlusive thrombosis) as the endpoint, 0.501 $\mu\text{g/ml}$ using fresh or retracted thrombus weights as an endpoint and 0.705 $\mu\text{g/ml}$ using protein content of the clot as an endpoint.

BEST POSSIBLE COPY

BEST POSSIBLE COPY

Antidote Testing for Antithrombotic Effects of r-Hirudin

Rats: Tests in rats investigated the potential of the commercially available activated prothrombin complex concentrate (APC) to antagonize the antithrombotic effects of r-hirudin in terms of its effects on r-hirudin's induction of increased venous bleeding times in small mesenteric veins following needle puncture. In this experiment, i.v. bolus injection of r-hirudin at doses of 3.0 and 30 mg/kg increased venous bleeding times from control values of respectively. In turn, APC administered at doses of 6, 18, 54 and 180 U/kg at 1 min after the 3.0 mg/kg dose of r-hirudin effectively neutralized the r-hirudin induced increased venous bleeding times. Likewise, APC at concentrations of 20 and 60 U/kg dose-dependently reduced bleeding times induced by a 30 mg/kg i.v. dose of r-hirudin from 575 sec down to values of 352 sec and 222 sec, respectively. These data suggest that APC can neutralize the antithrombotic effects of r-hirudin in the rat.

Rabbits: Tests in rabbits investigated the potential of the commercially available activated prothrombin complex concentrate (APC) at doses of 0.1, 0.4, 1.0, 2.5, 7.5, or 15 U/kg to antagonize the antithrombotic effects of r-hirudin, administered via i.v. infusion at doses of 0.1, 0.3, 1.0, and 3.0 mg/kg over a 1 hour period. At 5 min after the end of each r-hirudin infusion, three separate doses of APC (administered as a bolus i.v. injection) were tested for their ability to neutralize the antithrombotic effects of r-hirudin (gauged as effects on coagulation times and thrombin times). In this study, r-hirudin produced dose-dependent increases in coagulation times up to 3 hours of more at the 1.0 and 3.0 doses. Addition of APC effectively neutralized the prolongation of coagulation times within 5 min following administration. APC doses and % reductions in the r-hirudin induced increases in coagulation times are provided in Table 2, on the following page.

APPEARS THIS WAY
ON ORIGINAL

APPEARS THIS WAY
ON ORIGINAL

BEST POSSIBLE COPY

Table 2. Activated Prothrombin Complex (APC) Doses which Effectively Neutralized the Effects of r-Hirudin on Coagulation Time (measured at 5 min after administration of APC)

APPEARS THIS WAY
ON ORIGINAL

r-Hirudin (mg/kg)	Coagulation Times with r-Hirudin (sec)	Effective APC Doses (U/kg)	% Reduction in Coagulation Times
0.1	3227 ± 351	0.4	32
		1.0	45
0.3	4576 ± 1796	1.0	53
		2.5	54
		5.0	69
1.0	10964 ± 6800	1.0	65
		2.5	79
		5.0	83
3.0	>18000	3.0	68
		7.5	87
		15.0	88

BEST POSSIBLE COPY

The data in Table 2 show that APC was an effective antidote to r-hirudin when gauged in terms of its effects on coagulation times. In contrast, APC showed no evidence of reversal of r-hirudin's prolongation of thrombin times. Thus, effects on coagulation times would be the best index to gauge the effects of APC in reversing the effects of r-Hirudin.

In a second study in rabbits, the long term effects of antagonism of r-hirudin with APC on the numbers of erythrocytes, leukocytes, and thrombocytes, clotting time, mean corpuscular value of erythrocytes (MCV) and hematocrit were investigated. In this study, r-Hirudin (0.3 mg/kg) was infused over one 1 hour followed by bolus injection of APC (2.5 U/kg) at 5 min after the end of the r-hirudin infusion. At the end of the r-hirudin infusion coagulation times were prolonged from the control range of approximately 30 min to 175 min. APC reversed the prolonged coagulation times, within 5 min after bolus injection, with coagulation times remaining within the normal range to the end of the 72 hour observation period. No effects on any of the hematological parameters examined were observed and macroscopic and histological examinations showed no formation of blood clots in the vessels or capillaries of heart, kidney, or lungs. Thus, these results suggest that the anticoagulant effects of r-hirudin can be fully antagonized with APC without the occurrence of adverse events in rabbits.

SECONDARY PHARMACOLOGY:

APPEARS THIS WAY
ON ORIGINALIn Vitro Studies:

In vitro pharmacological effects of r-hirudin on smooth and/or cardiac muscle contraction were studied using various tissues isolated from the guinea pig and rats. Results from the studies using isolated guinea pig tissues showed that in isolated guinea pig ileum, r-hirudin at concentrations of 10^{-5} M had no spasmolytic effects on the contraction induced by carbachol (4×10^{-8} g/ml); histamine (2×10^{-7} g/ml) and BaCl. Studies using isolated guinea pig atria also showed that r-hirudin had no effects on either the force or rate of the spontaneous atrial contraction. Finally in studies using isolated guinea pig trachea, r-hirudin produced no relaxant effects.

Studies which used isolated tissues from rats showed the following: r-hirudin (10^{-5} g/ml) had no antagonistic effects on either oxytocin (3 mU/ml)-induced contractions in isolated rat uterus or on norepinephrine (2×10^{-6} g/ml)-induced contractions in isolated rat vas deferens, whereas positive controls epinephrine and phentolamine (10^{-7} g/ml) effectively antagonized (66% and 75%) the induced contraction in each preparation, respectively.

In Vivo/Ex Vivo Effects:APPEARS THIS WAY
ON ORIGINAL

Safety Assessment of r-Hirudin (i.v. Bolus) in Dogs: The safety of r-hirudin (single i.v. bolus doses of 0, 0.1, 1.0 and 10.0 mg/kg) was assessed in beagle dogs (1/sex) in terms of its effects on body weights, clinical observations, hematology, coagulation/fibrinolysis parameters and clinical chemistry, during a 5 day observation period following dosing. R-hirudin produced no clinical signs of toxicity and had no effects on body weights, hematology, or clinical chemistry at any dose tested, with the exception of a dose-dependent reduction in platelet aggregation capacity and higher urea values (the latter also in one control dog) at the 1 and 10 mg/kg dose. The main effects of r-hirudin were marked dose-dependent effects on coagulation parameters being most prevalent at the 1 and 10 mg/kg doses of r-hirudin and generally lasting from 60 to 120 min after dosing. In this regard, r-hirudin produce and prolonged thrombin times, prolonged PTT (1 and 10 mg/kg; 21 to 240 sec), prolonged thromboelastography times (TEG R and TEG R+K; at all three doses;), prolonged clotting times (marked at 1 and 10 mg/kg; 810 and 2220 sec and persisting through day 5 of the study) and reduced Quick values and thrombus stability.

APPEARS THIS WAY
ON ORIGINAL

Safety Assessment of i.v. Bolus r-Hirudin in Monkeys: The safety of r-hirudin, administered as single i.v. bolus injections at doses of 0 (1 male), 1.0 (1 female), and 10.0 mg/kg (1 female), was assessed in cynomolgus monkeys in terms of effects on circulation, respiration, hematology, coagulation, fibrinolysis and clinical chemistry over a period of 60 min after dosing. At i.v. bolus doses of 1 and 10 mg/kg, r-hirudin produced no treatment related effects on circulation, respiration, hematology or clinical chemistry values compared to control values, with the exception of a transient reduction in leukocyte numbers (-35%) at the 10 mg/kg dose (recovering by the end of the 1 hour observation period). The main effects of r-hirudin were marked dose-dependent effects on coagulation/fibrinolysis parameters (marked increases in PTT, TT (> 240 min), and thromboelastography [TEG] times (to > 6000 sec) and reduced Quick times, down to 0%). Effects on coagulation parameters occurred immediately following dosing at both the 1 and 10 mg/kg doses and were partly reversed (except for TT) after 1 hour at the 1 mg/kg low dose, but were maintained through the end of the 1 hour observation period at the 10 mg/kg dose.

APPEARS THIS WAY
ON ORIGINAL

Cardiovascular/Respiratory Effects:

Effects of r-Hirudin on Mean Arterial Pressure and Heart Rate in Anesthetized Rats. In studies conducted in anesthetized male rats (n=6), r-hirudin (10 mg/kg, i.v. bolus) had no effects on either heart rate or blood pressure relative to baseline control values of 106.5 ± 7.86 mm Hg and 349.67 ± 13.93 b.p.m. for mean arterial pressure and heart rate, respectively.

Effects of r-Hirudin on Cardiovascular and Pulmonary Function in Dogs. Studies conducted in anesthetized dogs (1/sex) investigated the effects of r-hirudin (1, 10, and 100 mg/kg, given as single i.v. bolus doses in an escalated dosing regimen) on cardiovascular and pulmonary function. Treatment-related effects on cardiovascular parameters appeared limited to the following: 1) mild increases in systolic and diastolic blood pressures (11 and 15%, relative to mean baseline pressures of 156.4 and 127.1 mm Hg, respectively, at the end of the observation period); 2) moderate increases in pO₂ at the end of 2nd and 3rd treatment until the end of the observation period; and 3) moderate decrease in pCO₂ at 30 and 60 min after 3rd treatment. Increases in cardiac output and cardiac power (31%) and decreased cardiac resistance (18%) did not appear to be treatment related since the changes were transient and only occurred at the end of the second treatment period. No effects on heart rate, body temperature, Lead I ECG, dp/dt-curve, pneumatograms, or respiratory rates were observed. R-Hirudin produced marked effects on coagulation parameters including:

increased TT (>300 sec); PTT (>300 sec), TEG r (>9000 sec), TEG r+k (> 9000 sec), and decreased TEG maximum amplitude (from 57 down to 1 mm) beginning at the end of the first treatment and continuing through the end of the observation period. Assessment of effects on hematological parameters revealed a mild reduction in Hemoglobin (in both dogs at the end of the 3rd treatment) and reduced hematocrit and increased mean cell hemoglobin and mean cell hemoglobin concentration in the male from the end of the first treatment to the end of the observation period. Finally, analysis of blood chemistry parameters only revealed a moderate decrease in plasma lactate in the female) at the end of the 1st treatment through the end of the observation period.

In another study in female beagle dogs (1 dog/dose level), the safety of r-Hirudin, (0.1, 1.0, or 10 mg/kg/h) infused intravenously over a 4 hour period was assessed in terms of its effects on circulation, respiration and coagulation/fibrinolysis during the infusion period and for 2 hours after the end of the infusion. At the 10 mg/kg dose, r-hirudin produced massive oozing hemorrhages at the sites of catheterization. At doses of 1.0 and 10.0 mg/kg, r-Hirudin produced circulatory effects which included: dose-dependent reductions in: stroke volume (up to 58%), heart efficiency (up to 63%), and cardiac output (up to 55%) and increases in total peripheral resistance (> 2 fold increase). No treatment-related effects on arterial pressure, heart, respiratory, and pulse rates, ECG, dp/dt, and transcutaneous pO₂ and pCO₂ were observed. Finally, qualitative changes in coagulation/fibrinolysis, parameters included: at the 0.1 mg/kg dose; distinct increases thrombin times, but only marginal or no effects on PTT, Quick times and thromboelastography (TEG); at the 1.0 mg/kg dose; moderate prolongation of PTT and marked prolongation of Thrombin and TEG times, with reduced thrombus stability and quick times; at the 10 mg/kg dose, massive alterations in all clotting tests, with complete inhibition of coagulation. Changes in coagulation parameters return to normal after the end of the infusion, except for thrombin times which remained markedly elevated through the end of the 2 hour observation period.

Effects of r-Hirudin on Circulation and Coagulation in Cats: A study in two anesthetized cats investigated the effects of r-hirudin (i.v. bolus injection) on arterial blood pressure (systolic, diastolic and tonogram +) and coagulation parameters (thromboelastography [TEG], Quick time, PTT, and thrombin time [TT]). R-hirudin, given intravenously at a dose of 1.0 mg/kg i.v. dose resulted in an increased systolic and diastolic blood pressure in 1 of 2 cats studied (up to 54.54% and 87.87%, respectively at 30 min after dosing). Maximum effects on coagulation parameters occurred at 15 min after dosing and

included: prolonged TEG r (3.85 fold) and TEG r+k (3.74 fold) and reduced TEG maximum amplitudes (-36%, i.e. reduced thrombus stability); reduced Quick times (51%), and prolonged PTT (60%) and TT (>180 min). All of the said parameters showed either full recovery or a tendency for recovery by the end of the 60 min observation period, except for TT which remained (> 180 min).

In other studies in anesthetized cats (n=2) examined the effects of r-Hirudin on blood pressure and coagulation parameters (Thromboelastography, [TEG: r-, r+k-time, and maximum amplitude]); Quick time, Prothrombin Time (PTT), and Thrombin Time (TT). In this latter study, r-hirudin produced a slight increase in systolic and diastolic blood pressures (18 and 24%, relative to baseline values of 115.8 and 82.7 mm Hg, respectively). Treatment-related changes in coagulation parameters included: marked increases in TEG reaction time (3.8 x baseline values of 420 sec) and TEG coagulation time (3.7 x baseline values of 585 sec), reduced TEG maximum amplitude (36% relative to baseline amplitude values of 69 MM); prolonged PTT times (62% relative to control values of 15 sec) and marked increases in TT (10.8 fold, relative to control values of 16.7 sec). Prolonged TT were maintained throughout the 60 min observation period, whereas other parameters returned toward predose values by the end of the observation period.

Effects of r-Hirudin on Cardiovascular and Pulmonary Function and other Parameters in Anesthetized Monkeys.

Studies in anesthetized monkeys (1 monkey/dose level) investigated the effects of r-hirudin on cardiovascular and pulmonary function, with effects on various hematological, clinical chemistry and coagulation parameters following i.v. bolus injection of hirudin at single doses of 1 and 10 mg/kg. At the 10 mg/kg i.v. dose, r-hirudin reduced systolic and diastolic blood pressures (29-64%) beginning 15 min after dosing through the end of the 60 min observation period. However, no treatment-related effects on heart rate were reported and no ECG data was available. The 10 mg/kg high dose monkey also showed increased respiratory rate (3 breaths/min) and respiratory volume (56%, relative to baseline values of 0.99 L/min). Notable changes in coagulation parameters occurred immediately after dosing in both the 1 and 10 mg/kg dosed monkeys and included: marked prolongation of PTT (up to 125 sec and > 240 sec, relative to baseline values of 28.7 and 25.3 sec); marked prolongation of TT (> 240 sec at both doses immediately after injection through the end of the 60 min observation period); marked increases in thromboelastography TEG r and TEG r+k values and complete inhibition of TEG maximum amplitude at the 10 mg/kg dose. Effects on coagulation values tended to return toward normal in the monkey treated with the 1 mg/kg dose but showed no evidence of reversal at the 10 mg/kg

dose over the 60 min observation period. Finally no effects on hematological or blood chemistry parameters were noted with the exception of a transient reduction in white blood cells (48%, relative to control baseline values of $7.89 \times 10^9/L$) which returned to normal by 60 min after dosing.

Effects of r-hirudin On Blood Glucose: A study conducted using male Wistar rats (n=7/group) showed that i.v. bolus injection of r-hirudin at a dose of 1 mg/kg had no effects on blood glucose levels relative to control or baseline values

In a second such study conducted in rabbits (7/group, both sexes) administration of a 1 mg/kg i.v. bolus dose of r-hirudin also had no appreciable effects on blood glucose levels, relative to control groups or base line values

Anti-inflammatory Effects of r-Hirudin in the Carrageenan Paw Edema in Rats: In this study, r-hirudin (1 mg/kg, i.v. bolus) injected into the left paw of rats immediately prior to the carrageen injection had no effects on the subsequent increase in paw volume (control increases of 0.55 ± 0.09 ml and 0.39 ± 0.11 ml at 3 and 6 hours after the carrageen challenge, respectively). In comparison, treatment with the anti-inflammatory agent, indomethacin (3 mg/kg i.v.), reduced the increases in paw volume by 36 and 23%, relative to the said control values, respectively.

APPEARS THIS WAY
ON ORIGINAL

Drug Interactions:

Interaction of Hirudin with Heparin and Low Molecular Weight Heparin in Rats: The effects of r-hirudin on bleeding time and as well as PTT was investigated in rats pretreated with either standard heparin (Liquemin; 100, 120, 150, and 200 U/kg) and low molecular weight heparin (Fraxiparin; 2.5, 5.0, and 10 mg/kg) doses selected to lead to a doubling of PTT and slight to moderate prolongation of bleeding time. Administration of Liquemin (120 mg/kg) alone increased bleeding times and PTT by 48% and 80%, relative to control times of 153 and 122 sec, respectively. R-Hirudin alone at doses of 1 and 3 mg/kg also produced dose-dependent increases in bleeding times (63 and 157%) and PTT (3.4 and 3.8 fold), relative to the said control values, respectively. The combination of r-Hirudin (1.0 and 3.0 mg/kg) and Liquemin (120 U/kg) produced additive effects on bleeding time (increased 70%) and PTT (increased 2.7 fold) relative to bleeding times of 227 sec and PTT of 219 sec observed with Liquemin (120 U/kg) alone, respectively. In contrast, Fraxiparin alone at doses of 2.5, 5.0, and 10.0 mg/kg produced a dose dependent increase in PTT (up 30, 92, and 272%, relative to a

control value of 122 sec), but had no effects on bleeding times. The combination of Fraxiparin (2.5 and 5.0 mg/kg) and r-Hirudin at doses of 1 and 3 mg/kg resulted in bleeding times, which were increased relative to Fraxiparin alone but slightly less than that seen with r-hirudin alone. The combination of r-Hirudin (1 and 3 mg/kg) and Fraxiparin (2.5 and 5.0 mg/kg) also produced increased PTT (166 and 214% at the 2.5 mg/kg dose of Fraxiparin and 109 and 114% at the 5.0 mg/kg dose of Fraxiparin), relative to values of 159 and 234 sec at the 2.5 and 5.0 mg/kg dose of Fraxiparin alone, respectively. However, the increases in PTT seen with the combinations were only slightly greater than the increases observed with r-hirudin alone. Thus, while the combination of (standard heparin) Liquemin and r-Hirudin produced additive effects on bleeding times and PTT, weak or no additive effects on the said parameters were observed with the combination of r-Hirudin and low molecular weight heparin (Fraxiparin).

Effects of r-Hirudin on the Action of Thrombolytic Agents:

Attempts to evaluate the possible interaction/effects of r-Hirudin with streptokinase using the rat wire coil thrombus model were inconclusive since, streptokinase, alone (doses up to 500,000 mg/kg i.v. bolus + 500,000 mg/kg i.v. infusions for 55 min) or in combination with r-Hirudin at doses up to (0.5 mg/kg i.v. bolus + 0.5 mg/kg i.v. infusion) administered 5 min after thrombus induction failed to elicit any thrombolytic effects.

In other studies, the interaction/effects of r-hirudin on the thrombolytic action of recombinant human tissue plasminogen activator (Actilyse®) were evaluated in the rat wire coil thrombus model. In this study, administration of r-hirudin (0.1, 0.25, or 0.6 mg/kg)

each given as one i.v. bolus dose immediately followed by a second dose given as a 55 min i.v. infusion) produced dose-dependent reductions in thrombus weights

Co-administration of the high dose of 0.5 mg/kg, with r-hirudin at the 0.1, 0.25, and 0.6 mg/kg doses resulted in reduced mean thrombus protein weights (-58.8, -74.1, and -43.2%) relative to that seen with r-Hirudin alone. The mid dose of , in combination with the mid and high doses of r-hirudin (0.25 and 0.6 mg/kg) also provided additional thrombolytic benefit (i.e. reduced mean thrombus protein weights 17 and 20% relative to that seen with r-hirudin alone). However, earlier experiments in which Actilyse was administered alone at doses of 0.5 and 0.05 mg/kg resulted in mean thrombus weights of 471.73 and 1563.29 µg, respectively. Relative to the latter weights, only the combinations of the high dose of with the mid dose of r-hirudin (0.25 mg/kg) and the mid dose of with the mid (0.25 mg/kg) and high (0.6 mg/kg) doses of r-hirudin resulted in reduced thrombus weights (25, 50.1 and 38%, respectively).

BEST POSSIBLE COPY

APPEARS THIS WAY
ON ORIGINAL

Finally, the effects of r-Hirudin in combination with another recombinant plasminogen activator (BM 06.022) on coronary blood flow was investigated in a study using a canine model of coronary artery thrombosis. In this model, all dogs received continuous infusion with heparin (1000 IU/kg/h beginning 10 min post occlusion) then either hirudin (1 mg/kg/hour continuous i.v. infusion); acetylsalicylic acid (ASA, 5 or 35 mg/kg i.v. bolus); or Sulotroban (10 mg/kg, i.v. bolus) was administered at 30 min post occlusion and finally BM 06.022 (140 kU/ml, i.v. bolus) or saline were administered at 1 h post occlusion. Results showed that the combination of hirudin and BM 06.022 prolonged the cumulative patency time (2.7 fold), produced a higher reperfusion rate (33%), and significantly reduced the residual thrombus weights (64%), relative to control (BM 06.022 + Saline) values of 47.5 ± 13.1 min, 66%, and 7.4 mg, respectively. These results suggest that r-hirudin could effect potential benefit in maintaining coronary blood flow following reperfusion.

APPEARS THIS WAY
ON ORIGINAL

MISCELLANEOUS PHARMACOLOGY:

Effects of r-Hirudin on Carbon Tetrachloride Intoxication on Rabbits (Study # 134-04.2): r-Hirudin (10 mg/kg/day for 5 days, i.v.) had no significant effect on carbon tetrachloride induced toxicities in rabbits.

Effects of r-Hirudin on Thromboplastin Induced Coagulation in Rabbits (Study # TPK 2/94): Thromboplastin (tissue factor) activates the extrinsic pathway of coagulation (the formed thrombin splits fibrinogen and activates platelets via its receptor, resulting in a marked decrease in fibrinogen levels and platelet counts). r-Hirudin (0.15 mg/kg/hr) significantly inhibited thromboplastin (250 mg/kg/hr) induced decreases in platelet counts and fibrinogen levels.

Influence of r-Hirudin and Concomitant Treatment With Coumadin and Aspirin on Different Hematology/Coagulation Parameters in the Dogs (Study # P-245): r-Hirudin (2 mg/kg, s.c., t.i.d. for 6 days) slightly (increased APTT and quick ratio values in dogs who are already receiving coumadin [0.17 mg/kg (day 1), 0.08 mg/kg (days 2-6), p.o.] and aspirin [4 mg/kg/day (days 1-6), p.o.]. Hence, r-hirudin did not produce remarkable effects on coagulation parameters in presence of concomitant treatment with warfarin and aspirin in dogs.

Effects of Helixate on r-Hirudin/Aspirin Induced Skin Bleeding in the Pig (Study # SBS 02/96): Helixate (recombinant Factor VIII, which contains no VWF: 30 u/kg, i.v.) had no significant effect on skin bleeding time and APTT in pigs treated with r-hirudin (0.3 mg/kg/hr for 3 hr i.v.) and aspirin (20 mg/kg, i.v.).

BEST POSSIBLE COPY

Effect on Haemate on r-Hirudin/Aspirin Induced Skin Bleeding in the Pigs (Study # SBS01/96): Haemate (Factor VIII and Von Willebrand factor [VWF] containing product: 30 u/kg) significantly decreased prolongation of skin bleeding time induced by r-hirudin (0.5 mg/kg x hr for 3 hr) and aspirin (20 mg/kg, i.v.) in pigs (data presented graphically). Haemate has no significant effect on APTT, PT and on r-hirudin plasma levels. Similar results were seen in study # SBS 1/95.

Effect Defrinase (Batroxobin) on r-Hirudin Induced Prolongation of Bleeding Time in Rats (Study # P-067): Defrinase (15 u/kg, i.v.) had no significant effect on r-hirudin (30 mg/kg, i.v.) induced increase in bleeding time in rats.

Antithrombotic Effect of r-Hirudin in Combination With Acetylsalicylic Acid in Rats (Study # V-848): In wire coil induced thrombosis model in rats, r-hirudin (0.1, 0.3 or 1.0 mg/kg, i.v.) and acetylsalicylic acid (10, 30 or 90 mg/kg, i.p.) both dose-dependently inhibited thrombus formation when given individually. When both drugs were given then effect was additive.

Special Antidote-Study With r-Hirudin in Rabbits (Study # V-833): In rabbits, Feibo. (25, 50, 75 or 100 u/kg, i.v.) or Autoplex T500E (1, 2.5, 5 or 25 u/kg, i.v.) both inhibited r-hirudin (0.1 mg/kg, i.v.) induced prolongation of coagulation time and complete inhibition was achieved at 75 and 2.5 u/kg respectively. In another study (# V-918), Autoplex (activated prothrombin complex) was shown to neutralize the effect of r-hirudin in rabbits, as measured by its effect on coagulation time, however, APC did not influence the hirudin induced prolongation of TT and PTT.

Interaction With Nitrate Solution in Dogs (Study # P-236): r-Hirudin (0.15 mg/kg/hr for 2 hr, i.v.) did not alter the effects of glycerin trinitrate (0.14 mg/kg/hr, i.v.) or isosorbid dinitrate (0.14 mg/kg/hr, i.v.) in dogs.

Effects of r-Hirudin on Spontaneous Motility and Spasmolysis in the Isolated Ileum of the Guinea Pig (Study # P-226): r-Hirudin (1.3 mg/ml) did not induce contraction in isolated guinea pig ileum. Furthermore, contraction induced by histamine, acetylcholin, barium chloride or serotonin was not affected by the presence of r-hirudin in this experiment.

Effect on Lipopolysaccharide (LPS) Induced Mortality in Rats (Study # LMR 29/89): r-Hirudin significantly decreased LPS-induced mortality in rats (mortality: control = 9/10 and test = 4/10).

BEST POSSIBLE COPY

Effect on Carrageenan Induced Paw Edema in the Rat (Study # OED 1-90 and # 2.3.3.1): r-Hirudin (3 mg/kg, i.v.) had no significant effect on carrageenan induced paw edema in rat.

Effects on General Behavior and Central Nervous System in Mice (Study # V-813): r-Hirudin (10 or 100 mg/kg, i.v.) had no significant effect on behavior, spontaneous motor activity, cardiazol-induced convulsion or hexobarbitone narcosis in male mice.

Effects on Intestinal Transport in Mouse (Study # P-227): r-Hirudin (1, 10 or 100 mg/kg, i.v.) had no significant effect on intestinal transport in mice.

Effects on Water and Electrolyte Metabolism in Rat (Study # P-228): r-Hirudin (1 - 100 mg/kg, i.v.) had no significant effect on water or electrolyte excretion in rats.

In summary, Lepirudin (r-hirudin) is a recombinant protein derived from the naturally occurring thrombin inhibitor hirudin in yeast. It is identical to hirudin except for the substitution of leucine for isoleucine at the N-terminal end of the molecule and the absence of a sulfate group on the tyrosine at position 63. r-Hirudin binds selectively to thrombin and does not require the presence of antithrombin III cofactor. It dose dependently prolonged activated partial thromboplastin time (APTT) and thrombin time (TT) in various species (rats, dogs, cats and monkeys). It has antithrombotic activity in various models of thrombosis.

APPEARS THIS WAY
ON ORIGINAL

APPEARS THIS WAY
ON ORIGINAL

BEST POSSIBLE COPY

ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION (ADME):

Absorption:

Rats:

APPEARS THIS WAY
ON ORIGINAL

Pharmacokinetics of HBW-023-¹²⁵I After I.V. Injection.

Animals:

WISKf(SPF71) rats.

Methods: Six rats (3 males and 3 females) were intravenously administered approximately 1.0 (148.3 μ Ci/mg) mg/kg of HBW-023-¹²⁵I via the tail vein; vehicle was 0.9% saline solution. Blood samples were obtained via the retrobulbar vein plexus at 0.083, 0.25, 0.50, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0 and 24.0 h after injection.

Radioactivity in plasma specimens was determined by a gamma counter. Pharmacokinetic parameters were derived

Results: As shown in the following table, plasma C_{max} values for HBW-023-¹²⁵I were similar in males (5.16 μ g/ml) and females (4.71 μ g/ml) and peaked rapidly after i.v. injection (t_{max} = 0.083 h). Plasma levels of HBW-023-¹²⁵I declined in a biphasic manner in males and in a triphasic manner in females; $t_{1/2\alpha}$ and $t_{1/2\beta}$ values were similar in males (0.07 and 6.35 h) and females (0.10 and 5.42 h). $AUC_{0-\infty}$ s were greater in males (25.41 μ g•h/ml) than females (13.57 μ g•h/ml).

Pharmacokinetic parameters for i.v. HBW-023-¹²⁵I (1.0 mg/kg) in male and female rats

Pharmacokinetic Parameter	Males (1.14 mg/kg)	Females (1.01 mg/kg)
C_{max} (μ g/ml)	5.16	4.71
t_{max} (h)	0.083	0.083
$t_{1/2\alpha}$ (h)	0.07	0.10
$t_{1/2\beta}$ (h)	6.35	5.42
$AUC_{0-\infty}$ (μ g•h/ml)	25.41	13.57

Note: Irrespective of the sex about 70% of administered radioactivity was excreted in urine during 0 - 24 hr period.

BEST POSSIBLE COPY

**Pharmacokinetic Study in Rats Following Single
and Repeat Daily I.V. Dose**
(Study # 134.4-02)

Methods: Groups of rats (3/sex/group) were given daily i.v. doses of 1, 10 and 100 mg/kg/day of r-hirudin for 28 days. Blood samples (0.4 ml) were collected from retroorbital vein plexus at 0, 5, 15, 30 min and 1, 2, 3, 6, and 24 hr after drug administration on day 1 and 28 of the study. Levels of r-hirudin in plasma samples were measured by

Results: Pharmacokinetic data for r-hirudin obtained by using two different methods were comparable. The $t_{1/2}$, β values ranged and AUC values increased with increasing dosages. Data also indicated increase in clearance and volume of distribution after 28 daily doses (this may account for decreased AUC values on day 28 compared to the values on day 1).

APPEARS THIS WAY
ON ORIGINAL

Pharmacokinetics After Single and Repeat I.V. Dose in Rats (r-Hirudin in Plasma)						
Parameters	1 mg/kg		10 mg/kg		100 mg/kg	
	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28*
AUC _{0-∞} (ng.hr/ml)	2,126	1,018	15,400	8,700	131,500	142,000
Cl (ml/min/kg)	8.8	18.0	11.7	20.4	13.2	11.7
V _d (L/kg)	0.44	0.69	0.55	1.13	1.20	0.42
t _{1/2} α	0.04	0.03	0.09	0.08	0.17	0.03
t _{1/2} β	0.84	0.64	0.68	0.77	1.5	0.71

* = only 1 rat was used

APPEARS THIS WAY
ON ORIGINAL

BEST POSSIBLE COPY

Pharmacokinetics After Single and Repeat I.V. Dose in Rats (r-Hirudin in Plasma)						
Parameters	1 mg/kg		10 mg/kg		100 mg/kg	
	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28*
AUC _{0-∞} (ng.hr/ml)	2,075	1,074	23,600	12,000	185,000	200,000
Cl (ml/min/kg)	10.1	17.7	7.2	14.1	9.2	8.3
V _{ss} (L/kg)	0.38	0.83	0.53	0.66	0.77	0.2
t _{1/2α} (hr)	0.03	0.04	0.11	0.05	0.12	0.02
t _{1/2β} (hr)	0.66	1.00	1.23	0.78	1.29	0.43

* = only 1 rat was used

APPEARS THIS WAY
ON ORIGINAL

Anti-hirudin IgG antibodies were detected in treated rats (low dose = 1/6, mid dose = 5/5 and high dose = 5/6). In subsequent experiments it was shown that mid (10 mg/kg) and high dose (100 mg/kg) treated rats had sufficiently high levels of anti-hirudin antibodies and potentially will interfere with immunoassay of r-hirudin. No effect on hepatic drug metabolizing activities were seen when rats were treated with up to 100 mg/kg/day for 28 days.

APPEARS THIS WAY
ON ORIGINAL

Absorption, Distribution and Excretion After a Single S.C. (1 mg/kg) Dose to Male and Female Rats (Study # 011219)

Methods: Wistar rats (both sexes) were given a single s.c. (1 mg/kg) dose of ¹²⁵I-r-hirudin. Blood samples were collected (from retrobulbar venous plexus) at 0.083, 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8, 24 and 48 hr after drug administration. Urine samples were collected over 0 - 5, 5 - 24, 24 - 48 hr after drug administration. Feces samples were collected during 0 - 24 and 24 - 48 hr after drug administration. At 48 hr after drug administration rats were killed and various organs were collected. Levels of radioactivity in various samples were measured by gamma counter.

APPEARS THIS WAY
ON ORIGINAL

Results: Irrespective of the sex T_{max} was Sponsor reported t_{1/2} values and distribution of radioactivity at 48 hr after drug administration. Since deiodinization of ¹²⁵I-r-hirudin occurs very rapidly therefore, these data represents iodine kinetics and distribution, no usable information can be obtained from this study. About 82% of the administered radioactivity was excreted in urine in 0 - 48 hr and fecal excretion was negligible.

BEST POSSIBLE COPY

Rabbits:

Pharmacokinetics of r-Hirudin in Rabbits following I.V. (Bolus and Infusion), I.M. and S.C. Injection (Report No. V-708)

Methods: Four groups of rabbits (3/group) were administered r-Hirudin via intravenous bolus (group 2), intravenous infusion (group 3), subcutaneous injection (group 4) or intramuscular injection (group 5), all at a dose level of 10 mg/kg, except for the i.v. infusion group, where a dose of 10 mg/kg/hour was infused over a 4 hour period (i.e. total dose 40 mg/kg). Hirudin was dissolved in isotonic saline at a concentration of 10 mg/ml. The volume of administration was presumably 1 ml/kg in Groups 2, 4, and 5 and at volume of 1 ml/kg/hour in the infusion group 3. A vehicle control group (group 1) received equal volumes of isotonic saline administered via i.v. bolus injection. Blood samples from groups 1 (control), 4 (s.c.), and 5 (i.m.) were collected at 0, 10, 20, 30, 60, 90, 120, 150, 180, 240, 300, 360, and 430 min following dosing for determination of r-Hirudin

APPEARS THIS WAY
ON ORIGINAL

BEST POSSIBLE COPY

plasma levels. Likewise, blood samples were collected from group 2 (i.v.) at 0, 5, 10, 30, 60, 90, 120, 150, 180, 240, and 300 min following dosing and from group 3 (i.v. infusion) at 0, 5, 10, 30, 60, 90, 120, 240, 250, 270, 300, 330, 360, and 390 min following dosing. The methodology for measuring r-Hirudin plasma concentrations was not indicated.

Results: Excessive pharmacological activity (i.e. enormous s.c. hemorrhages at the s.c. injection sites and intramuscular bleeding at the i.m. injection site) resulted in the death of one animal each in the s.c. and i.m. injection groups. Table 3 below, shows the calculated mean pharmacokinetic values for rabbits following administration of 10 mg/kg doses of r-Hirudin via i.v. bolus, s.c. and i.m. injection and following i.v. infusion of a 10 mg/kg/h dose for 4 hours.

Table 3. Range of pharmacokinetic parameters in rabbits given r-Hirudin at doses of 10 mg/kg (i.v. bolus, s.c. and i.m. injections) and at a dose of 10 mg/kg/hr for 4 hours (i.v. infusion) (Data taken from Sponsor's Summarized Pharmacokinetics report, Vol 15, pg. 16)

Group #	I.V. bolus (10 mg/kg)	I.V. Infusion (10 mg/kg)	S.C. (10 mg/kg)	I.M.† (10 mg/kg)
<u>Parameter</u> C _{max} (µg/ml) T _{max} (h) AUC (µg/ml·hr) Cl (ml/min) Vd (L) t _w (h)	0.08			5.1 1.0 6.76

†Values only available for one animal in the i.m. group

* Values for 2 of the three animals were 167.44 h and 180.04 h versus 1.07 h for the other animal.

The data in Table 3 show that Cmax values ranged from 83 to 89 $\mu\text{g/ml}$ at 5 min after i.v. bolus injection. In comparison, maximal plasma levels, attained at 1-2 h following infusion, were slightly less and even less following s.c.

attained at after dosing) and i.m. (5.1 $\mu\text{g/ml}$) attained at 1 h after dosing, only one animal available). The elimination of r-hirudin was fit best by dual and three compartment models of elimination following i.v. and s.c. dosing, respectively. Terminal half-life values were in the range of 1 h following i.v. bolus dosing, but were slightly longer following s.c. administration and even longer following i.m. dosing (6.76 h, in the single animal which was available in the i.m. group). The Sponsor stated that post infusion data did not correlate with infusion data and that only the calculated half-life value of 1.07 h in one animal correctly reflected the terminal elimination of r-Hirudin (Note $t_{1/2}$ values of 167.44 and 180.04 h and corresponding AUC values of 392.76 and 734.18 $\mu\text{g/ml}\cdot\text{hr}$ were observed in the other two rabbits which received the i.v. infusions).

APPEARS THIS WAY
ON ORIGINAL

Concurrent analyses of the effects on the coagulation parameters, Thrombin Time (TT) and Partial Thromboplastin Time Showed that regardless of the route of administration, after 5-10 min, TT increased to over 300 sec and did not decline for the remainder of the 420 min study period. Following the i.v. bolus injection, PTT was immediately increased from basal values of

whereas following i.v. infusion, increases were less prominent and occurred later. Even less dramatic changes were noted in animals dosed via s.c. and i.m. injections

compared to i.v. However, PTT values tended to remain elevated throughout the 420 min time period following s.c. and i.m. dosing, but were generally returned to baseline values in the i.v. bolus dose groups.

APPEARS THIS WAY
ON ORIGINAL

**Pharmacokinetics Study in Rabbits Following
Single and Repeat I.V. Dose**
(Study # 134.4-04)

Methods: Groups of rabbits (3/sex/group) were given daily i.v. doses of 1, 10 and 30 mg/kg/day of r-hirudin for 28 days. Blood samples were collected at 0, 5, 15, 30 min, 1, 2, 3, 6 and 24 hr after drug administration on day 1 and 28 of the study. Levels of r-hirudin in plasma samples were measured

Results: Pharmacokinetic data for r-hirudin obtained by using two different methods were comparable. The $t_{1/2\beta}$ and AUC values increased with increasing dosages. Plasma clearance values after single dose did not change with increasing dosages, hence, a saturation can be excluded. Volume of distribution values indicates limited distribution. Pharmacokinetic parameters remained similar after 28 daily doses, hence, there were no accumulation. In rabbits, no anti-hirudin antibodies were induced after 28 daily i.v. doses.

Pharmacokinetics After Single and Repeat I.V. Dose in Rabbits						
Parameters	1 mg/kg		10 mg/kg		30 mg/kg	
	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28
AUC _{0-24 hr} (ng.hr/ml)	1,741	1,899	17,480	18,739	50,000	56,194
Cl (ml/min/kg)	9.8	9.0	9.4	9.1	10.3	9.7
V _d (L/kg)	0.45	0.45	0.42	0.40	0.46	0.45
t _{1/2α} (hr)	0.13	0.14	0.14	0.13	0.14	0.12
t _{1/2β} (hr)	0.80	0.89	0.71	0.70	0.74	0.75

BEST POSSIBLE COPY

APPEARS THIS WAY
ON ORIGINAL

APPEARS THIS WAY
ON ORIGINAL

Pharmacokinetics After Single and Repeat I.V. Dose in Rabbits						
Parameters	1 mg/kg		10 mg/kg		30 mg/kg	
	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28
AUC _{0-24h} (ng.hr/ml)	2,469	2,836	22,556	27,695	72,639	68,000
Cl (ml/min/kg)	6.9	6.3	7.5	6.2	7.2	7.9
V _d (L/kg)	0.29	0.26	0.33	0.27	0.26	0.36
t _{1/2α} (hr)	0.11	0.09	0.13	0.10	0.11	0.15
t _{1/2β} (hr)	0.89	0.70	1.02	0.86	0.62	0.90

Dogs:

APPEARS THIS WAY
ON ORIGINAL

Pharmacokinetics of HBW-023-¹²⁵I After I.V. Injection.

Subjects: Two male beagle dogs (18 and 21 kg; approximately 8 years of age).

Methods: Two dogs were intravenously administered approximately 0.6 (44.0 μCi/mg) and 1.4 (5.43 μCi/mg) mg/kg of HBW-023-¹²⁵I, respectively, via the saphenous vein; vehicle was 0.9% saline solution. Blood samples were obtained via the jugular vein at 0.083, 0.167, 0.25, 0.333, 0.50, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 9.0, 24.0, 48.0, 72.0 and 96.0 h after injection.

Radioactivity in plasma specimens was determined
Pharmacokinetic parameters were derived on a personal

Results: As shown in the following table, there were dose-related increases in C_{max} for plasma specimens (1.80 and 6.90 μg/ml, respectively); t_{max} values were 0.083 and 0.166 h, respectively. Plasma levels of HBW-023-¹²⁵I declined in a triphasic manner; t_{1/2β} values were 0.28 and 0.49 h, respectively. There were dose-related increases in AUC_{0-5h} values for plasma specimens

APPEARS THIS WAY
ON ORIGINAL

BEST POSSIBLE COPY

APPEARS THIS WAY
ON ORIGINAL

Pharmacokinetic parameters for i.v. HBW-023-¹²⁵I in male dogs

Pharmacokinetic Parameter	Dog H2 (0.60 mg/kg)	Dog H1 (1.39 mg/kg)
C _{max} (µg/ml)	1.80	6.90
t _{max} (h)	0.083	0.166
t _{1/2} (h)	0.28	0.49
AUC _{0-5 h} (µg•h/ml)	1.55	3.46

Note: About _____ of the administered radioactivity was excreted in urine during _____ period. APPEARS THIS WAY ON ORIGINAL

I.V. Pharmacokinetics Pilot Study with r-Hirudin in Dogs.
(Report No. P-187)

Methods: The pharmacokinetics of r-Hirudin along with the time course of r-Hirudin's effect on different coagulation factors (bleeding times and APTT) was studied in Beagle dogs male (n=4) and female (n=6) following i.v. infusion of r-Hirudin at a dose level of 0.15 mg/kg/hr at a rate of 1.0 ml/kg/hr (i.e. 0.15 mg/ml) for a 6 hour infusion period. For comparison, another group of 5 dogs were administered 30000 U streptokinase as a one hour infusion. Blood samples were collected at predosing, and at 15, 30 min, 1, 2, 3, 4, 5, 6, and 8 hours after the start of infusion for determination of thrombin times (TT) and activated partial thromboplastin times (APTT) and for determination of plasma r-Hirudin concentrations.

Results: Infusion of r-Hirudin resulted in increased mean bleeding times (150 to 412 sec, relative to control values of 24 sec), slight elevations in PTT relative to mean baseline values of 15.24 sec) and increased TT values

Changes in the aforementioned coagulation parameters were evident by 15 min after dosing and were associated with mean plasma concentrations of around 180 ng/ml at the 15 min time point and about 747 ng/ml at the 5 hour time point. By 2 hours after the end of infusion bleeding times, PTT and TT had generally returned to the normal range, except for one dog which still showed prolonged TT (>300 sec). The results from this study suggest a good correlation between r-Hirudin plasma levels and changes in coagulation parameters.

BEST POSSIBLE COPY

Pigs:

APPEARS THIS WAY
ON ORIGINAL

I.V. and S.C Pharmacokinetic Study with r-Hirudin in Pigs
(Report No. V-745)

Methods: Two groups of three female adult Göttinger mini pigs were administered r-Hirudin at a dose of 10 mg/kg (dissolved in isotonic saline in a total volume of 0.1 ml/kg) via either i.v. or s.c. injections. Blood samples were collected at 0, 10, 20, 30 min, 1, 1.5, 2, 2.5, 3, 4, 5, 6, and 7 hours after dosing for determination of plasma r-Hirudin concentrations, PTT, TT and effects on hematology (hemoglobin, hematocrit, mean corpuscular hemoglobin, and the numbers of leukocytes, erythrocytes, and platelets).

Results: Injection of r-hirudin, at the 10 mg/kg i.v. dose resulted in C_{max} levels of $172.11 \pm 2.5 \mu\text{g/ml}$, a volume of distribution of $2621 \pm 2.5 \text{ ml}$, and an AUC value of $74.16 \pm 4.73 \mu\text{g/ml} \times \text{h}$. Elimination following i.v. dosing was biexponential with a half life value for the alpha phase of 0.167 ± 0.01 and a terminal elimination half-life value of $1.22 \pm 0.15 \text{ h}$. Following s.c. dosing, peak plasma levels of occurred between 90 min and 7 hours post dosing and ranged from 2.57 to $4.99 \mu\text{g/ml}$. No significant elimination was evident during the 7 hour observation period following s.c. dosing. Thus, pharmacokinetic parameters were not available for the s.c. dose. PTT increased to $> 240 \text{ sec}$ from 10 min through the first hour after i.v. dosing, decreased steadily thereafter to a level of _____ at the end of the 7 hour period. In the s.c. dosed group PTT values attained a maximal of 44.3 sec in one pig, but steadily increased in the other two pigs and ranged from _____ at the end of the observation period. TT values increased to $> 240 \text{ sec}$ at 10 min after both the i.v. and s.c. injections and remained elevated through the end of the 7 hour observation period. Finally, hematological analysis showed increased leukocytes in both the i.v. dosed group _____ and following s.c. dosing ($10^9/l$).

APPEARS THIS WAY
ON ORIGINAL

BEST POSSIBLE COPY