CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

20-873

PHARMACOLOGY REVIEW

DURKAY

NDA 20,873

PHARMACOLOGIST'S REVIEW OF NDA 20,873
(Amendment Dated August 5, 1999)

SEP 14 1999

Sponsor & Address: The Medicines Company
Cambridge, MA

Reviewer: Timothy W. Robison, Ph.D.
Pharmacologist, HFD-180

Date of Submission: August 5, 1999

Date of HFD-180 Receipt: August 9, 1999

Date of Review: September 8, 1999

Drug: Angiomax™ (Hirulog®; BG8967/bivalirudin)

Category: Anticoagulant/Thrombin Inhibitor.

Submission Contents:

1. Twenty-four hour continuous infusion study in the rat.

TOXICOLOGY:

Acute Toxicity in Rats

A 24-Hour Continuous Infusion Study of Hirulog® in the Rat (Project number P8967-98-01).

Testing Laboratory: [redacted]

Study Started: September 22, 1998

Study Completed: June 4, 1999

GLP Compliance: Statements of compliance with GLP Regulations and the Quality Assurance Unit were included.

Animals: Sprague-Dawley®SD® rats were received from [redacted] Sprague Dawley, [redacted] Animals were approximately 12-weeks old at the start of treatment.

Drug Batch: Hirulog®, Lot number 97-2069 and 97-2031.

Methods: Toxic effects of hirulog were evaluated in Sprague-Dawley rats in response to a 24-hr intravenous infusion. Hirulog was administered at dose levels of 0, 100, 500, and 2000 mg/kg/24 hr. There were two control groups: a saline control group that received 0.9% NaCl and a vehicle-control group that received [redacted] The sponsor's selection of the high dose was based upon a 14-day continuous intravenous infusion toxicity study (P8967-93-02) in which a dose of 1000 mg/kg/day was found to produce mild nephropathy, segmental zones of necrosis, acute and chronic inflammation, interstitial fibroplasia, and tubular epithelial regeneration in the kidney and an increased incidence of Kupffer cell hypertrophy in the liver. For the present study with regard to toxicology assessment, there were 12 rats/sex/group. For the toxicokinetic portion, there were three groups of 10 rats/sex/group. Saline, vehicle, or drug solution was administered once over an approximate 24 hr period by continuous intravenous infusion into the left femoral vein at a dose volume of 50 mL/kg/24 hr using an indwelling catheter. Animals were allowed a recovery period of approximately two weeks prior to initiation of infusion. For 20 days prior to the initiation of the infusion, animals were observed twice daily for mortality, general appearance, and behavior. Physical examinations were conducted and body weights were measured during the week prior to the initiation of the infusion. Urine was collected for analysis on days 1 and 15. For toxicokinetic groups at 100, 500, and 2000 mg/kg/24 hr, blood for determination of plasma hirulog levels was collected at 1.5, 3, 6, 12, 24, and 48 hr after the start of the infusion. Plasma concentrations of hirulog were determined using a competitive enzyme immunoassay (EIA). At completion of the 24-hr infusion, 6 animals/sex/group were sacrificed for toxicology assessment. Remaining animals were sacrificed after a 14-day recovery period. During sacrifice after the infusion or the recovery period, blood for determination of hematological and serum biochemistry parameters was collected. Animals were submitted to gross macroscopic examination. Absolute and relative organ weights were determined for the adrenal glands, brain, heart, kidneys, liver, lungs, ovaries, spleen, testes with epididymides, thymus, thyroid with parathyroids. Organs/tissues were collected and processed for microscopic examination as follows: adrenals (2), aorta (thoracic) bone marrow (sternebrae, and femur with knee joint), bone marrow smear (from femur), brain (forebrain, midbrain, hindbrain), exteriorization site, eyes with optic nerve, gastrointestinal tract (esophagus, glandular and non-glandular stomach, duodenum, jejunum, ileum, cecum, colon, rectum), Harderian glands (2), heart, infusion site including the surrounding tissue and the posterior vena cava at the catheter tip and approximately 2.5 cm proximal to the catheter tip, kidneys (2), lacrimal glands (exorbital), liver, lungs including bronchi, mesenteric lymph node, submandibular lymph node (2), mammary gland, ovaries (2), pancreas, peripheral nerve (sciatic), pituitary gland, prostate, salivary gland (mandibular, 2) seminal vesicles (2) skeletal muscle (caudal thigh), skin, spinal cord (cervical), spleen, testes with epididymides (2), thymus, thyroids with parathyroids (2), tongue, trachea, urinary bladder, uterus, vagina, and all gross lesions. Microscopic examinations were performed on all tissues from animals found dead and from all saline control, vehicle control, and 2000 mg/kg/24 hr group animals at scheduled necropsies. The infusion site (vena cava past the cannula tip),

liver, lungs, kidneys, and any gross lesions were examined from all animals in the 100 and 500 mg/kg/24 hr groups. For interpretation of results from statistical analysis, primary comparisons were between the saline control group and hirulog-treatment groups. If a statistically significant difference between the saline control group and a hirulog-treatment group was found, a comparison between the vehicle control group and hirulog-treatment groups was performed to determine if the difference was due to the vehicle or hirulog. If no statistical difference between the saline control group and hirulog treatment groups was found, any difference between the vehicle control group and hirulog treatment groups was considered irrelevant.

Results:

1. **Drug Administration:** Six animals in various groups received greater than 110% of the designated dose (110.7 to 113.7%) and four animals in various groups received less than 90% of the designated dose (48.1 to 89.8%). One female rat (#11168) in the 100 mg/kg/24 hr toxicokinetic group was not dosed due to equipment failure. These variations appeared to have little or no impact on the toxicology assessment.

2. **Observed Effects:** One male rat in the 2000 mg/kg/24 hr was observed to have a pale appearance at 1 hr post-dosing, prior to completion of the infusion, and during the recovery period.

3. **Mortality:** There were no treatment-related deaths in the toxicology assessment portion of the study. One saline control animal (#11082) in the toxicology assessment portion of the study was found dead on day 13. Death was attributed to renal failure, pulmonary inflammation, and cardiomyopathy. From the toxicokinetic portion of the study, 2 male rats and 1 female rat in the 500 mg/kg/24 hr group and 3 male rats and 4 female rats in the 2000 mg/kg/24 hr group were found dead or sacrificed in a moribund condition on day 1. Death was attributed to blood loss from the collection site. Necropsy examination of three animals revealed internal hemorrhage

4. **Body Weight and Food Consumption:** During the recovery period, there were no alterations in body weight gain that appeared to have any relationship to treatment. Body weights for male saline controls on days -1 and 15 were 311 and 370 g, respectively. Body weight gains for the male 0, 100, 500, and 2000 mg/kg/24 hr groups were 104.1, 112.2, 79.4, and 102.35% of the control, respectively. Body weights for female saline controls on days -1 and 15 were 241 and 267 g, respectively. Body weight gains for the female 0, 100, 500, and 2000 mg/kg/24 hr groups were 107.7, 110.6, 106.8, and 118.7% of the control, respectively.

5. Hematology and Blood Coagulation: There were small changes in erythrocyte counts, hemoglobin levels, and hematocrit for the male 2000 mg/kg/24 hr group, which indicated a small blood loss. Increases of prothrombin time and activated partial thromboplastin time (APTT) for the 2000 mg/kg/24 hr group at the end of the infusion were consistent with the pharmacological properties of hirulog. At the end of the infusion, erythrocyte counts, hemoglobin levels, and hematocrit for the male 2000 mg/kg/24 hr group were decreased to 87.9, 85.9, and 87% of control values (7.01 x $10^6/\mu L$, 13.5 g/dL, and 39.9%), respectively. At the end of the infusion, prothrombin time and APTT for the male 2000 mg/kg/24 hr group were increased to 130.6 and 191.85% of control values (14.7 and 23.3 sec), respectively. At the end of the infusion, APTT for the female 2000 mg/kg/24 hr group was increased to 121.9% of the control (22.4 sec). At the end of the infusion, neutrophil counts for the female 100, 500, and 2000 mg/kg/24 hr groups were decreased in a dose-related manner to 73.7, 42.1, and 36.8% of the control (1.9 x $10^3/\mu L$), respectively. At the end of the infusion, neutrophil percentages for the female 100, 500, and 2000 mg/kg/24 hr groups were decreased to 79.5, 54.5, and 54.5% of the vehicle control (44%), respectively. No changes in hematology or blood coagulation parameters were found following the 14-day recovery period.

6. Blood Chemistry and Urinalysis:

Blood Biochemistry: At the end of the infusion, glucose levels for the male 2000 mg/kg/24 hr groups were decreased to 76.6% of the vehicle control (179 mg/dL). At the end of the infusion, urea nitrogen levels for the female 2000 mg/kg/24 hr group were elevated to 110% of the control (21.9 mg/dL). On day 15, total protein levels for female treatment groups were slightly elevated to 107-109% of the control (5.6 g/dL).

Urinalysis: At the end of the infusion, the urine volume for the male 2000 mg/kg/24 hr group was decreased to 69.7% of the control (16.2 mL), respectively. At the end of the infusion, urine volumes for the female 0, 100, 500, and 2000 mg/kg/24 hr groups were decreased to 60.8, 37.2, 47.2, and 48.8% of the saline control (25.0 mL), respectively. The drug vehicle, [redacted] is an osmotic diuretic and might be expected to increase urine volume. At the end of the infusion, moderate to abundant amounts of blood were found for 2 of 6 (33.3%) male rats and 2 of 6 (33.3%) female rats in the 2000 mg/kg/24 hr treatment group as compared to negative or trace finding in all saline control and vehicle control groups. At the end of the infusion, the incidence and quantities of red blood cells in the urine for the male 500 and 2000 mg/kg/day groups and the female 2000 mg/kg/24 hr group were slightly increased.

Day 0 to 1 Urinalysis: Incidence and quantities of blood and red blood cells for male and female rats at 0 (saline), 0 [redacted] 100, 500, and 2000 mg/kg/24 hr (n = 6/group).

Dose, mg/kg/24 hr	Blood		Red Blood Cells	
	Male	Female	Male	Female
Saline control	3-Negative 2-Trace 1-1+	3-Negative 1-Trace 2-1+	4-Negative 1 at 1-2 1 at 4-6	3-Negative 2 at 4-5 1 at 8-10
Vehicle control	6-Negative	6-Negative	6-Negative	6-Negative
100	3-Negative 2-Trace	6-Negative	3-Negative 1 at 3-4 1 at 4-5	5-Negative 1 at 2-3
500	3-Negative 1-Trace 2-1+	5-Negative 1-2+	3-Negative 1 at 4-5 1 at 5-6 1 at 8-10	5-Negative 1 at 10-12
2000	3-Negative 1-1+ 2-3+	4-Negative 2-3+	3-Negative 3 at 6-8	4-Negative 1 at 10-12 1 at 15-18

Note for blood findings: 1+, trace to slight; 2+, slight to moderate; and 3+, moderate to abundant.

7. **Physical Examinations:** No reported findings.

8. **Organ Weights:** Changes in absolute and relative thyroid gland weight were observed at the primary and recovery necropsies; however, there were no histopathological correlations.

9. **Gross Pathology:** There were no treatment-related gross pathological findings in the tissues collected from the primary and recovery necropsies.

10. **Histopathology:** There were no treatment-related histopathological findings in the tissues collected from the primary and recovery necropsies. From lung tissue collected at primary necropsy, minimal granulomatous inflammation was observed for 1 saline control male rat, 1 female rat at 100 mg/kg/24 hr, 1 female rat at 500 mg/kg/24 hr, and 1 male rat and 4 female rats at 2000 mg/kg/24 hr. These lesions were characterized by minimal, focal to random intra-alveolar or interbronchiolar aggregates of macrophages. The distribution of these lesions was not consistent with a generally distributed test article-related effect and may have been related to inhalation of foreign material. There were no similar findings in lung tissues collected at the recovery necropsy. Spontaneous lung lesions are common in this rat strain and were not observed in other toxicology studies with hirulog.

11. **Plasma Drug Levels:** Plasma C_{max} and AUC values for hirulog in male and female rats increased in an approximate dose proportional manner. There were no gender-related differences in C_{max} or AUC values. Clearance values (7.81 to 13.1 mL/min/kg) exceeded the plasma glomerular filtration rate (3 mL/min/kg); however, they were less than values for renal plasma flow (21.3 mL/min/kg) or hepatic plasma flow (31.9 mL/min/kg) (Pharmaceutical Research 10: 1093-1095, 1993). Previous pharmacokinetic studies with hirulog in rats reviewed in NDA 20,873 (Document Room Date of July 27, 1998) suggested that hirulog and/or degradation products were distributed into the tissues beyond the central compartment (i.e., blood volume). Quality control samples were used to monitor the performance of the assay. The acceptance criteria outlined in the study protocol stated that not more than 3 of the 9 quality control samples could be greater than 15% inaccurate and not 2 of these at the same level. During the second analytical run, two of the quality control samples at the same level were greater than 15% inaccurate, which would indicate failure of the assay run. However, due to low sample volume, it apparently would have not been possible to repeat all samples; therefore, the sponsor agreed to extend this acceptance criterion to 20% and accept the assay run.

Plasma toxicokinetic parameters for hirulog in rats that received the test article by continuous intravenous infusion at dose levels of 100, 500, and 2000 mg/kg/24 hr.

Dose, mg/kg/24 hr	C_{max}, ng/mL		T_{max}, hr		$AUC_{0\text{-}24hr}$, Ng hr/mL		CL, mL/min/kg	
	Male	Female	Male	Female	Male	Female	Male	Female
100	6830	8890	6	3	110609	112218	10.2	7.81
500	32252	32339	12	3	589060	603269	10.8	10.7
2000	106308	152710	3	6	1835347	1989022	13.1	9.09

Hirulog was administered by continuous intravenous infusion to rats at doses of 0, 100, 500, and 2000 mg/kg/24 hr. For toxicology assessment, there were 12 rats/sex/group. At the end of the 24 hr infusion, 6 rats/sex/group were sacrificed. Following a 14-day recovery period, the remaining rats were sacrificed. The maximum tolerated dose appeared to be 2000 mg/kg/24 hr. There were no treatment-related deaths in the toxicology assessment portion of the study. There was not a target organ of toxicity. At the end of the infusion, there was evidence of slight blood loss for the 2000 mg/kg/24 hr group as indicated by decreased erythrocyte counts, hemoglobin levels, and hematocrit for male rats and blood in the urine for both male and female rats.

SUMMARY AND EVALUATION

Hirulog is a 20 amino acid peptide that directly inhibits thrombin. Hirulog is under development for use as an anticoagulant in patients undergoing percutaneous transluminal coronary angioplasty (PTCA). In the present amendment, the sponsor has submitted a 24-hr continuous intravenous infusion toxicology study in rats.

Hirulog was administered by continuous intravenous infusion to Sprague-Dawley rats at doses of 0, 100, 500, and 2000 mg/kg/24 hr. For toxicology assessment, there were 12 rats/sex/group. At the end of the 24-hr infusion, 6 rats/sex/group were sacrificed. Following a 14-day recovery period, the remaining rats were sacrificed. The maximum tolerated dose appeared to be 2000 mg/kg/24 hr. There were no treatment-related deaths in the toxicology assessment portion of the study. There was not a target organ of toxicity.

The outcome of this acute continuous intravenous infusion toxicity study in rats submitted in the present amendment does not alter the conclusions and recommendations from the review of NDA 20,873 submitted on December 23, 1997, April 24, 1998, and May 1, 1998 (Document Room Date of July 27, 1998).

RECOMMENDATIONS:

None.

/S/

Timothy W. Robison, Ph.D.

9-8-99

Date

cc:
Orig. NDA 20,873
HFD-180
HFD-181/CSO
HFD-180/Dr. Choudary
HFD-180/Dr. Robison

/S/ 9/14/99

R/D Init.: J. Choudary 8/24/99

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Sponsor: The Medicines Company
Cambridge, MA

REVIEW # 1

Reviewer: Timothy W. Robison, Ph.D.
Pharmacologist, HFD-180

JUL 27 1998

Date of Review: July 23, 1998

Date of Submission: Original: December 23, 1997
Amendment: April 24, 1998
Amendment: May 1, 1998

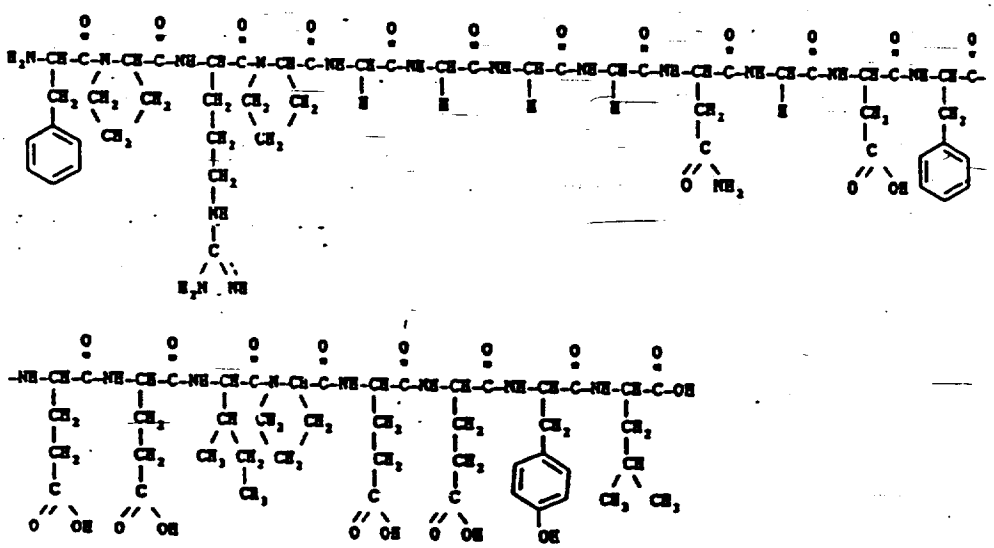
Date of HFD-180 Receipt: Original: December 23, 1997
Amendment: April 27, 1998
Amendment: May 4, 1998

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

ORIGINAL SUMMARY

Drug: Hirulog® (BG8967/bivalirudin)

Chemical Name and Structure: D-phenylalanyl-L-propyl-L-arginyl-L-propyl-glycyl-glycyl-glycyl-glycyl-L-asparagyl-glycyl-L-aspartyl-L-phenylalanyl-L-glutamyl-L-glutamyl-L-isoleucyl-L-prolyl-L-glutamyl-L-glutamyl-L-tyrosyl-L-leucine trifluoroacetate (salt)hydrate



Molecular Formula: $C_{98}H_{138}N_{24}O_{33}$
Molecular Weight: 2180.19 daltons
(anhydrous free base peptide)

Formulation: Hirulog is presented in single-use vials as a white lyophilized cake, which is sterile. Each vial contains 250 mg Hirulog, [redacted] sodium hydroxide, and 125 mg mannitol. Reconstitution with water yields a clear to opalescent, colorless to slightly yellow solution, pH 5.0-6.0.

Category: Anticoagulant/Thrombin Inhibitor

Related Drugs/INDs/NDAs/MFs: [redacted]

Proposed Marketing Indication: Hirulog is indicated for use as an anticoagulant in patients undergoing percutaneous transluminal coronary angioplasty (PTCA).

Dose: The recommended dosage of Hirulog is a 4 hr IV infusion at a rate of 2.5 mg/kg/hr with an IV bolus dose of 1.0 mg/kg administered immediately after initiation of the infusion, followed by an IV infusion at a rate of 0.2 mg/kg/hr for up to 20 hr as clinically warranted. Treatment with Hirulog should be initiated just prior to PTCA. The dose of Hirulog may need to be reduced, and anticoagulation status monitored in patients with renal impairment.

Preclinical Studies and Testing Laboratories:

The sponsor has used 3 different methods for preparation of hirulog as follows: a solid phase peptide method (SPPM), a homogenous phase pilot scale (HPPS), and a modified homogenous phase commercial scale (HPCS). The modified homogenous phase commercial scale is the form intended for marketing.

APPEARS THIS WAY
ON ORIGINAL

Study	Study #	Testing Laboratory	Batch #	Review Page #
PHARMACOLOGY:				7
ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION:				
ABSORPTION:				
Rats				
IV and SC pharmacokinetics in rats.	P8967-92-10		HPPS	21-22
4 hr IV infusion pharmacokinetic study in rats.	P8967-94-17		HPCS	22
Rabbits				
IV and SC pharmacokinetics study in rabbits.	P8967-92-11		HPPS	23
Monkeys				
Pharmacokinetics in baboons following IV and SC injections.	P90-033		SPPM	24
Pharmacodynamics and pharmacokinetics of Hirulog-S and Hirulog-H in baboons.	P8967-91-01		SPPM and HPPS	24-25
Pharmacokinetic study in cynomolgus monkeys.	P90-032		SPPM	25-26
Pharmacokinetic profile after a single 4 hr IV infusion in monkeys.	P8967-94-14		HPCS	26-27
A 4 hr intravenous infusion pharmacokinetic study in cynomolgus monkeys.	P8967-94-16		HPCS	28
DISTRIBUTION:				
Rats				
ADME study in rats administered ^{14}C and ^{3}H labeled hirulog by IV and SC injection.	P90-035		SPPM	29-31
Tissue distribution of $^{3}H/^{14}C$-labeled hirulog in rats after IV administration.	P8967-93-13		Radiolabel preparation	32-33
Tissue distribution of a ^{3}H-labeled dipeptide following IV administration to rats.	P8967-94-10		Radiolabel preparation	33-35

METABOLISM:				
In Vitro				
Stability in physiological fluids.	TR-10a		HPCS	35-36
Stability in culture media for mutagenicity studies.	TR-10-b		HPCS	36
Rats				
Effect on rat liver microsomal enzymes following a 28 day continuous IV infusion in rats.	P8967-94-06		HPCS	36-37
EXCRETION:				
Rats				
ADME study in rats administered ^{14}C and ^{3}H labeled hirulog by IV and SC injection.	P90-035		SPPM	38-39
Tissue distribution of $^{3}H/^{14}C$-labeled hirulog after IV administration to rats.	P8967-93-13		Radiolabel preparation	39-40
Pharmacokinetics of ^{3}H-labeled hirulog in partially nephrectomized rats.	P8967-92-03		Radiolabel preparation	40-41
Monkeys				
Metabolism and excretion of ^{3}H and ^{14}C-labeled hirulog following IV bolus administration in monkeys.	P8967-93-05		Radiolabel preparation	42-43
TOXICOLOGY:				
Acute Toxicity				
Acute toxicity in mice, rats, and monkeys.	P89-012 P90-002 P8967-93-10 P90-004 P90-003 P90-025 P8967-93-11 P90-005 P90-006	[redacted]	Unknown SPPM HPCS SPPM SPPM Unknown HPCS SPPM SPPM	46-48

Acute IV toxicity of hirulog and its major impurities in rats.	P8967-94-12	[redacted]	Hirulog Lot No. 67A03Z Degradation products: PB19-1 PB19-2 PB19-3	48-49
Acute toxicity of HirulogF and HirulogR after a 4 hr IV infusion in dogs.	P8967-92-06		HPPS	49-50
Acute toxicity of HirulogR and HirulogF after a 4 hr IV infusion in monkeys.	P8967-92-07		HPPS	50-52
<u>Subacute Toxicology</u>				
Rats				
14 day toxicity study of hirulog administered by continuous IV infusion to rats.	P8967-93-02		HPPS	52-58
14 day IV toxicity study of hirulog and partially degraded hirulog in rats.	P8967-94-13		HPCS Degradation product: PB19-4	58-62
14 day IV toxicity study of PB19-6 (D-PHE12-Hirulog) in rats.	P3967-94-14		ASF01130-P 70	63-67
28 day IV toxicity study in the rat.	P90-021		SPPM	67-68
28 day IV infusion toxicity study of hirulog in the rat with a 14 day recovery period.	P8967-94-02		HPCS	68-76
Monkeys				
7 day pilot toxicity study of hirulog administered IV to monkeys.	P90-007		SPPM	76-78
28 day toxicity study of hirulog administered SC to monkeys.	P90-008		SPPM	78-83
28 day IV infusion toxicity study of hirulog in the monkey with a 14 day recovery period.	P8967-94-01		HPCS	83-91

Reproductive Toxicology			
Rats			
Segment I Fertility and Reproductive Performance Study in Rats.	B3967-93-16	HPPS	92-98
Segment II Tetratogenicity Study in Rats.	B3967-93-17	HPPS	99-105
Rabbits			
Segment II Tetratogenicity Study in Rabbits.	B3967-94-07	HPCS	106-111
Rats			
Segment III Pre- and Postnatal Study in Rats.	B3967-97-01	HPCS	112-118
Genotoxicity			
Bacterial mutagenesis assay using pre-incubation method.	B30-19	SPPM	118-119
Bacterial mutagenesis assay using plate incorporation method.	B3967-93-09	HPCS	119-120
Forward gene mutation assay in Chinese hamster ovary cells.	B3967-94-04	HPCS	120-122
Chromosomal aberration test in human peripheral blood lymphocytes.	B3967-94-03	HPCS	122-123
Rat micronucleus test.	B3967-94-05	HPCS	123-124
Rat hepatocyte DNA repair test.	B30-20	SPPM	125

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Special Toxicity Tests				
Irritation studies in rabbits.	P91-123 P8967-92-05 P8967-93-12		Unknown HPPS HPCS	126-127
Hemolysis and plasma protein flocculation of human blood in vitro.	P8967-94-08		HPCS	128
Antigenicity study in guinea pigs.	P90-024		SPPM	128-129
Subacute SC study in rabbits.	P90-023 P91-009		SPPM SPPM	129

PHARMACOLOGY:

Hirulog is a direct thrombin inhibitor that was designed using the leech protein, hirudin, as a model. Hirulog is a 20 amino acid peptide that contains 3 structural domains: **1)** the NH_2-terminal sequence, D-Phe-Pro-Arg-Pro, that interacts with thrombin's active site, **2)** the COOH-terminal sequence, Asn-Gly-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu, derived from amino acid residues 53-64 in hirudin, that binds with the anion-binding exosite (formed by two surface loops (Met^{32}-Leu^{41} and Val^{56}-Ser^{63}), and **3)** an intervening tetraglycyl spacer that links the NH_2 and COOH-terminal sequences. Hirudin irreversibly inhibits thrombin activity. In contrast, the affinity of hirulog for thrombin is 1000-fold lower, which allows reversible binding. Pharmacology studies with hirulog examine its inhibition of thrombin activity, prolongation of the activated partial thromboplastin time in several species, inhibition of thrombin-induced platelet aggregation, anticoagulant activity as compared to heparin and hirudin, and its antithrombotic activity in several animal models.

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Primary Pharmacology

Inhibition by Hirulog of Thrombin Activity In Vitro.

The inhibitory activity of hirulog toward human α-thrombin was examined using a chromogenic substrate, hexahydrotyrosyl-L-alanyl-L-arginine-p-nitroanilide. Hirulog (100 nM to 125 μM) inhibited human α-thrombin activity in a concentration-dependent manner. Hirulog displayed a mixed competitive/non-competitive inhibition of the thrombin catalyzed reaction. For example, with hirulog at 10.1 nM, the K_m for thrombin cleavage of the chromogenic substrate increased from 1.5 to 5.5 μM and the K_{cat} decreased from 17.9 to 11.1 sec^{-1}. The K_i for hirulog inhibition of thrombin cleavage of the chromogenic substrate was 2.3 nM. Hirulog displayed no significant inhibitory action toward human plasmin, bovine trypsin amidolytic activities, or Factor X_a.

Anticoagulant Activity of Hirulog In Vitro in Human and Other Mammalian Plasmas.

The anticoagulant activities of hirulog were assessed in the activated partial thromboplastin time (APTT), prothrombin time (PT), and thrombin time (TT) tests in several species. For human plasma, the APTT was prolonged to 200% of the control by the concentration range of 0.112-0.225 μg/mL and the PT was prolonged to 200% of the control by the concentration range of 0.77-1.54 μg/mL. The TT was prolonged to 304.2 and 900% of the control by 0.115 and 0.867 μg/mL, respectively. Given the specificity of hirulog for thrombin, the order of sensitivity was as follows: TT > APTT > PT. At 0.2 μg/mL, the APTT was prolonged to 196% of the control for human, 168% for monkey, 182% for rat, 171% for murine, 122% for porcine, 116% for canine, 110% for bovine, and 113% for rabbit. Species specificity using APTT was as follows: mouse > rat > human > cynomolgus monkey > bovine > porcine > canine > rabbit with the concentration range of 0.2 to 1 μg/mL.

Species Specific Anticoagulant Activity of Hirulog.

The anticoagulant activity of hirulog, as assessed by APTT, was measured in citrated plasma from human, rabbit, rat, cynomolgus monkey, baboon, pig, and dog. In plasma samples from all species, hirulog displayed a dose-dependent prolongation of the APTT. Hirulog concentrations that prolonged the APTT to 300% of the control were as follows: human, 820 ng/mL; baboon, 350 ng/mL; cynomolgus monkey, 900 ng/mL; rat, 1800 ng/mL; dog, 3500 ng/mL; rabbit, 14000 ng/mL; and pig, 24000 ng/mL.

Effects of Hirulog Toward Soluble and Clot-Bound Thrombin.

The activity of hirulog toward soluble and clot-bound thrombin was assessed. Human α-thrombin was incubated in human plasma containing hirulog at final concentrations of 0.1, 0.5, or 1 μM. Following a 10 or 60 min incubation, samples were assayed for fibrinopeptide A (FPA) release. Washed ^{125}I-labeled fibrin clots were in plasma containing hirulog at 0, 0.5, or 1.0 μM. Following a 10, 60, or 90 min incubation, samples were assayed for FPA release. Hirulog produced a concentration-dependent inhibition of free thrombin-mediated release of FPA. Unlike heparin, hirulog also produced a concentration-dependent inhibition of clot-bound thrombin-mediated release of FPA.

Effects of Hirulog on Human Platelet Aggregation In Vitro.

The activity of hirulog toward human thrombin-induced platelet aggregation was assessed. Hirulog displayed a concentration-dependent inhibitory activity toward thrombin-induced platelet aggregation; although, it had no effect against collagen-induced platelet aggregation. Hirulog concentrations of 0.007, 0.020, 0.034, and 0.067 μg/mL inhibited thrombin-induced platelet aggregation by 21.9, 96.9, 100, and 100%, respectively.

Kinetic Mechanism for the Interaction of Hirulog with Human Thrombin.

A 4 step mechanism for complex formation between hirulog and thrombin has been proposed. The first step with hirulog involves binding of its COOH-terminal hirudin-like region to the anion-binding exosite to form EI^1 (Enzyme-Inhibitor Complex) with a dissociation constant of 0.75 μM. Second, an intramolecular conformational change is induced by this binding to form, EI^2 with a rate constant of 300 s^{-1}. In the third step, the P_1 arginine of hirulog interacts with the primary specificity pocket in a very rapid step to form EI^3. Finally, there is a second intramolecular conformational change to form EI^4 with a rate constant of 30 s^{-1}. Active site interactions of hirulog, described by the formation of EI^3 and EI^4, increase the stability of the hirulog-thrombin complex by 400-fold. Recombinant hirudin and hirulog induce similar conformational changes in thrombin. The K_i for hirulog was determined to be 1.9 nM.

Specificity of Hirulog for Human Alpha-Thrombin.

The specificity of Hirulog was assessed by measuring the ability of this peptide to inhibit the activity of 15 separate serine proteinases. The catalytic site of thrombin resembles that of many serine proteinases, having catalytic-site residues His^{57}, Asp^{102}, and Ser^{95}. However, the Tyr60a-Pro-Pro-Trp60d insertion sequence in thrombin confers restricted access of the thrombin active site to many proteinase inhibitors, thus explaining thrombin's limited activity toward a diverse array of Arg-containing protein or peptide substrates. The sequence of D-Phe-Pro-Arg in hirulog displays a high affinity for thrombin's catalytic site as this tripeptide sequence is the basis for highly specific substrates and active site directed inhibitors. In comparison with its significant inhibitory action on thrombin activity, hirulog had no effects on the activities of Human Factor Xa, Human leukocyte elastase, Bovine chymotrypsin, Human cathepsin G, Human sc-tPA, Human tc-tPA, Bovine trypsin, Human Factor XIIa, Human Protein Ca, Human plasmin, Porcine pancreatic kallikrein, Human plasma kallikrein, Human kidney urokinase, and Human Factor VII_a.

Effects of Hirulog on Tissue-Type Plasminogen Activator (tPA)-Induced Clot Lysis in Human Plasma.

The effects of hirulog toward tissue-type plasminogen activator (tPA)-induced fibrinolysis was examined in a plasma system. Washed ^{125}I-fibrin clots were added to pooled normal human plasma at 37°C containing human tPA at 0, 0.1, or 1.0 µg/mL and hirulog at 0, 1, or 20 µM. Aliquots were removed at time points between 0 and 120 min for measurement of fibrin degradation products. Hirulog had no significant effects on the kinetics or extent of fibrinolysis at any tPA concentration.

Studies on Hirulog Activity in Plasma Coagulation Assays.

The anticoagulant effects of hirulog were examined in vitro using the APTT, PT, and TT assays. Hirulog prolonged coagulation in a dose-dependent manner as indicated by the APTT, PT, and TT assays. Hirulog concentrations required to increase coagulation times by 300% of the control were 820 ng/mL for APTT (baseline value, 30.2 sec), 2600 ng/mL for PT (baseline value, 12.9 sec), and 86 ng/mL for the TT (baseline value, 16.3 sec) assay.

Studies on Hirulog Effects on Platelet Function.

The effects of hirulog on thrombin, ADP, and collagen-induced platelet aggregation were assessed to determine the specificity of this peptide. Hirulog inhibited thrombin-induced platelet aggregation and alpha granule secretion in a dose-dependent manner with an IC_{50} of 81.1 ng/mL; however, it had no effects on ADP or collagen-induced platelet aggregation at concentrations up to 217.8 µg/mL.

In Vitro Comparative Studies of the Anticoagulant Activities of Heparin and Hirulog.

The in vitro anticoagulant activities of heparin and hirulog were compared in APTT assays. The activities of these two agents were compared in plasma obtained from collagen-activated platelet rich plasma to examine the effects of platelet activation on these two agents. Both agents displayed dose-dependent prolongation of APTT. Heparin at 0.06 U/mL prolonged the APTT to 169.9% of the control; however, 0.13 U/mL resulted in a prolongation to 283.4%, suggesting a steep dose response curve. Anticoagulant activity produced by hirulog displayed a biphasic response: concentrations of 3 to 195 ng/mL prolonged the APTT from 103.9 to 200.9% of the control; however, higher concentrations produced a more shallow response (i.e., 781 ng/mL produced a 282.4% prolongation, while 12496 ng/mL produced a 820.1% prolongation). The activity of heparin was significantly reduced in collagen-activated platelet rich plasma (i.e., 0.13 U/mL produced a 233.9% prolongation), while the activity of hirulog was unaffected. The activity of heparin may be reduced by products of platelet activation, while hirulog is unaffected by these products.

Anticoagulant Activity of Hirulog in Plasma from Healthy Human Volunteers and Patients with Coronary Artery Disease.

The anticoagulant activities of hirulog (1.6 µg/mL) and heparin (0.13 U/mL) were tested in plasma obtained from healthy volunteers and patients with stable angina, unstable angina, and acute myocardial infarction. These concentrations are predicted to prolong the APTT to 400% of control. Platelet factor 4 levels were elevated in patient with coronary artery disease as compared to healthy volunteers; although, differences were not significant. Antithrombin III levels were similar in all 4 groups. Hirulog produced a more consistent prolongation of the APTT in healthy volunteers and patients with stable angina, unstable angina, and acute myocardial infarction than heparin.

Studies on Binding of Hirulog to Human Blood Cells and Plasma Proteins.

The potential interaction of hirulog with plasma proteins and blood cells was investigated with regard to inhibition of thrombin in various systems. In order to test for the binding of hirulog to plasma proteins, the concentration dependence for hirulog inhibition of the thrombin-catalyzed reaction was measured in the presence of 5, 10, and 25% citrated human plasma. Recovery of hirulog in dialysis buffer was similar in the absence or presence of plasma. Further, gel filtration experiments revealed no significant plasma protein binding for hirulog. Hirulog exists in a free or uncomplexed form. Binding to red blood cells was 7%. Potential binding of hirulog to plasma proteins or red blood cells appeared to play little role in its pharmacological inhibition of thrombin.

Effects of Hirulog on Recombinant Tissue-Type Plasminogen Activator-Induced Thrombolysis in a Rat Aorta Model: Comparisons to Heparin and Rec-Hirudin.

The effects of hirulog on recombinant tissue-type plasminogen activator (rtPA)-induced fibrinolysis was examined in a rat thrombolysis model and compared with saline, heparin, and recombinant hirudin. rtPA was administered as an intravenous bolus of 1 mg/kg followed by an intravenous maintenance infusion at 1 mg/kg/hr. Concomitant with tPA administration, animals received saline, heparin (10 U/kg, 1.5 U/kg/min), recombinant hirudin (1 mg/kg, 0.02 mg/kg/min), or hirulog (0.6 mg/kg, 0.02 mg/kg/min). Antithrombotic agents or saline were administered concomitant with tPA infusion (30 min duration) and for 50 min following the end of tPA infusion. Hirulog reduced reperfusion time as compared to the control and recombinant hirudin. All three anticoagulant treatments reduced reocclusion compared to the control. Vessel patency was increased by all 3 anticoagulants, with hirulog being the highest.

Antithrombotic Activity of Hirulog in a Rat Model of Carotid Endarterectomy.

The antithrombotic activities of hirulog and heparin were compared in a microsurgical carotid endarterectomy model in the rat. In the first set of experiments, hirulog was administered as 0.8 mg/kg IV bolus followed by a 2.2 mg/kg/hr intravenous infusion or heparin was administered as an intravenous bolus of 10 U/kg followed by a 90 U/kg/hr intravenous infusion. In a second set of experiments, hirulog was administered as 0.4 mg/kg IV bolus followed by a 1.0 mg/kg/hr intravenous infusion. Saline was used as the control in both sets of experiments. Low dose hirulog inhibited fibrin deposition, while the higher dose inhibited both platelet and fibrin deposition. Heparin also inhibited both platelet and fibrin deposition.

Antithrombotic Activity of Hirulog in a Rabbit Model of Platelet Embolization in the Cranial Vasculature.

The effect of hirulog was evaluated on bovine thrombin-induced (90 mU/kg, intracarotid administration) intracranial accumulation of ^{111}In-labeled platelets in the cranial vasculature of anesthetized rabbits. Hirulog was administered as an intracarotid bolus dose at 0.5, 0.1, or 0.2 mg/kg, 1 min prior to intracarotid administration of thrombin. Hirulog, in a dose-dependent manner, decreased platelet accumulation. Platelet aggregation increased by 83% in the control, but declined to 62.9, 19.8, and 2.0% with hirulog at 0.05, 0.1, and 0.2 mg/kg, respectively.

Antithrombotic Activity of Hirulog in a Porcine Carotid Artery Model of Recurrent Arterial Thrombotic Occlusion.

The effects of hirulog on arterial thrombus formation were examined in a porcine carotid artery model of recurrent arterial thrombotic occlusion. After a 30 min period of recurrent occlusive cycles, saline, heparin, or hirulog treatment was initiated. Hirulog was administered either as a 1.2 mg/kg intravenous bolus followed by a continuous intravenous infusion of 2.4 mg/kg/hr or a 2.4 mg/kg bolus followed by a continuous intravenous infusion of 4.8 mg/kg/hr. There were dose-dependent prolongations of the APTT and bleeding time. The higher dose of hirulog substantially inhibited recurrent arterial thrombosis and reduced occlusion frequencies with minimal effects on coagulation and bleeding time. Heparin was administered as follows: a 50 U/kg intravenous bolus followed by a 50 U/kg/hr infusion; a 250 U/kg bolus injection; or a 500 U/kg bolus injection. All heparin treatments prolonged the APTT; however, the two higher dose treatments prolonged bleeding time. Only the highest dose of heparin reduced the frequency of occlusions where coagulation was prolonged 20-fold and bleeding time was increased 4-fold.

Effects of Hirulog on Anticoagulation, Template Bleeding Time, and Platelet-Dependent Thrombus Time in Baboons.

The anticoagulant effect of hirulog was examined in baboons (Papio anubis) at doses ranging from 0.12 to 12.0 mg/kg/hr administered as a continuous intravenous infusion. For studies of template bleeding time, hirulog was administered at doses of 0.12 to 24.0 mg/kg/hr as a continuous intravenous infusion. The effects of hirulog on platelet-dependent thrombosis were determined in a baboon AV shunt model. Thrombogenic surfaces included an endarterectomized baboon aorta, collagen-coated Gortex, Dacron vascular graft, and a two chamber device designed to simulate both

arterial and venous thrombosis. Hirulog in a dose-dependent manner prolonged the APTT. At doses of 0.12 and 12 mg/kg/hr, the APTT was 173.2 and 900% of the control, respectively. Hirulog doses ≤3.0 mg/kg/hr had no effect on bleeding time; however, higher doses increased bleeding time. For the endarterectomized baboon aorta, doses ≤ 0.6 mg/kg/hr interrupted deposition of platelets and fibrin in a dose-dependent manner. For the collagen-coated Gortex and the Dacron vascular graft, deposition of platelets and fibrin was interrupted by a dose of 12 mg/kg/hr. Platelet deposition in models of arterial and venous-like thrombus formation were interrupted at a dose of 6 mg/kg/hr. Hirulog is an effective agent for prevention of high shear, platelet-dependant thrombus formation.

Hirulog Pharmacokinetics and Pharmacodynamics of Anticoagulant Activity in Baboons Following Intravenous or Subcutaneous Administration.

The pharmacokinetics and pharmacodynamics of 1 mg/kg Hirulog were examined in baboons (Papio anubis) following intravenous bolus or subcutaneous administration. A chronic arteriovenous shunt between the femoral artery and vein was prepared. Following intravenous or subcutaneous administration, pharmacological activity (i.e., prolongation of the APTT) paralleled plasma levels of hirulog. Pharmacological activity and plasma hirulog levels peaked at 2 min after intravenous administration and returned to baseline within 2 hr after administration. Pharmacological activity and plasma hirulog levels peaked at 30-180 min after subcutaneous administration and returned to baseline within 24 hr after administration.

Interactions of Hirulog with Aspirin, Aspirin and tPA and Aspirin, Urokinase, and Warfarin.

The interaction between aspirin and hirulog was examined in male and female Sprague Dawley rats. Rats were pretreated with aspirin (0, 600 or 800 mg/kg) at 1 hr prior to intravenous treatment with hirulog (0 or 100 mg/kg). Clinical signs (i.e., decreased activity, abnormal gait, abnormal stance, body drop, dyspnea, red anal discharge, prostration, tremors, and convulsion), incidence of mortality, and findings at necropsy (i.e., dark liver, aspirin in the stomach, red nasal discharge, enlarged, pale and mottled kidneys, hyperemia of the mucosa of the glandular portion, and dark red linear areas of the glandular mucosa) were similar among comparable treatment groups (i.e., aspirin alone versus aspirin + hirulog).

This study compared the substitution of hirulog for heparin in conjunctive thrombolytic therapy in rabbits and subsequent development of toxicity. Aspirin (25 mg/kg oral) and [redacted] (25,000 IU/kg intravenous for 1 hr) were administered in conjunction with either hirulog (5 mg/kg/hr intravenous for 48 hr) or heparin (100 U/kg intravenous bolus and an intravenous infusion of 50 U/kg/hr for 48 hr). Hirulog administered in the presence of aspirin and [redacted] did not produce any unexpected toxic effects.

The toxicity of hirulog at 4 and 40 mg/kg/day administered by continuous intravenous infusion was examined in male beagle dogs that received the study drug by continuous intravenous for 7 days in conjunction with oral doses of 50 mg aspirin on days 1, 3, and 5. The control group received saline and empty gelatin capsules. There were no significant gross or histopathological changes for the 4 or 40 mg/kg/day groups. The observed increases in PT and APTT following treatment with hirulog + aspirin were expected given the anticoagulant natures of these compounds. Differences between the control group and the 4 and 40 mg/kg/day groups were minor and appeared to have little biological significance.

Interactions between either hirulog (administered by intravenous infusion at 3 mg/kg/hr for 48.25 hr starting 15 min prior to the tPA infusion) or heparin (100 U/kg intravenous bolus loading dose followed by a 48 hr continuous intravenous infusion at 50 U/kg/hr) with tPA (administered as a 3 hr intravenous infusion at 0.75 mg/kg/hr for the first hr and at 0.25 mg/kg/hr for the remaining 2 hr) and aspirin (40 mg administered by the oral route on day -2 and ~30-45 min prior to the start of the tPA infusion) were examined in rhesus monkeys. There was no unexpected toxicity in monkeys that received hirulog + tPA + aspirin as all observed effects appeared to be exaggerated pharmacological actions of these compounds.

Potential interactions between hirulog (administered by intravenous bolus injection of hirulog at 3 mg/kg followed immediately by intravenous infusion of hirulog at 7.5 mg/kg/hr for 4 hr and then at 0.6 mg/g/hr for 20 hr) and urokinase (2 hr infusion (3 mL/hr) of urokinase at 0 or 1000 U/min) were examined in cynomolgus monkeys. Treatment with hirulog led to a significant increase of the APTT; although, addition of urokinase had minimal effect on the hirulog-induced increase of APTT.

Potential interactions between, the two anticoagulants, hirulog (administered as a 3 mg/kg intravenous bolus loading dose followed immediately by a continuous infusion for 4 hr at 7.5 mg/kg/hr and 20 hr at 0.6 mg/kg/hr) and warfarin (0 or 0.15-2 mg/kg) were examined in cynomolgus monkeys (Macaca fascicularis). The combination of hirulog and warfarin did not further prolong the PT and APTT as compared with hirulog alone. Pharmacokinetic parameters (T_{max}, C_{max}, and AUC_{0-last}) for hirulog were not altered by combination with warfarin.

Hirulog inhibited human α-thrombin activity in a concentration-dependent manner. This inhibition was of a mixed competitive/non-competitive nature. The formation of the Hirulog-thrombin complex has been postulated to involve a 4 step mechanism. The first step with hirulog involves binding of its COOH-terminal hirudin-like region to the anion-binding exosite to form EI^1 (enzyme-inhibitor complex^1) with a dissociation constant of 0.75 μM. Second, an intramolecular conformational change is induced by this binding to form, EI^2 with a rate constant of 300 s^{-1}. In the third step, the P_1 arginine of hirulog interacts with the primary specificity pocket in a very rapid step to form EI^3. Finally, there is a second intramolecular conformational change to form EI^4 with a rate constant of 30 s^{-1}. Active site interactions of Hirulog, described by the formation of EI^3 and EI^4, increase the stability of the Hirulog-thrombin complex by 400-fold. The K_i for Hirulog was determined to be 1.9 nM. Using the activated partial thromboplastin time (APTT) as an index of blood coagulation, species specificity for prolongation of the APTT by hirulog was as follows: mouse > rat > human > cynomolgus monkey > bovine > porcine > canine > rabbit. Hirulog (0.1-1 μM) produced a concentration-dependent inhibition of free thrombin-mediated release of fibrinopeptide A (FPA). Unlike heparin, hirulog produced a concentration-dependent inhibition of clot-bound thrombin-mediated release of FPA. Hirulog inhibited thrombin-induced platelet aggregation and alpha granule secretion in a concentration-dependent manner (IC_{50} = 81.1 ng/mL). The activity of heparin may be reduced by products of platelet activation, while Hirulog was unaffected by these products. Binding to plasma proteins or interaction with red blood cells play no role in the pharmacological action of hirulog. In a rat thrombolysis model, rtPA-induced fibrinolysis in combination with hirulog significantly reduced reperfusion time as compared to recombinant hirudin. Hirulog was superior to hirudin or heparin with regard to increasing vessel patency in this model. In a microsurgical carotid endarterectomy model using the rat, hirulog inhibited both platelet and fibrin deposition. Hirulog, in a dose-dependent manner, inhibited bovine thrombin-induced intracranial accumulation

of ^{111}In-labeled platelets in the cranial vasculature of anesthetized rabbits. In a porcine carotid artery model of recurrent arterial thrombotic occlusion, hirulog inhibited recurrent arterial thrombosis and reduced occlusion frequencies with minimal effects on coagulation and bleeding time. For the endarterectomized baboon aorta, collagen-coated Gortex and Dacron vascular graft, or in models of arterial and venous-like thrombus formation, hirulog interrupted deposition of platelets and fibrin. Hirulog produced no unexpected toxic interactions when administered in combination with either aspirin, [redacted] and aspirin, tPA and aspirin, urokinase, or warfarin.

Safety Pharmacology

Neuropharmacological Profile.

The effects of Hirulog on alertness, spontaneous motor activity, ataxia, and convulsions were observed in CD-1 mice over a period of 24 hr following intravenous treatment with 5 mg/kg. An additional 4 mice received Hirulog by the intravenous route at 5 mg/kg followed by electroshock treatment 10 min later. No significant clinical signs or anticonvulsant activity were observed following intravenous treatment with Hirulog at 5 mg/kg.

Phenylquinone (PPQ) Writhing Assay.

The effects of Hirulog on phenylquinone (PPQ)-induced writhing were examined in CD-1 mice following intravenous treatment with 5 mg/kg. Mice were treated with PPQ by the intraperitoneal route at 15, 30, and 60 min following treatment with Hirulog, or 60 min following the control treatment. Hirulog had no effects on PPQ-induced writhing at 5 mg/kg.

Gastrointestinal Propulsion Assay.

The effects of Hirulog on gastrointestinal propulsion were examined in male CD-1 mice following intravenous treatment with 5 mg/kg. A 10% suspension of activated charcoal was administered by the oral route 10 min after treatment with Hirulog. Mice were sacrificed 30 min after charcoal treatment and the distance traveled by the charcoal in the small intestine was determined. Hirulog had no significant effects of transit of a charcoal meal in the small intestine.

Barbiturate Sleep Time Assay.

The effects of Hirulog on pentobarbital-induced sleeping time were examined following intravenous treatment with 5 mg/kg in mice. Pentobarbital was administered by the intraperitoneal route at 10 min after Hirulog treatment. The time of loss of righting reflex was determine. Mice were observed until the righting reflex was recovered. Hirulog increased the pentobarbital-induced sleeping time by 68%; although, this effect was not considered biologically significant.

Antiulcerogenic Assay.

The antiulcerogenic effects of Hirulog were assessed in a rat ulcerogenic model. Pyloric ligation was performed on male Sprague Dawley rats followed by oral administration of 300 mg/kg aspirin. Rats were treated by the intravenous route with either Hirulog at 1 or 5 mg/kg or citmedine at 1 or 5 mg/kg. Hirulog at 1 or 5 mg/kg reduced gastric ulcerations by 8 or 76%, respectively. The positive control, citmedine at 1 or 5 mg/kg reduced ulcerations by 56 or 85%, respectively.

Cardiovascular Pharmacodynamic Effects in Rats.

The effects of Hirulog administered by intravenous route at 1 and 5 mg/kg on heart rate and blood pressure were assessed in male Sprague Dawley rats anesthetized with isoflurane. Positive control agents included epinephrine (1 and 2 µg/kg), norepinephrine (1 and 2 µg/kg), isoproterenol (2 µg/kg), acetylcholine (10 µg/kg), and histamine (100 µg/kg). Each group of 6 rats received all 5 agonists. At 5 and 30 min following treatment with Hirulog, animals were treated with agonists. Pre-dose-agonist responses were compared to post-dose agonist responses. Intravenous treatment with 1 or 5 mg/kg did not produce changes in mean pressor or depressor responses to agonists that would suggest antagonism or potentiation. A dose-related blood pressor effect was observed following intravenous treatment with Hirulog; although, no effect on heart rate was observed. At 1 mg/kg, blood pressure increased from 88/53 to 99/64. At 5 mg/kg, blood pressure increased from 98/67 to 133/93.

Cardiovascular Effects in Dogs.

The effect of Hirulog at 5 mg/kg infused over a 15 min period on cardiovascular responses (i.e., systolic arterial pressure, diastolic arterial pressure, mean arterial pressure, heart, cardiac output, left ventricular pressure, left ventricular end diastolic pressure, dP/dt, contractile force) were assessed in 2 male and 2 female beagle dogs anesthetized with sodium thiamylal. The vehicle

had no effect on cardiovascular parameters. Infusion of Hirulog increased systolic pressure (increase of 28-51% over baseline of 93 mm Hg), diastolic pressure (increase of 16-98% over baseline of 48 mm Hg), mean arterial pressure (increase of 13 to 77% over baseline of 64 mm Hg), left ventricular pressure (increase of 18 to 40% over baseline of 94 mm Hg), and left ventricular end diastolic pressure (increase of 26 to 74% over the baseline of 7.2 mm Hg). Following the 15 min infusion period, all parameters returned to predose values.

Effect of Hirulog on Diuresis in Rats.

Hirulog at 1 or 5 mg/kg had no effects on urinary volume and pH and electrolyte concentrations of Na^+ and K^+ in Sprague Dawley rats at 4 hr after dosing.

Effect of a Single Four-Hour Intravenous Infusion of Hirulog on the Glomerular Filtration Rate of Male Dogs.

The effect of Hirulog on the glomerular filtration rate (GFR) was evaluated in six male beagle dogs. GFR was measured by monitoring the clearance of ^{99m}Tc-radiolabeled diethylenetriamine pentaacetic acid (Tc-DPTA), which is cleared from the blood solely by GF. Hirulog was administered by continuous intravenous infusion (1 mL/kg/hr) over a 4 hr period at a dose rate of 1.7 mg/kg/hr. Tc-DPTA was administered 1 hr after the start of the infusion. Blood for determination of creatinine clearance, renal function, and other clinical chemistry parameters was collected immediately prior to the start of the infusion on each treatment day and 24 hr later. Blood for determination of the APTT and plasma hirulog levels was collected prior to the start of the infusion and immediately prior to the end of the infusion. There was no mortality or clinical signs of toxicity. APTT values were slightly prolonged during treatment with hirulog (27.0 sec) as compared to saline (16.2-19.3 sec). Hirulog did not have any significant effects on clinical chemistry parameters or renal function. Clearance of hirulog in dogs was 859 mL/kg/hr. Prior to the end of the drug infusion, the mean plasma hirulog level was 1991 ng/mL with a range of 224 to 5247 ng/mL.

Effect of Hirulog on Mean Arterial Blood Pressure and Heart Rate Following Intravenous Infusion in Baboons.

The possible pressor effects of Hirulog infusion at doses of 1 mg/kg/15 min and 5 mg/kg/15 min were examined by measurement of mean arterial blood pressure and heart rate in two juvenile male baboons (Papio anubis). Each baboon was treated first with a 15 min infusion of Hirulog at 1.0 mg/kg and mean blood pressure and heart rate were monitored during the infusion. Fifteen min after completion of the first dose, each baboon was treated with 5 mg/kg infused for 15 min. Infusion of Hirulog at doses of 1 or 5 mg/kg had no significant effects on mean arterial blood pressure or heart rate.

A Pharmacokinetic Study of Hirulog in Cynomolgus Monkeys.

This study examined the effects of Hirulog administered by either the intravenous or subcutaneous route at 4 mg/kg/day for 7 days on pharmacokinetics and blood pressure in two adult cynomolgus monkeys (1 male and 1 female). Monkeys were treated by either the intravenous or subcutaneous route during the first week and by the alternate route during the third week. Bleeding time was not affected at 1 hr after intravenous or subcutaneous treatment. Following intravenous administration, the APTT was significantly increased within 2 min after administration from a baseline average of 19.9 sec to 180.8 sec and returned to baseline between 120 and 180 min following administration. Following subcutaneous administration, the APTT was increased within 2 min after administration from a baseline average of 21.3 sec to 35.1 sec and returned to baseline between 4 and 6 hr after administration. The sponsor stated that there were no significant changes in mean arterial blood pressure following either subcutaneous or intravenous treatment with Hirulog in the presence of ketamine anesthetia. The male monkey died on day 21 due to acute septicemia and/or bacteremia possibly related to a tooth abscess or the indwelling catheter. The female animal died on day 26 due to acute nephritis and hepatitis. Staphylococcus aureus was cultured from tissue fluids in both animals. Hirulog was quantified by an inhibitory enzyme immunoassay. The mean C_{max} (29380 and 23569 ng/mL on days 1 and 7, respectively) with intravenous administration were significantly higher than the mean C_{max} (2606.5 and 1635 ng/mL on days 1 and 7, respectively) with subcutaneous administration. Further the T_{max} with IV administration was 2 min as compared to a T_{max} ≥30 min with subcutaneous administration. Mean AUC values (10287 and 10465 ng*hr/mL on days 1 and 7, respectively) with intravenous administration were greater than AUC values (8189 and 2636 ng*hr/mL on days 1 and 7, respectively) with subcutaneous administration.

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION:

Radiolabeled hirulog used in metabolism and excretion studies:

a. ^{3}H-labeled:
D-Phe-(^{3}H-Pro)-Arg-Pro-Gly-Gly-Gly-Gly-Asg-Gly-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu

b. ^{14}C-labeled (the label could be located in any of the glycine residues):
D-Phe-Pro-Arg-Pro-(^{14}C-Gly)-(^{14}C-Gly)-(^{14}C-Gly)-(^{14}C-Gly)-Asg-(^{14}C-Gly)-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu

Absorption

Rats

Hirulog: Intravenous and Subcutaneous Pharmacokinetics Study in Rats (Biogen Study No. P8967-92-10).

Methods: Hirulog (homogenous phase pilot scale) was administered to male Sprague Dawley rats by either the subcutaneous route at doses of 50 and 250 mg/kg or the intravenous route at a dose of 50 mg/kg for assessment of plasma hirulog levels, APTT values, and PT values. Blood samples were collected from the retroorbital sinus of each rat prior to dosing and from 4 rats at each time point. Plasma hirulog levels were measured by an enzyme-linked immunoassay that detects the peptide through its ability to inhibit the binding of a specific monoclonal antibody to a bovine serum albumin (BSA)-peptide conjugate bound to the solid phase. The BSA-peptide conjugate is hirulog analog, H133, that is covalently coupled to BSA using a heterobifunctional reagent.

Results: One animal, that received hirulog by the subcutaneous route at 250 mg/kg, was found dead 9 hr after dosing due to bleeding from the nose and eyes. Following intravenous administration of hirulog at 50 mg/kg, the APTT was elevated to >300 sec at 2 and 15 min after dosing, and returned to baseline (~35 sec) by 2 hr. Following subcutaneous administration of hirulog at 50 mg/kg, the APTT was elevated to 195 and 210 sec at 15 and 30 min after dosing, respectively, and returned to baseline (~35 sec) between 4 and 24 hr. Following subcutaneous administration of 250 mg/kg, APTT values were elevated to >300, >300, and 248 sec at 15, 30, and 60 min after dosing, and returned to baseline (~35 sec) between 4 and 24 hr. Changes in PT values following intravenous or subcutaneous administration occurred in a similar manner as described for APTT. Following subcutaneous

administration of 50 or 250 mg/kg to rats, C_{max} values were not proportional to dose. The C_{max} following intravenous administration of 50 mg/kg was 10 times that observed with subcutaneous administration of 50 mg/kg. AUC values were not reported.

Plasma hirulog C_{max} and T_{max} values following intravenous administration of 50 mg/kg or subcutaneous administration of 50 or 250 mg/kg to male rats.

Group	Route	Dose, mg/kg	C_{max}, ng/mL	T_{max}, hr
1	IV	50	381,500	Immed. postdose
2	SC	50	37,738	30 min
3	SC	250	81,750	30 min

A Four Hour Intravenous Infusion Pharmacokinetic Study of Hirulog in the Albino Rat (Biogen Study No. P8967-94-17).

Methods: Plasma pharmacokinetics of hirulog (homogenous phase pilot scale)were determined in rats that received a 4 hr intravenous infusion with doses of 1.04, 3.12, or 10.42 mg/kg/hr. The dose rate was 2 mL/kg/hr. Each group was composed of 5 male and 2 female rats. Blood for determination of plasma hirulog levels and APTT was collected prior to treatment, at time points between 0.083 and 12 hr after dosing.

Results: Plasma hirulog C_{max} and AUC values were approximately proportional to dose. Plasma hirulog levels returned to baseline at 1.5 to 2 hr after discontinuation of the infusion. For male rats, elevations of APTT prior to the end of the infusion were approximately proportional to dose; however, no measurements were made for female rats.

Plasma pharmacokinetics of hirulog determined in rats that received a 4 hr intravenous infusion with doses of 1.04, 3.12, or 10.42 mg/kg/hr.

Dose mg/kg/hr	T_{max}, hr	C_{max}, ng/mL	$AUC_{0-4.33}$ ng·hr/mL	$AUC_{0-5.5}$ ng·hr/mL	AUC_{0-6} ng·hr/mL	k_{el}, hr^{-1}	$T_{1/2}$, hr
1.04	1	2468	6970	7575	7791	0.321	2.2
3.12	1	4525	11772	-	-	-	
10.42	1	19484	60341	62668	-	1.693	0.4

Rabbits

Hirulog: Intravenous and Subcutaneous Pharmacokinetics Study in Rabbits (Biogen Study No. P8967-92-11).

Methods: Plasma hirulog levels and APTT values were determined for male rabbits that received hirulog (homogenous phase pilot scale) by the intravenous route at 50 mg/kg or the subcutaneous route at 50 or 250 mg/kg. There were 3 male rabbits per group. Following intravenous administration, blood for determination of plasma hirulog levels was collected prior to treatment, immediately after dosing, and at 0.25, 0.5, 1, 2, 4, 6, 12, and 24 hr after dosing. Blood for determination of APTT values was collected prior to dosing and at 0.25, 1, 4, 6, and 24 hr after dosing. Following subcutaneous administration, blood for determination of plasma hirulog levels was collected prior to treatment and at 0.25, 0.5, 1, 2, 4, 6, 12, and 24 hr after dosing. Blood for determination of APTT values was collected prior to treatment and at 0.5, 2, 4, 6, and 24 hr after treatment.

Results: Following intravenous administration of hirulog at 50 mg/kg, the plasma C_{max} was 31 times that observed by the subcutaneous route. Plasma C_{max} values for hirulog following subcutaneous administration were proportional to dose. Plasma AUC values were not reported. Following intravenous administration of 50 mg/kg, the APTT was elevated to 225.7 and 174.3 sec at 0.25 and 1 hr after dosing, respectively, and by 4 hr had returned to baseline (26.7 sec). Following subcutaneous administration of 50 mg/kg, the APTT was elevated 39.7 and 33.5 sec at 0.5 and 2 hr after dosing, and returned to baseline (18.7 sec) between 6 and 24 hr after dosing. Following subcutaneous administration of 250 mg/kg, the APTT was elevated to 51.5, 100, 51.5, and 50.0 sec at 0.5, 2, 4, and 6 hr after dosing and returned to baseline by 24 hr.

Plasma hirulog C_{max} and T_{max} values in male rabbits that received hirulog by the intravenous route at 50 mg/kg or the subcutaneous route at 50 or 250 mg/kg.

Dose	C_{max}, ng/mL	T_{max}, min
50 mg/kg, intravenous route	635,478	2
50 mg/kg, subcutaneous route	20,400	35
250 mg/kg, subcutaneous route	110,398	45

Monkeys

Hirulog Pharmacokinetics in Baboons Following Intravenous and Subcutaneous Injections (Biogen Study No. P90-033).

Methods: Plasma hirulog levels and APTT values were determined in baboons (Papio anubis/cynocephalus) that received hirulog (solid phase peptide method) by the subcutaneous or intravenous route at a dose of 1 mg/kg. These animals were prepared surgically with a chronic arteriovenous shunt between the femoral artery and vein. There were 4 and 2 animals for the intravenous and subcutaneous studies, respectively.

Results: The C_{max} value following intravenous administration was approximately 12.6 times that following subcutaneous administration; however, cumulative AUC values were approximately equivalent. The rise in cumulative AUC following subcutaneous administration was much slower than that observed for intravenous administration. The maximum cumulative AUC values were achieved at approximately 50 and 350 min following intravenous or subcutaneous administration, respectively. Bioavailability of hirulog when administered by the subcutaneous route to monkeys is approximately 97.3%. Following intravenous administration, the APTT was elevated to 600% of the control at 2 min after dosing and declined to baseline by 120 min. Following subcutaneous administration, the APTT was elevated to ~200% of the control at 15 min after dosing and gradually declined to ~150% by ~350 min.

Plasma pharmacokinetics of hirulog in baboons (Papio anubis/cynocephalus) that received hirulog by the subcutaneous or intravenous route at a dose of 1 mg/kg.

Injection Route	$T_{½}$ min	T_{max} min	C_{max} ng/mL	Cumulative AUC min·ng/mL
Intravenous	11	2	12,200	185000
Subcutaneous	220	15	970	180000

Pharmacodynamics and Pharmacokinetics of Hirulog-S and Hirulog-H in Baboons (Biogen Study No. P8967-91-01).

Methods: The pharmacodynamics and pharmacokinetics of hirulog produced by solid-phase (Hirulog-S) or homogeneous-phase (Hirulog-H) synthesis were compared in baboons. Two groups of 4 baboons (2 males and 2 females) received Hirulog-S and Hirulog-H by the intravenous or subcutaneous routes at 1 mg/kg in a crossover

design. Following intravenous administration, blood for determination of plasma hirulog levels and APTT values was collected at 0, 2, 5, 10, 15, 30, 60, 120, 180, 360, and 1440 min after dosing. Following subcutaneous administration, blood for determination of plasma hirulog levels and APTT values was collected at 0, 15, 30, 60, 90, 180, 360, and 1440 min after dosing.

Results: Following intravenous administration of either hirulog-H or hirulog-S, APTT values were elevated to 273.5 and 278.8 sec at 2 min after dosing, respectively. Values declined to baseline (36.3-37.3 sec) within 2 hr after dosing. Following subcutaneous administration of Hirulog-S, APTT values were elevated to 176.8-253% of the control (35.3 sec) between 15 and 180 min after dosing. Following subcutaneous administration of Hirulog-H, APTT values were elevated to 162-209% of the control (38.7) between 15 and 90 min after dosing. Following intravenous administration of Hirulog-S or Hirulog-H, plasma drug levels reached maximum levels at 2 min after dosing of 22,748 and 26,562 ng/mL, respectively. Following subcutaneous administration of Hirulog-S, a maximum plasma level of 1215 ng/mL was obtained at 30 min after dosing. Following subcutaneous administration of Hirulog-H, a maximum plasma level of 1116.7 ng/mL was obtained at 45 min after dosing. Plasma AUC values were not provided following intravenous or subcutaneous administration for either hirulog preparation. Following intravenous administration of either Hirulog-H or Hirulog-S, plasma drug levels displayed a high degree of correlation with anticoagulant activity (as reflected by APTT values). The pharmacological action of hirulog is prolonged following subcutaneous administration as compared with intravenous administration; although, bolus intravenous administration yields a C_{max} value that is ~20 times that observed with subcutaneous administration.

A Pharmacokinetic Study of Hirulog in Cynomolgus Monkeys (Biogen Study No. P90-032).

Methods: Plasma pharmacokinetics and blood pressure were evaluated in cynomolgus monkeys (1 male and 1 female) following intravenous or subcutaneous administration of hirulog (solid phase peptide method). Hirulog was administered by intravenous bolus to the male monkey at a dose of 4 mg/kg/day for 7 days, while the female monkey received hirulog by the subcutaneous route at a dose of 4 mg/kg/day for 7 days. On day 15, the route of administration was alternated. Body weight was measured on days 1, 8, 15 and 22. Bleeding times were determined prior to the start of treatment and at 1 hr after treatment with hirulog on days 1, 7, 15, and 21. Blood for determination of plasma drug levels and APTT values were collected on days 1, 7, 15, and 21 immediately prior to treatment and at 2, 5, 10, 15, 30, 60, 120, 180, 240, 360, and 1440 min after dosing.

Results: Following intravenous administration of hirulog, maximal elevations of APTT (161.4-195.2 sec) occurred at 2 min after dosing on the first or seventh day of treatment and returned to baseline (17.7-22.9 sec) within 4 to 6 hr. Following subcutaneous administration of hirulog, APTT values were elevated to 159-345% of the baseline from 2 min to 4 hr after dosing and returned to baseline (18.4-26.2 sec) by 6 hr. Following intravenous treatment with hirulog, the maximal plasma level was observed at 2 min after dosing. For the male, AUC values following intravenous and subcutaneous administration of hirulog were similar. However, for the female, AUC values following intravenous administration were 2 to 3 times AUC values observed following subcutaneous administration. The male monkey was found dead, 1 day prior to the completion of the in-life phase of the study. The female monkey was found dead, 4 days after completion of the in-life phase of the study. Death for both animals was attributed to systemic Staphylococcus aureus infection, possibly related to placement of catheters for drug administration and blood collection.

Plasma pharmacokinetics of hirulog in cynomolgus monkeys (1 male and 1 female) following intravenous or subcutaneous administration.

Parameter	Intravenous Route				Subcutaneous Route			
	Male		Female		Male		Female	
	Day 1	Day 7	Day 15	Day 21	Day 15	Day 21	Day 1	Day 7
AUC ng*hr/mL	12046	11439	8528	9491	11469	-	4909	2636
C_{max}, ng/mL	37188	24102	21572	23036	3382	-	1831	1635
T_{max}, min	2	2	2	2	120	-	30	30

Acute Pharmacokinetic and Pharmacodynamic Profile of Hirulog After a Single Four-Hour Intravenous Infusion in Cynomolgus Monkeys (Biogen Study No. P8967-94-14).

Methods: The dose-dependent pharmacokinetic and pharmacodynamic profiles of a commercial chemistry lyophilized formulation of hirulog (homogenous phase commercial scale) were assessed in cynomolgus monkeys (Macaca fascicularis). Three male and three female monkeys were used in this study. Hirulog was infused intravenously over a 4 hr period at doses of 1.7, 6.25, and 17 mg/kg/hr on days 1, 15, and 29, respectively. Dosing solution concentrations were 1.7, 6.25, and 17.0 mg/mL, respectively. Hirulog was administered by a percutaneous catheter placed in the right or left saphenous vein at an infusion rate of 1.0 mL/kg/hr. Animals were observed for 14 day periods between each dose level.

Blood for determination of plasma drug levels was collected prior to the start of the infusion and at 15, 30, 60, 120, 230, 245, 250, 155, 260, 270, 280, 300, 330, 360, and 420 min after the start of the infusion. Blood for determination of APTT values was collected prior to the start of the infusion and at 60, 230, 250, 260, 280, 300, and 360 min after the start of the infusion. Blood for determination of hematological and clinical chemistry parameters was collected on days -1, 14, and 28 prior to treatment.

Results: Plasma C_{max} and AUC values for hirulog were proportional to dose. Clearance values (349.4 to 875.7 mL plasma/hr/kg) exceeded the glomerular filtration rate (76 mL plasma/hr/kg); however, they were less renal plasma flow (1010 mL plasma/hr/kg) or liver plasma flow (1595.8 mL plasma/hr/kg). The volume of distribution (210 to 586 mL/kg) exceeded blood volume (73.4 mL/kg) by approximately 3- to 8-fold suggesting distribution into tissues. The half life was <1 hr. Increases in APTT occurred in a dose-dependent manner. For the low dose, 1.7 mg/kg/hr, APTT values reached a maximum of 58.5 sec between 60-230 min and then returned to baseline (18.3-22.4 sec, within 2 hr after discontinuation of the infusion. For 6.25 mg/kg/hr, APTT values reached a maximum of 72.6 sec between 60-230 min and then returned to baseline (18.9-21.6 sec) within 4 hr after discontinuation of the infusion. For the high dose, 17 mg/kg/hr, APTT values reached a maximum of >212 sec between 60-230 min and then returned to baseline (19.2-23.5 sec) at approximately 6 hr after discontinuation of the infusion. One female died on day 15 at 2.5 hr after completion of the infusion. Gross necropsy found a pale liver and a purple subcutaneous focus around the left saphenous vein where hirulog was administered.

Pharmacokinetic parameters for hirulog in cynomolgus monkeys that received a 4 hr intravenous infusion at doses of 1.7, 6.25, and 17 mg/kg/hr on days 1, 15, and 29, respectively.

Parameter	1.7 mg/kg/hr		6.25 mg/kg/hr		17 mg/kg/hr	
	Male	Female	Male	Female	Male	Female
C_{max}, ng/mL	2799	5145	17679	27096	62333	54110
T_{max}, hr	3.84	2.62	3.85	3.41	3.84	4.08
AUC_∞, ng*hr/mL	8235	15873	61322	80970	198118	197260
$T_{1/2eff}$, hr	0.46	0.50	0.44	0.52	0.49	0.41
Cl_{tot}, mL/hr/kg	875.7	503	425.9	395.2	366.9	349.4
V_{ss}, mL/kg	586	307.7	274.7	317.7	256.3	210

A 4-Hour Intravenous Infusion Pharmacokinetic Study of Hirulog in the Cynomolgus Monkey (Biogen Study No. P8967-94-16).

Methods: Plasma and urinary levels of hirulog and its metabolites in cynomolgus monkeys were evaluated following a 4 hr intravenous infusion with hirulog (homogenous phase commercial scale) at a dose of 7.5 mg/kg/hr. A catheter was placed in the femoral vein and the tip reached the approximate level of the kidneys. There was one group consisting of 2 male and 2 female monkeys. The dose rate was 1 mL/kg/hr. Blood for determination of plasma hirulog and metabolite levels was collected prior to the start of treatment, immediately prior to the end of the infusion, and at 1 hr after the end of the infusion. Urine was collected prior to the start of the infusion, at 0-4 hr during the infusion period, and for a 4 hr period following the end of the infusion. One female did not produce urine samples, and contamination of the urine with water was suspected for 2 other animals. The day following the last sample collection, animals were sacrificed without further examination.

Results: Plasma hirulog levels are shown in the table below. No data was presented regarding plasma metabolites or urinary levels of hirulog or its metabolites.

Plasma hirulog levels (ng/mL) in cynomolgus monkeys in response to a 4 hr intravenous infusion at a dose of 7.5 mg/kg/hr (Hirulog Lot No. 67A03Q).

Animal No.	101 (Male)	102 (Male)	151 (Female)	152 (Female)
0 hr (pre)	BLQ	BLQ	BLQ	BLQ
Prior to end of the infusion				
1 hr post-infusion				

BLQ = below limit of quantitation

APPEARS THIS WAY
ON ORIGINAL

Distribution:

Rats

Absorption, Distribution, Metabolism, and Excretion Study in Albino Rats Administered ^{14}C and ^{3}H Labeled Hirulog by Intravenous and Subcutaneous Injection (Biogen Study No. P90-035).

Methods: The absorption, distribution, metabolism, and excretion of labeled hirulog (^{3}H and ^{14}C) was examined in rats following intravenous or subcutaneous administration of a dose at 3 mg/kg. ^{3}H was located on $(Pro)_2$, while ^{14}C was located on $(Gly)_{5, 6, 7, 8, 10}$. (See structures at beginning of ADME section). Radiolabel in blood or plasma was examined in 6 rats (5 males and 1 female) following intravenous or subcutaneous administration. Radiolabel levels in organs were examined in three rats (2 males and 1 female) following intravenous or subcutaneous administration. Radiolabel levels in urine and feces were examined in 9 rats (7 male and 2 female) following intravenous or subcutaneous administration. Following intravenous administration, blood for determination of radiolabel levels was collected at 0.083, 0.25, 0.75, 1, 2, 4, 6, and 24 hr. Radiolabel levels in blood and organs were determined at 24 hr after intravenous administration. Following subcutaneous administration, blood was collected at 0.25, 0.5, 2, 4, 6, 24, 48, and 72 hr. Radiolabel levels in blood and organs were determined at 72 hr after subcutaneous administration. Radioactivity in the organs was examined as follows: brain, heart, blood, kidneys, liver, spleen, lymph nodes, lungs, pituitary, thymus, and pancreas. Elimination of radiolabel into urine and feces is reported in the Excretion section.

Results: Following intravenous administration of dual labeled hirulog, ^{3}H and ^{14}C radiolabel concentrations in blood and plasma paralleled one another for the first hr; however, by 2 hr, ^{3}H had disappeared, while ^{14}C levels maintained a relatively constant level through 24 hr. Similar results were obtained following subcutaneous administration; although, ^{3}H disappeared after 4 hr and ^{14}C levels maintained a relatively constant level through 72 hr. Drug half life was significantly longer with subcutaneous administration (1-2.3 hr) as compared to intravenous administration (~0.3 hr). AUC values for ^{14}C radiolabel were considerably higher following subcutaneous administration as compared to intravenous administration; although, for ^{3}H radiolabel, the difference was not as great. With intravenous administration, the volume of distribution of ^{14}C radiolabel suggested a wide distribution into tissues, while for ^{3}H radiolabel, the distribution was less extensive. There was no evidence of tritium exchange occurring *in vivo*. Total clearance of ^{14}C-radiolabel was relatively low