

Table 2 (continued)

Test item	Animals used	Administration route and dosage	Results	Reference
[Effect on the heart]				
Left intra-ventricular pressure	Anesthetized open chest dogs (P)	0.3-30mg/kg, i.v.	Decrease at 10 mg/kg or above	
dp/dt max	Anesthetized open chest dogs (P)	0.3-30mg/kg, i.v.	Decrease at 10 mg/kg or above	E-21
Cardiac output	Anesthetized open chest dogs (P)	0.3-30mg/kg, i.v.	Slight increase at 30 mg/kg	
Right intraatrial pressure	Anesthetized open chest dogs (P)	0.3-30mg/kg, i.v.	Slight increase or decrease at 30 mg/kg	
[Effect on blood flow]				
Blood flow volume of femoral artery	Anesthetized dogs (P)	1-10mg/kg, i.v.	Increase at 3 mg/kg or above	E-23
		30-1000 µg, i.a.	Increasing tendency at 300 µg or above	
Blood flow volume of local skeletal muscles in the acral regions of the hind legs	Anesthetized dogs (P)	1-10mg/kg, i.v.	Increase at 10 mg/kg	E-22
		30-1000 µg, i.a.	Increase at 30 µg or more	
Blood flow volume of internal carotid artery	Anesthetized dogs (P)	1-30mg/kg, i.v.	Decreasing tendency at 10 mg/kg or more	
Blood flow volume of vertebral artery	Anesthetized dogs (P)	1-30mg/kg, i.v.	Increasing tendency at 10 mg/kg or more	
Local blood flow volume of cerebral cortex	Anesthetized dogs (P)	1-30mg/kg, i.v.	Slight decrease at 30 mg/kg	
Urine volume and electrolyte secretion	Wistar rats	3-30mg/kg, i.v.	No effect	E-21

Note: P : Pentobarbital anesthesia

E. Other General Pharmacological Effects.

Argatroban at a 3% solution showed a slight inhibitory effect on the corneal reflex of guinea pig.

Argatroban at 1-30 mg/kg i.v. had no effect on the contraction induced by stimulation of either the nerve or the muscle in a sciatic nerve-gastrocnemius preparation in rats.

Argatroban (1-30 mg/kg, s.c.) had no inhibitory effect on vascular permeability in mice.

F. Pharmacological Effects of Metabolites.

The pharmacological effects of the metabolite M-1 of argatroban were summarized on Table 4 (page 27). The thrombin inhibitory effect of metabolite M-1 was 1/40 of that of argatroban. The metabolite M-1 (1 mg/kg or above, i.v.) produced a slight piloerection in rats and had a slight increase in heart rate of dogs at 10 mg/kg i.v. or above. Other pharmacological effects of the metabolite M-1 were either the same as or lower than those of argatroban.

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TABLE 3

(SPONSOR'S Table 7.25) Effect on the Autonomic Nervous System and Gastrointestinal System

Test item	Animals used	Administration route and dosage	Results	Reference
[Isolated smooth muscles]				
Acetylcholine	Ileum of Hartley guinea pigs	10 ⁻⁷ - 10 ⁻⁴ M	No effect	
Histamine constriction	Ileum of Hartley guinea pigs	10 ⁻⁷ - 10 ⁻⁴ M	No effect	
BaCl ₂ constriction	Ileum of Hartley guinea pigs	10 ⁻⁷ - 10 ⁻⁴ M	No effect	
Serotonin constriction	Isolated stomach of Wistar rats	10 ⁻⁷ - 10 ⁻⁴ M	Suppression at 10 ⁻⁶ or above	E-21
Spontaneous motility of uterus	Uterus of Wistar rats			
	Estrus period	10 ⁻⁷ - 10 ⁻⁴ M	Slight increasing tendency at 10 ⁻⁶ M or above	
	Non-estrus period	10 ⁻⁷ - 10 ⁻⁴ M	No effect	
	Pregnant uterus	10 ⁻⁷ - 10 ⁻⁴ M	No effect	
Norepinephrine constriction	Seminiferous tubules of Wistar rats	10 ⁻⁷ - 10 ⁻⁴ M	No effect	
[Gastro-intestinal system]				
Intestinal transport function	ddy mice	10-100mg/kg, i.v.	No effect	
Gastrointestinal movement	Anesthetized Japanese white rabbits (P)	1-30mg/kg, i.v.	Suppression of gastric movement (transient) and increase in ileum movement at 10 mg/kg or more	E-21
Secretion of gastric acid	Wistar rats	3-30mg/kg, i.v.	No effect	

Note: P: pentobarbital anesthesia

TABLE 4

(SPONSOR'S TABLE 7.27) Pharmacological Effect of Main Metabolites (M-1)

Test item	Animals used	Administration route and dosage	Results for M-1	Result for argatroban
Thrombin inhibitory effect	Bovine thrombin	in vitro	$I_{50}=1.2 \mu\text{M}$	$I_{50}=0.032 \mu\text{M}$
Acute spontaneous electroencephalogram	Cats	150 $\mu\text{g}/\text{kg}/\text{min}$, i.v.	Causing slightly slow electroencephalogram	Causing slightly slow electroencephalogram
General behavior	Wistar rats	1-30 mg/kg , i.v.	Slight piloerection at 1 mg/kg or above	Slight changes at 10 mg/kg or more
Prolongation of hexobarbital sleeping time	ddY mice	10-100 mg/kg , i.v.	No effect	Slight reduction at 10 mg/kg
Normal body temperature	Japanese white rabbits	3-30 mg/kg , i.v.	No effect	No effect
Blood pressure/heart rate				
Blood pressure	Anesthetized dogs (P)	0.3-30 mg/kg , i.v.	Decrease at 10 mg/kg or above	Decrease at 10 mg/kg or above
Heart rate	Anesthetized dogs (P)	0.3-30 mg/kg , i.v.	Slight increasing tendency at 10 mg/kg or more	Increase at 10 mg/kg or more
Respiration	Anesthetized dogs (P)	0.3-30 mg/kg , i.v.	Slight increase at 30 mg/kg or more	Increase at 10 mg/kg or more
Electrocardiogram	Anesthetized dogs (P)	0.3-30 mg/kg , i.v.	No effect	No effect
Isolated heart				
Contractile force	Left atrium of Hartley guinea pigs	10^{-7} - 10^{-3} g/ml	Suppression at 10^{-3} g/ml	Slight decrease at 10^{-7} g/ml or above

G. Effects on Tumor Colonization:

Mice were injected with sarcoma or Lewis lung carcinoma cells (tumor inoculation), and lung tumor colonies were examined, on days 20-22. Argatroban, at high doses (1 or 5 mg pellets, implanted ip, prior to inoculation, or given as 2 mg/ml oil suspension, or given 500 µg/ml water soluble solution) increased the number of lung colonies, induced by iv injections of above murine neoplasia. The drug did not increase the tumor cell multiplication, or tumor cell uptake (using ¹²⁵I-UdR). It did not affect the phagocytic activity of mice treated in vivo, or tumor cytotoxicity of spleen cells in vitro. The drug, when given at low doses, for extended periods (as a soluble compound by osmotic minipump implanted sc), caused the effect on metastasis to disappear. These studies suggest that the drug at high doses may enhance vascular attachment of tumor cells.

H. IV Effects on Cardiovascular Systems in Anesthetized Beagle Dogs: (Study # 91-159-1600)

Three groups of anesthetized male dogs (n=3-5, implanted with two catheters, one in the left ventricle for cardiovascular assessment, and the second one in the pulmonary artery) were given iv bolus dose (0.4 ml/kg, lot numbers W9026AX and R9096AX), followed by a continuous iv infusion of the drug (2.5 ml/kg/hr) for 2-hrs. The low and high doses were 0.07 mg/kg bolus + 0.84 mg/kg, and 0.2 mg/kg + 2.5 mg/kg resp. The dose selection was based on a 4-week cont iv toxicity studies in dogs, and on the solubility of the drug. The control group received saline only (similar volumes of bolus and iv infusion rates). Cardiovascular monitoring was carried out 15 min prior to the drug treatment, until the end of the study (2-3 hrs). The following parameters were recorded continuously every 30 seconds: systolic, diastolic and mean arterial blood pressures, heart rates, left ventricular and pulmonary pressures (pulmonary wedge pressure (PWP) and cardiac output), and electrocardiography (RR, QRS, ST, QT, PR, R and T wave heights). After 2 hrs of monitoring (except, one dog was observed for an additional 1 hr recovery period), the dogs were sacrificed and hearts were examined in gross pathology. There were no mortalities, and no clinical signs in any animal. Both, electrocardiograms and hemodynamic data were not significantly different from saline treated controls. In gross pathology, 3 of all 3, and 4 of all 4 animals, in low and high dose groups resp, showed endocardial dark areas in the endocardium of the hearts (vs 2 of 5 in controls). This was attributed to the pharmacological activity of the drug.

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION (ADME)

Pharmacokinetics:

Following studies were submitted under IND : _____ initial submission (dated December 8, 1988 and were reviewed on 6/1/1989), along with a 24-hr PK study in dogs (amendment, dated September 11, 1992 and reviewed on 12/15/1993). These are reproduced below.

V. ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION (ADME):

The ADME of argatroban were studied in rat, rabbits and dogs after administration of ^{14}C -argatroban. Unchanged argatroban and its metabolites in plasma, urine and feces were determined by _____ method. The radioactivity in organs and tissues except fatty tissues were measured by combusting samples of tissue to $^{14}\text{C}\text{O}_2$. Fatty tissues were dissolved with _____ prior to counting.

A. Absorption:

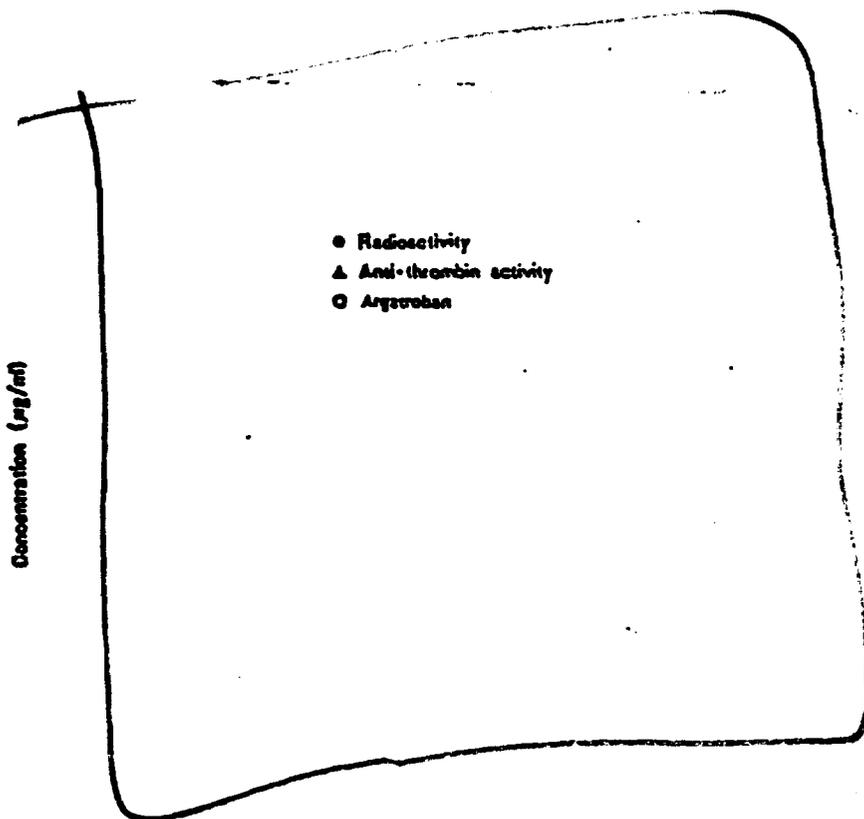
Figure 1 (page 32) shows plasma level-time curves of radioactivity, unchanged argatroban, and anti-thrombin activity in male rats after single or daily administration of ^{14}C argatroban/kg. Anti-thrombin activity in the plasma was similar to plasma level profile of argatroban. Time to peak level was 5 minutes after i.v. administration of ^{14}C -argatroban in both male and female rats. Plasma levels of radioactivity in male rats measured 30 min after the last dose of argatroban following daily administration of argatroban for 10, 15 and 21 days were 0.239-0.296 ug/ml which were comparable to the level (0.285 ug/ml) after a single dose. Similarly, the plasma levels of radioactivity at 15 min and 1 hr after administration were approximately constant from the first day to the final (21) day (15 min: 2.63-3.64 ug/ml; 1 hr:0.44-0.68 ug/ml).

After administration of ^{14}C -argatroban to rabbits, radioactivity rapidly disappeared from blood and it was no longer detectable 2 hr after administration.

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FIGURE 1

(Sponsor's Figure 7.4) Plasma levels of total radioactivity, unchanged argatroban and anti-thrombin activity after single intravenous administration of ^{14}C -argatroban (3 mg/kg) to male rats



Radioactivity and anti-thrombin activity were converted to µg equivalent of argatroban. Each point represents the mean \pm S.D. (n=4).

Figure 2 (page 34) shows the plasma levels of radioactivity, unchanged argatroban, metabolites and anti-thrombin activity after single i.v. administration of ^{14}C -argatroban to male dogs. Peak concentration of radioactivity reached peak 5 min after injection of the compound. Unchanged argatroban was detected for only 30 min.

No differences were noted between males and females with respect to any of the parameters measured in both rat and dog studies.

Pharmacokinetic parameters following administration of ^{14}C -argatroban to rats and dogs are presented in Table 5 (page 35) and Table 6 (page 35) respectively.

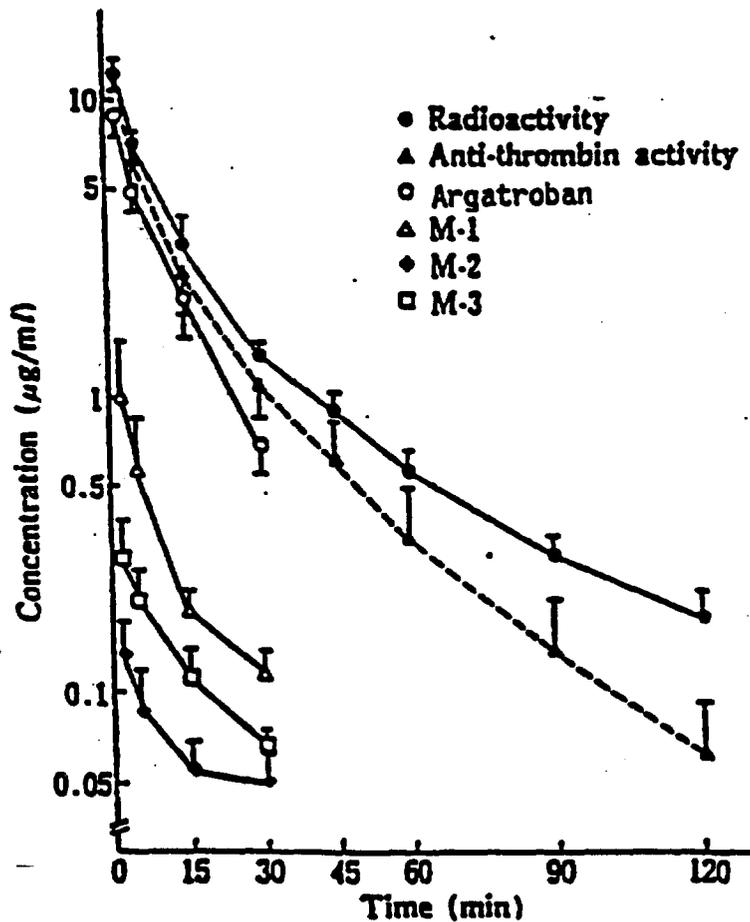
On i.v. infusion of 0.02, 0.2 and 0.5 mg argatroban/kg/hour in male dogs, plasma levels reached steady state levels of 29-34, 239-296 and 734-789 ng/ml respectively by about 2 hours. The dose proportionality of plasma concentrations of argatroban was maintained throughout the infusion time of 5 hours. After the end of infusion, the pharmacokinetics of argatroban were similar to those obtained with single dose i.v. bolus administration.

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FIGURE 2

(Sponsor's Figure 7.7) Plasma levels of radioactivity, unchanged argatroban, metabolites and anti-thrombin activity after single intravenous administration of ^{14}C -argatroban (3 mg/kg) to male dogs.



Radioactivity, metabolites and anti-thrombin activity were expressed at μg argatroban. Each point represents the mean \pm S.D. (n=5).

TABLE 5
(Sponsor's Table 7.3) Pharmacokinetic parameters obtained from a two-compartment model after intravenous administration of ¹⁴C-argatroban in rats

Parameter	Radioactivity		Argatroban		Anti-thrombin activity	
	Male	Female	Male	Female	Male	Female
A (µg/ml)	8.50	8.99	9.27	8.28	17.29	17.72
B (µg/ml)	0.19	0.19	0.30	0.23	0.43	0.42
α (hr ⁻¹)	10.46	10.12	15.11	13.92	17.44	21.58
β (hr ⁻¹)	0.52	0.48	2.50	1.93	2.77	2.91
T _{1/2α} (min)	4.0	4.1	2.8	3.0	2.4	1.9
T _{1/2β} (min)	79.6	84.6	16.6	21.6	15.0	14.4
Vd ⁰ (l/kg)	0.35	0.33	0.31	0.35	0.17	0.17

1) : The volume of distribution of the central compartment (Vd=dose/(A+B)).
Two-compartment model : C = A e^{-αt} + B e^{-βt}.

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TABLE 6
(Sponsor's Table 7.10:) Pharmacokinetic parameters obtained from analysis of plasma level-time curves following administration of ¹⁴C-Argatroban in dogs

	Radioactivity		Argatroban		Anti-thrombin Activity	
	Male	Female	Male	Female	Male	Female
Level at 2 min (µg/mL)						
t _{1/2α} (min)						
t _{1/2β} (min)						
AUC 0→∞ (µg·hr/mL)						

*Data were fit to a one-compartment model.
N.D. = not determined.

24-Hour Continuous I.V. Infusion Pharmacokinetic Study in Dogs
(Study # 91-052-1600)

Methods: Groups of male beagle dogs (3/group) were given continuous I.V. infusion (2.5 ml/kg/hr) of 0.125, 0.417 or 1.25 mg/kg/hr of Argatroban for 24 hours (3, 10 or 30 mg/kg/day). The vehicle for Argatroban was:

Blood samples were withdrawn at 0 (pretreatment), 10, 20, 30, 45, 60, 90 and 120 min., and 4, 8, 12 and 24 hours after the start of infusion (24 hr sample was taken prior to stoppage of infusion). Blood samples were also collected at 2, 5, 10, 20, 30, 45, 60, 90, 120 and 240 min. after the stoppage of infusion. Plasma concentrations of Argatroban were measured by _____ methods.

Results: The decline of plasma concentrations of Argatroban was mono-phasic at low and mid dose levels with $t_{1/2}$ of 11-14 min. At high dose the disappearance of Argatroban from plasma was biphasic with $t_{1/2}$ alpha of 6.9 min. and $t_{1/2}$ beta of 56.7 min. Other pharmacokinetic parameters were as follows:

Parameters	Total Dose (mg/kg/day)		
	3	10	30
*cl (ml/min/kg)	8.0 ± 1.8	14.9 ± 2.1	11.0 ± 6.7
**V _c (ml/kg)	155.0 ± 16.0	225.1 ± 27.2	223.0 ± 212.7
t _{1/2} alpha (min)	14.0 ± 4.6	10.7 ± 2.4	6.9+
t _{1/2} beta (min)	-----	-----	56.7+

- * = normalized plasma clearance
 ** = normalized volume of central compartment
 + = mean of 2 dogs

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B. Distribution:

1. Radioactivity levels in tissues of male rats after i.v. administration of ^{14}C -argatroban (3 mg/kg) are shown in Table 7 (page 37). The radioactivity levels in each tissue and the distribution pattern were similar in male and female rats. High levels of radioactivity were detected only in the metabolic and excretory organs (liver, kidney, intestine). The radioactivity in tissues declined quickly with time.

TABLE 7

(Sponsor's Table 7.4) Distribution of radioactivity in the tissues after single intravenous administration of ^{14}C -argatroban (3 mg/kg) to male rats.

Organ	Time	Concentration ($\mu\text{g eq./g wet tissue}$)					
		5 min	30 min	1 hr	6 hr	24 hr	192 hr
Blood		2.62 ± 0.61	0.192 ± 0.032	0.105 ± 0.037	0.006 ± 0.007	N. D.	N. D.
Plasma		3.75 ± 0.82	0.285 ± 0.030	0.116 ± 0.046	0.007 ± 0.008	N. D.	N. D.
Thymus		1.02 ± 0.32	0.257 ± 0.023	0.109 ± 0.032	0.009 ± 0.008	N. D.	N. D.
Heart		0.371 ± 0.074	0.095 ± 0.017	0.033 ± 0.004	0.025 ± 0.031	N. D.	N. D.
Lung		1.30 ± 0.44	0.239 ± 0.025	0.086 ± 0.015	0.021 ± 0.014	0.004 ± 0.003	N. D.
Liver		27.4 ± 2.8	2.85 ± 0.72	1.58 ± 0.56	0.240 ± 0.054	0.082 ± 0.012	0.027 ± 0.011
Stomach		1.63 ± 0.94	0.398 ± 0.186	0.115 ± 0.031	0.028 ± 0.004	0.005 ± 0.001	N. D.
Spleen		0.463 ± 0.062	0.199 ± 0.015	0.085 ± 0.012	0.022 ± 0.006	N. D.	N. D.
Pancreas		3.06 ± 0.41	0.524 ± 0.060	0.127 ± 0.054	0.021 ± 0.010	N. D.	N. D.
Kidney		23.4 ± 4.0	1.64 ± 0.13	0.699 ± 0.219	0.147 ± 0.058	0.056 ± 0.003	0.005 ± 0.003
Adrenal		0.828 ± 0.268	0.151 ± 0.037	0.048 ± 0.062	N. D.	N. D.	N. D.
Testis		0.401 ± 0.102	0.118 ± 0.025	0.076 ± 0.014	0.015 ± 0.004	0.002 ± 0.001	0.001 ± 0.001
Small intestine		4.48 ± 2.29	17.6 ± 10.6	22.6 ± 7.6	2.63 ± 4.66	0.065 ± 0.036	0.002 ± 0.002
Large intestine		1.55 ± 0.43	0.172 ± 0.031	0.294 ± 0.143	4.61 ± 2.51	0.905 ± 0.770	0.008 ± 0.009
Muscle		0.369 ± 0.111	0.053 ± 0.010	0.079 ± 0.040	0.022 ± 0.007	0.003 ± 0.004	N. D.
Skin		1.42 ± 0.61	0.191 ± 0.033	0.152 ± 0.129	0.055 ± 0.038	N. D.	N. D.
Bone		0.363 ± 0.042	0.057 ± 0.013	0.092 ± 0.035	N. D.	N. D.	N. D.
Brain		0.038 ± 0.011	0.005 ± 0.003	0.019 ± 0.026	0.003 ± 0.003	N. D.	N. D.
Spinal cord		0.032 ± 0.004	N. D.	0.046 ± 0.071	N. D.	N. D.	N. D.
Eye ball		0.237 ± 0.049	0.073 ± 0.005	0.040 ± 0.028	N. D.	N. D.	N. D.
Fat pad		0.527 ± 0.196	N. D.	0.038 ± 0.018	0.021 ± 0.024	0.016 ± 0.022	N. D.
Stomach contents*		0.429 ± 0.725	1.52 ± 0.96	0.337 ± 0.302	0.034 ± 0.011	0.004 ± 0.005	N. D.
Intestinal contents*		27.7 ± 15.9	424 ± 157	265 ± 32	19.7 ± 25.9	0.725 ± 0.666	0.016 ± 0.010

Radioactivity was converted to μg equivalent of argatroban. Each value represents the mean + S.D. (n=4 or 5).

N.D.: Not detected.

*: Total amount.

2. Placental Transfer: Whole body autoradiograms were examined at 30 min and 24 hr after administration of ^{14}C -argatroban (3 mg/kg, i.v.) on gestation days 13 and 19. Radioactivity was transferred to the fetus, but there was no apparent accumulation of radioactivity in the fetus.

3. Excretion into Milk: On intravenous administration (3 mg/kg, i.v.) to lactating rats, large concentrations of argatroban and/or its metabolites were excreted in milk.

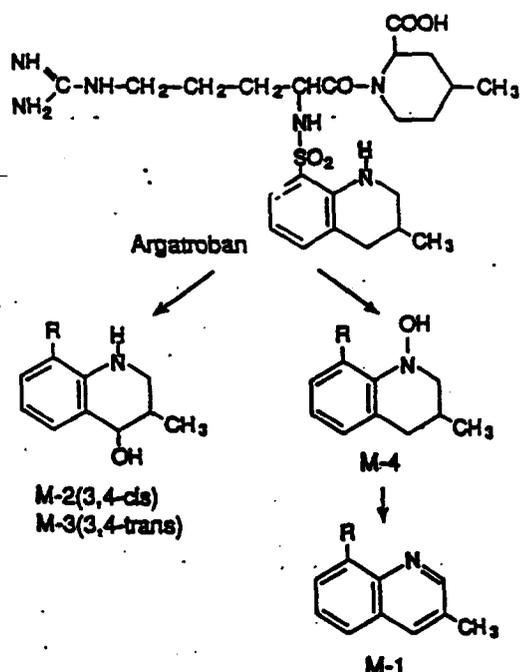
4. Serum Protein Binding and Blood Cell Binding: After i.v. administration of argatroban, the drug was bound to serum proteins in rats (47.1% at 5 min and 54.4% at 30 min) and in dogs (45.2% at 5 min and 47.8% at 30 min). The in vitro serum protein binding of argatroban (0.5 μM) in rats, dogs and human were 44.4%, 62.1% and 53.7% respectively. The in vitro binding of argatroban (0.5 μM) to albumin (HSA) and alpha 1-acid glycoprotein (alpha 1-AGP) in human serum were 20.3% and 34.0%, respectively. After single i.v. administration of ^{14}C -argatroban to male rats, 16.2% and 32% of the radioactivity was bound to blood cells at 30 min and 60 min after dosing, respectively. When argatroban (3 mg/kg, i.v.) was given to male rats once daily for 10, 15 and 21 days, it was bound to blood cells 28.3-29.0% at 30 min after last dosing. When argatroban (3 mg/kg) was administered i.v. to male dogs, it was bound to blood cell at 7.2%.

C. Metabolism:

Four metabolites of argatroban (M-1, M-2, M-3 and M-4) were isolated from rats' bile. The two major metabolic pathways (Figure 3, page 38) of argatroban are hydroxylation at the 4-carbon of the 3MTHQ ring and N-hydroxylation of the 3MTHQ ring. Metabolite M-4 was converted to M-1 when added to rat hepatic microsomes.

FIGURE 3

(Sponsor's FIGURE 1.3) METABOLIC PATHWAYS OF ARGATROBAN IN RATS



The 4 metabolites identified from rats' bile were also found in urine and feces of rabbits and dogs. M-1 was the major metabolite as judged from the excretory patterns in rats, rabbits and dogs.

As mentioned in the section of pharmacological effects of argatroban metabolites, the thrombin inhibitory effect of the main metabolite M-I was only 1/40 of that of argatroban.

Single (6 mg/kg, i.v.) or repeated (6 mg/kg/day x 21 days, i.v.) administration of argatroban to male rats produced no effects on hepatic drug-metabolizing enzyme activity as measured in vitro and in vivo with hexobarbital and zoxazolamine as model compounds.

D. Excretion:

After i.v. administration of ^{14}C -argatroban (3 mg/kg) to male rats, the biliary radioactivity for each of the unchanged drug and metabolites M-1, M-2, M-3 and M-4 were 22.18%, 33.75%, 11.7% and 7.1%, respectively. In male rats i.v. administration of 3 mg. ^{14}C -argatroban/kg, 11.5% and 84.9% of radioactivity was excreted into urine and feces, respectively, during the 0-24 hr period while the corresponding values in female rats were 13.6% and 72.4%, respectively. In male rats, the percent of the administered dose excreted into the urine during the 0-24 hr as unchanged drug, metabolite M-1, M-2 and M-3 were 7.1, 0.8, 0.3 and 0.5, respectively, (totaling to about 8%). The corresponding fecal excretion rates were 17.9%, 12.2%, 3.8% and 1.2% of the injected dose respectively (totaling to 47.2%). In the urine and feces collected 0-24 hr after administration, 3.1% and 33.1% of the dose were excreted as unknown highly polar metabolites, respectively. There were no differences in the metabolic patterns between single and repeated administrations in rats.

In rabbits, the 0-24 hr samples of urine and feces contained 34% and 31%, respectively, of the administered dose (3 mg/ ^{14}C -argatroban/kg, i.v.). During the 0-192 hr, 36% and 61% of the administered dose were excreted into urine and feces, respectively.

In male dogs, 16.15% and 62.17% of the administered dose (3 mg ^{14}C -argatroban/kg, i.v.) was excreted into urine and feces, respectively, during the 0-24 hr while the corresponding values in female dogs were 16.28% and 59.54%, respectively. There was no difference in the excretory pattern between a single and repeat dose administrations.

Following 4-hr iv infusion study in dogs, submitted to IND amendment, dated September 11, 1992 (reviewed 12/15/1993) is reproduced below.

4-Hour I.V. Infusion Interaction Study In Dogs:
Argatroban, (Recombinant Human Tissue Plasminogen Activator
(rt-PA) and Aspirin
(Study # 91-051-1600)

Methods: The purpose of this study was to assess the effects of rt-PA alone or in combination with aspirin on the pharmacodynamic effects of Argatroban in dogs. Effects of co-administration of these compounds on pharmacokinetic parameters of Argatroban in dogs were also assessed. There were 5 treatment groups (3 dogs/group). Group 1 (Saline Control): received a bolus injection of saline 0.21 ml/kg followed by saline infusion of 1.1 ml/kg/hr for 4 hours via one catheter and 0.8 ml/kg/hr for the first hr. and 0.3 ml/kg/hr for the remaining 3 hours via second catheter. Group 2 (Argatroban): received a bolus injection of Argatroban 0.06 mg/kg (0.12 ml/kg) followed by a Argatroban infusion of 0.55 mg/kg/hr (1.1 ml/kg/hr) for 4 hours, and saline was infused via second catheter (0.8 ml/kg/hr for the first hour and 0.3 ml/kg/hr for the remaining 3 hours). Group 3 (rt-PA): received a bolus injection of rt-PA (0.09 mg/kg, 0.09 ml/kg) followed by a rt-PA infusion of 0.8 mg/kg/hr for the first hour and 0.3 ml/kg/hr for the remaining 3 hours. Saline was infused into the second catheter (1.1 ml/kg/hr for 4 hours). Group 4 (Argatroban + rt-PA): dogs received Argatroban via catheter 1 and rt-PA via catheter 2 at the same bolus + infusion doses as mentioned in group 1 and 2. Group 5 (Argatroban + rt-PA + Aspirin): dogs were treated similarly as group 4 except all dogs were also given aspirin (162.5 mg orally in capsule) at 26 and 2 hours before the start of bolus + infusion schedule. All dogs were observed for clinical signs twice daily and mortality check was done daily for 14 days. Body weights were recorded weekly and food consumptions daily. Blood samples were collected from overnight fasted dogs twice at pretest, and at 1, 4, 6, 8 and 24 hours after the start of infusion and preterminally for hematological (including PT and APTT) tests. Blood samples were also collected at 0 (pretest), 10, 30, 60, 120, 180 and 240 min. after the start of infusion, and at 5, 10, 20, 30, 45, 60, 90, 120, 180 and 240 min. after the stoppage of infusion for measuring levels of Argatroban and rt-PA in plasma. At the end of observation period all animals were sacrificed and subjected to complete necropsy.

Results:

1. **Observed Effects:** Hematomas/contusions at the injection sites were seen in treated dogs and the severity was greater in dogs which received Argatroban + rt-PA (with or without aspirin).

2. **Mortality:** None

3. **Body Weight/Food Consumption/Water Consumption:** Numerical data are not meaningful.

4. **Hematology/Coagulation/Bone Marrow:** No treatment related effects were seen except increases in PT and APTT and TT were seen in Argatroban treated dogs, and these values returned to baseline values within 2-4 hours after stoppage of infusion. The PT and APTT values were not affected when dogs were given rt-PA alone. Addition of rt-PA &/or aspirin to Argatroban regimen had no significant effect on PT and APTT values obtained in the presence of Argatroban. Furthermore, alpha-2-antiplasmin levels were decreased by $\geq 50\%$ in dogs treated with rt-PA &/or Argatroban or aspirin. This is an expected pharmacodynamic effect of rt-PA. However, there was no exaggeration of this effect by the presence of Argatroban &/or aspirin. Thus co-administration of Argatroban and rt-PA by I.V. infusion with or without orally administered aspirin did not increase the toxicity/pharmacodynamic effects of Argatroban or rt-PA alone.

Effects on Coagulation Parameters

Treatment	PT (sec)		APTT (sec)		TT (sec)	
	4 Hr ¹	8 Hr ²	4 Hr	8 Hr	4 Hr	8 Hr
Group 1 (saline)	7.6 ± 0.45	7.9 ± 0.44	9.9 ± 0.64	9.5 ± 0.87	7.3 ± 0.58	7.1 ± 0.75
Group 2 (Argatroban)	10.0 ± 1.42	8.2 ± 0.90	13.9 ± 0.93	9.0 ± 0.12	>19	7.6 ± 1.48
Group 3 (rt-PA)	7.7 ± 0.32	7.8 ± 0.30	11.2 ± 0.56	10.5 ± 0.74	9.3 ± 3.16	6.6 ± 0.55
Group 4 (Argatroban + rt-PA)	9.9 ± 1.30	8.3 ± 0.74	15.3 ± 2.18	9.4 ± 0.52	>22	6.7 ± 0.95
Group 5 (Argatroban + rt-PA + Aspirin)	11.9 ± 2.78	8.3 ± 0.40	17.9 ± 3.58	9.2 ± 0.52	12.7 ± 2.52	7.8 ± 0.07

¹ = infusion terminated at 4 hr

² = 4 hours after the stoppage of infusion

5. **Gross Pathology:** No treatment related effects were seen.

6. Plasma Levels of Argatroban and rt-PA: Argatroban plasma levels were measured by methods and rt-PA plasma concentrations were measured by ELISA. Sponsor has provided a summary table on page 011 of volume 1.

Parameter	Group		
	2 (Argatroban)	4 (Argatroban/rt-PA)	5 (Argatroban/ rt-PA/Aspirin)
C _{obs} (ng/mL) ^a	843.5 ± 70.2	1284.5 ^b	865.1 ± 34.2
CL/W (mL/min/kg) ^c	10.8 ± 1.0	10.0 ± 4.9	11.3 ± 0.7
V ₁ /W (mL/kg) ^d	94.2 ± 25.3	132.1 ± 96.2	140.4 ± 34.9
V _{ss} /W (mL/kg) ^e	219.4 ± 51.1	197.8 ± 55.0	233.5 ± 25.8
t _{1/2α1} (min) ^f	3.3 ± 2.2	3.5 ^b	5.2 ± 1.6
t _{1/2α2} (min)	22.6 ± 3.5	25.1 ^b	22.5 ± 2.4

- ^a Observed argatroban plasma concentration at 240 minutes post-infusion start.
- ^b Average of animals 4034 and 4112 for which the data were described by bi-exponential equations. Animal 4023 received only 83.7 % of the nominal dose, and the data were described by a mono-exponential equation.
- ^c Weight-normalized clearance.
- ^d Weight-normalized volume of the central compartment.
- ^e Weight-normalized steady state volume of distribution, assuming elimination from the central compartment.
- ^f Half-life.

Sponsor did not provide a explanation for t_{1/2α1} and for t_{1/2α2}. However, the data indicates that co-administration of Argatroban with rt-PA or rt-PA + aspirin had no effect on pharmacokinetic parameters of Argatroban.

Following new ADME studies were submitted in the current NDA 20,883.

1. Comparative Pharmacokinetics of Argatroban, and its Major Metabolite M1, in Male Primates:

The comparative pharmacokinetics of argatroban, and its major metabolite M1 were also studied in male primates (Macacca Mulatta, n=2) in a preliminary study, following a single iv dose of 0.5 mg/ml of the drug, or an M1 compound (batch numbers of both were not indicated). Blood samples were collected at 5, 15, 30, 60 and 120 min after dosing. The concentration of the drug and M1 metabolite were measured by a validated methods. Anticoagulant effects (HMT, aPTT, ECT, TT, PT, and Heptest) of the 2 drugs were also measured.

Results as indicated in Table I (reproduced from volume 17, page 396 of the submission), show that M1 had faster clearance (K_e), shorter half life, and produced much weaker anticoagulant response, in terms of AUC (the details on how various parameters were measured or calculated were missing).

Table I. Comparative pharmacokinetics of argatroban and its major metabolite M1 in primates.

	Argatroban	M1 Compound
k_e (1/min.)	0.039075	0.062965
$t_{1/2}$ (min.)	17.7	11.0
$AUC_{0-\infty}$ ($\mu\text{g}\cdot\text{min}/\text{mL}$)	25.0	7.3

2. The Distribution and Excretion of ^{14}C -Argatroban in Pregnant and Nursing Rats:

The distribution and excretion of ^{14}C -argatroban (3 mg/kg, iv), were also examined in 6 pregnant rats (on day 19 of gestation), and in 3 lactating rats (on day 8) resp. The proportion of unchanged argatroban at 5 min in the plasma, liver, kidney, and placenta of dams was 71%, 63%, 74% and 68% resp. Thirty min later these values significantly decreased (32, 21, 33, and 32% resp). Very little radioactivity was detected in fetuses at 5 or 30 min (0.003% and 0.002% resp), suggesting no accumulation of the drug occurs in the fetus. However, iv administration of ^{14}C -argatroban to lactating rats, significant (32-43%) radioactivity was found in the milk from 0.5-6 hrs as an unchanged drug, these levels declined gradually over time.

3. The PK/Metabolism Studies in Rats After Single or Repeated IV Administrations:

The PK/metabolism studies in rats after single (3 mg/kg, ^{14}C 16.4-28.7 μCi , iv to males and females n = 3-4) or repeated (3 mg/kg, 11.8 μCi , for 21 days, iv) administrations, indicate a decline with α and β elimination half lives of 4 and 80 min resp, and major urinary and fecal metabolite was M1. Approximately, 90% of the administered drug was recovered from urine and feces within 24 hrs (both after single and repeated doses), and the biliary excretion was 90% within 48 hrs. Liver, kidney and GI tract had the highest activity. After 21 days, no accumulation of activity was noted in any tissues.

4. Studies of Sex Differences in Argatroban Metabolism by Hepatic Microsomes of Rats:

The metabolism studies in hepatic microsomes of male and female rats showed that the sex differences in argatroban metabolism exist. The synthesis of 4 major metabolites (M1, M2, M3, M4) was 1.4-2.8 fold higher in males than in females.

5. Metabolism of Argatroban by Rat Liver and Kidney S-9 Preparations, and Hydrolysis by Various Proteases and Arginase:

Using rat liver and kidney S-9 preparations (as well as trypsin, thrombin, plasmin and arginase), the studies indicated, that neither the amide bond, nor the guanidine bond in argatroban (which are thought to be the products of the amide bond hydrolysis, or deurea/deamination of the guanidine group) is hydrolyzable or is further metabolized.

6. Determination of the Principal Cytochrome P-450 Enzymes Involved in the Formation of 4 Major Metabolites of Argatroban in Human Liver Microsomal Incubates:

In human liver microsomes (where CYP3A4 is the major liver microsomal cytochrome P450 enzyme), all 4 major metabolites of argatroban were formed by the action of CYP3A4 and CYP3A5 (using 2 inhibitors of CYP3A, nifedipine and troleandomycin). The metabolites M1, M2, M3 and M4 accounted for 20%, 47%, 29% and 4% resp, of total drug metabolism. In vitro, CYP3A4 catalyzed the formation of 86-94%, and CYP3A4/5 of 98%-99% of 4 major metabolites.

These studies indicate that pharmacokinetics (PK) of argatroban, after iv dosing, are similar in rats, rabbits, and dogs. The half life of argatroban were comparable in rats ($T_{1/2\alpha}$ 4.1 min, $T_{1/2\beta}$ 80-85 min), rabbits ($T_{1/2\alpha}$ 5.5 min, $T_{1/2\beta}$ 36 min), dogs ($T_{1/2\alpha}$ 2.4-3 min, $T_{1/2\beta}$ 20-29 min), and humans ($T_{1/2\alpha}$ 7 min, $T_{1/2\beta}$ 54 min).

Argatroban is rapidly distributed to tissues, peak levels were attained at 5 min, very little (<1%) remained by 24 hours. Liver, kidney, and small intestine (at 5 min) had 7.3, 6.2, and 1.2 fold higher conc resp, than the plasma. The drug crosses the placental barrier, but no apparent accumulation of the radioactivity was noted in the fetus by 1 hr. However, significant, quantities of the drug (when given iv to lactating mothers) are excreted in the milk in rats, within 0.5 hrs of dosing, and decrease gradually by 24 hrs. The mean in vitro protein binding of argatroban at 0.5 μ M in rat, dog, and human plasma was 44.4%, 62.1%, and 53.7% resp.

The drug is metabolized to 4 major metabolites in liver (M1, M2, M3 and M4), which are also observed in urine and feces of rats, rabbits and dogs. In human liver microsomes, these 4 metabolites were formed by the action of cytochrome P450 isoenzymes CYP3A4 and CYP3A5. The microsomes from male rats produced 1.4-2.8 fold higher metabolites (M1 to M4) than female rats. The major metabolite M1 of argatroban had 1/40th the thrombin inhibitory effects of the drug. In primates, the major metabolite M1 had faster clearance, shorter half life, and produced 3.4 fold weaker anticoagulant response. In rats 80-90% of the administered drug was excreted by fecal route, and mostly in the first 24 hrs. None of the M1 to M4 metabolites of the drug (or the more polar unidentified compounds) were conjugated with sulfates or glucuronides. In urine + feces, 96.4%, 65%, and 78% of administered drug was excreted within 24 hrs in rats, rabbits and dogs resp.

TOXICOLOGY:

ACUTE TOXICITY

Acute toxicity studies with the drug were carried out in ddy mice (by bolus iv, ip, sc, and oral), Wistar rats (by bolus iv, ip, sc, and oral), and in Beagle dogs (by iv). For oral, ip, and sc administration in mice and rats, the drug was suspended in 0.5% (w/v) tragacanth aqueous solution. For the iv injection, the drug was mixed in 0.9% saline (at a conc of 2.7 mg/ml), and the highest dose of 81 kg was given to mice and rats. For dog iv studies (due to poor solubility in saline), the drug (10% w/v) was dissolved in 30% sorbitol w/v (containing 40% ethanol w/v, and remainder distilled water). Higher iv doses could be given in ddy mice and Wistar rats (along with additional studies in Japanese white rabbits and Beagle dogs), using HCl as a diluent.

Acute Toxicity of Argatroban in Mice, Rats, Rabbits and Dogs:

Methods: The acute toxicity of argatroban (batch #: not stated) after iv administration was studied in male ddy mice, male Wistar rats, male Japanese white rabbits, and male beagle dogs. Argatroban was dissolved in HCL solution, pH 2-4 for iv dosing (for rabbits in 20% propylene glycol solution). Mice received 10 mg/ml solution at a rate of 0.1-0.34 ml/10 g/second, rabbits 50 mg/ml at a rate of 2-4 ml/kg/min, and dogs 3-10 mg/ml, at a rate of 2 ml/kg/6 seconds (the volumes and rates for rats were not given). In the same study, oral (to mice and dogs) or ip (to mice) argatroban (in 0.5-1% tragacanth aqueous solution) was also given to animals, however doses were not specified. All animals were observed for toxic signs and mortality daily.

Results: After iv administration, the minimum lethal doses in mice, rats and rabbits were 200, 124, and 150 mg/kg resp, Table II. The highest administered iv doses (65.8 mg/kg) in dogs were not lethal. The major toxic symptoms in mice, rats and rabbits were clonic convulsion and/or respiratory paralysis, in dogs atonia and collapse (which continued for a short time). The LD₅₀ values for oral in mice and dogs were indicated as 6,200 and >1,000 mg/kg resp, and LD₅₀ for ip in mice were 600-800 mg/kg. Oral and ip doses caused similar toxic signs (clonic convulsion, respiratory paralysis and death) in animals. The minimal lethal doses could not be defined with oral or ip drug in this study, as only limited data were provided.

Table II. Highest tolerated, and minimum lethal doses of argatroban in mice, rats, rabbits and dogs in acute iv toxicity studies.

Species (strain)	No/Sex/ Group	Doses (mg/kg/day)	Highest Tolerated Doses (mg/kg/day)	Minimum Lethal Doses (mg/kg/day)
<u>I.V.</u> Mice (Males, ddy)	1-5	174, 200, 230	174	200
Rats (Males, Wistar)	4-7	100, 124, 136.4, 150, 200	100	124
Rabbits (Males, Japanese, white)	3	100, 150, 200	100	150
Dogs (Males, beagle)	1	30, 50, 65.8	65.8	>65.8

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Following acute toxicity studies were submitted to IND initial submission, dated December 8, 1988, and were reviewed on 6/1/1989. These are reproduced below.

Acute Toxicity of Argatroban in Rats and Mice

<u>Species</u>	<u>Strain</u>	<u>Sex</u>	<u>Body weight (g)</u>	<u>Route</u>	<u>Dose range (mg/kg)</u>	<u>LD 50-mg/kg (95% conf. limit)</u>	<u>Maximum non-lethal dose,mg/kg</u>
Mouse	ddy	Male & Female	21-35	i.v.	Single dose	>81	>81
Mouse	ddy	Male	21-35	i.p.	167-864	475 (402-561)	167
		Female	"	"	"	640 (568-721)	241
Mouse	ddy	Male	21-35	s.c.	108-14,860	3750 (2190-6420)	108
		Female	"	"	"	3900 (3000-5080)	470
Mouse	ddy	M & F	21-35	oral	-	>15,000	>15,000
Rat	Wistar	M & F	81-146	i.v.	-	>81	>81
Rat	Wistar	Male	81-146	i.p.	68-726	320 (282-363)	68
		Female	"	"	"	409 (370-452)	68
Rat	Wistar	Male	81-146	s.c.	50-7,000	620 (383-1005)	>50
		Female	"	"	"	1565 (282-1735)	>50
Rat	Wistar	M & F	81-146	oral	Single dose	>15,000	>15,000

1. The acute i.v. LD50 in mice could not be determined due to volume and concentration limitations.

2. In the acute i.p. toxicity study in mice, deaths occurred within 4 hr to 2 days postdose. Prior to death, clinical signs were reduced spontaneous activity, ptosis, vessel dilation, convulsions, piloerection and bradypnea. Intraperitoneal hemorrhage, anemia and subcutaneous bleeding were found at necropsy in dead and moribund animals.

3. In the acute s.c. toxicity study in mice, deaths occurred within 1-2 hr to 1 day postdose. Signs of anemia and piloerection persisted in survivors to the end of the study. Enlarged spleen and anemia were also noted.
4. In the acute i.p. toxicity study in rats, deaths were observed within 2 hr to 2 days after treatment. Clinical signs included reduced spontaneous activity, prostration, ptosis, anemia, bradypnea, and hemorrhage at the site of injection. Evidence of intraperitoneal hemorrhage, anemia, peristernal bleeding, enlarged liver and spleen were observed at necropsies. In the acute s.c. study in rats, deaths occurred between 4 hr to 5 days postdose. Clinical signs prior to death included reduced spontaneous activity, balling, ptosis, anemia, and piloerection. Anemia was also evident in animals treated with 50 mg/kg. Clinical symptoms developed 2-4 hr post dose with recovery noted by 2-9 days. Expanded subcutaneous hematoma, crusting, erosion and ulcer at the injection site, anemia, enlarged spleen and intragastric hemorrhage were observed at necropsy.

Acute Toxicity of Argatroban by Intravenous Administration in Dogs

1. Animals: Ten to 11-month old male (11.0-13.3 kg) and female (8.8-12.8) Beagle dogs.
2. Methods: Groups of 3 male and 3 female dogs each were treated by a single i.v. injection of 0 (20 ml/kg 5% sorbitol solution, pH 1.61), 100 or 200 mg argatroban/kg on day 1. General observations and food consumption were recorded once daily for 14 days. Body weights were recorded on days 0, 1, 10 and 14. Blood samples were collected from cephalic vein on days 0, 1, 7 and 14. Autopsy and histopathological examinations were performed on dead animals upon discovery and on day 14 on survivors.
3. Results:
 - a. Mortalities: One male and one female of the 200 mg/kg group were found dead 24 and 48 hours after dosing.
 - b. General Observations: The two dead animals had exhibited vomiting, paralysis of the hind limbs, conjunctival hyperemia, tremor, inability to stand, and coma prior to death. Vomiting and paralysis of the hind limbs were also observed in the surviving animals in both 100 and 200 mg/kg groups.
 - c. Food Consumption and Body Weight: Slight transient decreases in food consumption and body weight were observed in one female of the 200 mg/kg group.
 - d. Hematology Examinations: There were no argatroban-treated trends on the hematology values except some sporadic changes in the white blood cell count and platelet count.

e. Serum Biochemical Examinations: Increases in SGPT in all argatroban-treated animals (+283% in male 100 mg/kg group, +2465% in male 200 mg/kg group, +690% in female 100 mg/kg group) were found on the first post-administration day. These increases were accompanied by increases in SGOT values in some animals. Slight increases were observed in ALP in all animals of the 200 mg/kg group. Slight to moderate increases in BUN were observed in all males and two females of the 200 mg/kg groups. The serum biochemical parameters returned to normal by day 14. The actual values of serum biochemical examinations in female 200 mg/kg group were missing from the tables.

f. Gross Pathological Examinations: Autopsy of the dead animals showed severe hemorrhages in the thoracic and abdominal cavities and in the subendocardium and diaphragm.

g. Histopathological Examinations: The dead animals had liver cell necrosis, hemorrhage in the perivascular area of the liver, and necrosis, vacuolation, or atrophy of the kidney tubular epithelium. In the surviving animals, proliferation of kidney tubular epithelium was found.

4. Conclusion: Acute i.v. administration of Argatroban to dogs caused CNS toxicity symptoms such as tremor and coma. It produced histopathological changes such as necrosis of liver cells and necrosis of renal tubular epithelium in animals that died. The "no effect level" was not established since both doses (100 and 200 mg/kg, i.v.) produced toxicity.

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Following 24 hrs acute i.v. infusion toxicity studies were submitted to IND amendment dated September 11, 1992, and were reviewed on 12/15/1993. These are reproduced below.

24-Hour Continuous I.V. Infusion Toxicity Study in Rats
(Study # 869/002)

Methods: Groups of rats (5/sex/group) were given continuous I.V. infusion (2.5 ml/kg/hr) of 0.9% saline, vehicle, 0.7, 2.075 and 6.25 mg/kg/hr of Argatroban for 24 hours. The vehicle contained

It

should be noted that clinical formulation does not contain Tween 20. Additionally, heparin was added to all test and control preparations such that each ml contains 101 units of heparin. The selection of highest tested dose was based on the maximum amount which can be infused and solubility of the drug. All animals were observed for clinical signs throughout the infusion period and once daily during 14 days of observation period after stoppage of the infusion. Body weight and food intake were recorded weekly. Blood samples were withdrawn from retro-orbital sinus for hematological tests. Hematological parameters (including PT and APTT) were monitored at pretest, at the end of 24-hour infusion and at the end of 14 days of observation period. At the end of observation period, all animals were sacrificed and subjected to gross pathological examinations. No histopathological examinations were done.

Results:

1. **Observed Effects:** Severe irritation and swelling were seen in the tail of animals treated with vehicle or drug. No tail abnormalities were seen in animals treated with 0.9 saline.
2. **Mortality:** One female from mid dose group died due to severe bleeding from the site of infusion.
3. **Body Weight/Food Consumption/Water Consumption:** In treated males, body weight gains were increased by 3.8% at low dose while body weight gains were decreased by 12% and 7% in mid and high dose treated groups respectively, when compared to vehicle control values. Additionally, body weight gains in vehicle control group males were decreased by 10.5% compared to saline treated male rats. In treated females, body weight gains were decreased by 10%, 20% and 23% at low, mid and high dose respectively when compared to vehicle control group. Body weight gains of vehicle treated females were increased by 5.6% when compared to saline treated females. No significant effect on food intakes were evident.

4. Hematology/Coagulation/Bone Marrow: At the end of 24 hr. infusion, dose related increase in PT and APTT were seen in treated animals. At the end of observation period PT and APTT values returned to normal in all treated rats. The effects on PT and APTT in treated rats are exaggerated pharmacological effects of the drug.

<u>Dose</u>	<u>Sex (M/F)</u>	<u>PT (sec)</u>	<u>APTT (sec)</u>	
saline control	F		12.8 ± 0.4	16.1 ± 1.8
	M		12.3 ± 0.3	18.1 ± 1.7
vehicle control	F		13.4 ± 0.3	17.9 ± 2.4
	M		13.0 ± 1.0	20.1 ± 1.3
low dose	F		16.6 ± 3.7	23.5 ± 7.7
	M		14.2 ± 1.2	26.8 ± 4.2
mid dose	F		16.7 ± 1.4	24.7 ± 4.1
	M		18.0 ± 1.5	38.2 ± 6.5
high dose	F		22.0 ± 5.1	36.0 ± 12.8
	M		23.7 ± 6.2	46.5 ± 9.4

5. Blood Chemistry/Urinalysis: Not done.

6. Vital Signs/Physical Examination/Ophthalmic Examination: Not done.

7. Organ Weights: Not reported.

8. Gross Pathology: Vehicle formulation caused severe local irritation of the tail.

9. Histopathological Examinations: Not done.

The data indicated that vehicle used in this study produced severe irritation at the injection sites. No target organ of toxicity was identified. Drug only produced exaggerated pharmacological effects.

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24-Hour Continuous I.V. Infusion Toxicity Study in Dogs
(Study # 869/001)

Methods: Groups of dogs (2/sex/group) were given continuous I.V. infusion (0.46 - 0.58 ml/kg/hr) of 0.9% saline, vehicle control, 0.13 - 0.14, 0.40 - 0.44 and 1.15 - 1.22 mg/kg/hr of Argatroban for 24 hours. The selection of highest tested dose was based on the "maximum" amount which can be infused and solubility of the drug. All animals were observed at pretest, at 0.5, 1, 2, 4, 6, 10 and 24 hours after start of infusion for clinical signs and daily thereafter. Animals were observed twice daily for mortality. Body weights were recorded weekly and food consumptions were monitored daily. Blood samples were collected from cephalic vein for hematological tests. Hematological parameters (including PT and APTT) were monitored twice during pretest, just before start of infusion, at 3, 6 and 24 hours of infusion, and at 24 and 48 hours after the stoppage of infusion. At the end of 14 days of observation period all dogs were returned to stock.

Results:

1. **Observed Effects:** Clinical signs such as peripheral vasodilation, edema, subdued behavior and/or dyspnea were seen in all dogs treated with vehicle and/or drug. Severity of clinical signs were greater in treated dogs. These signs were not seen in saline treated dogs.
2. **Mortality:** None
3. **Body Weight/Food Consumption/Water Consumption:** Since there were only 2 dogs/sex/group therefore, no meaningful statistical evaluation can be done.
4. **Hematology/Coagulation/Bone Marrow:** At the end of 24 hours infusion, dose related increase in PT (males: 5.8 to 9.9 sec and females 5.9 to 10.9 sec) and APTT (males: 12.3 to 43.5 sec and females: 14.0 to 47.4 sec) were seen in treated dogs. Twenty-four hours after stoppage of infusion, PT and APTT values returned to normal.
5. **Blood Chemistry/Urinalysis:** Not done.
6. **Vital Signs/Physical Examination/Ophthalmic Examination:** Not done.
7. **Plasma Clearance:** The average clearance for males was 4.9 ± 1.3 ml/kg/min and for females was 3.6 ± 0.4 ml/kg/min.

Drug only produced clinical signs (peripheral vasodilation, edema, subdued behavior and/or dyspnea) and exaggerated pharmacological effects.

Following acute toxicity studies were submitted to IND ~~initial~~ initial submission, dated December 8, 1988, and were reviewed on 6/1/1989. These are reproduced below.

Acute Toxicity Test of Degradation Products of Argatroban in Rats

Animals: Male SPF rats (96.5-108.8 g) of Wistar-Slc strain.

Methods: Two groups of 5 rats each were treated i.v. with a single injection of degradation products G (1, 2, 3, 4-tetrahydro-3-methyl-8-quinoline sulfonic acid) and H (3-methyl-8-quinoline sulfonic acid) of argatroban dissolved in normal saline at a dose of 450 mg/kg and 60 mg/kg, respectively. No control group was employed. The selection of the doses was based on a preliminary test. General conditions were observed intermittently for 4 hr after administration of the test solution and subsequently once daily for 14 days. Body weight was recorded on the day of administration and days 1, 3, 7, 10 and 14 after administration. The date of autopsy was not specified. Histopathological examinations on heart, lung, liver, kidney, spleen and injection site of tail were performed on one rat from each group.

Results:

1. General conditions: No mortality was found. A decrease in spontaneous movement, crouching, lying, increased limb tone, tonic convulsion, Straub tail reaction, ataxic gait, bradypnea, lacrimation and red snivel were observed 5 min to 2 hr after administration of compound G. No abnormal reactions were observed after administration of compound H.

2. Body Weight: The mean body weights in both groups after administration of the test compounds were higher than those on the day of treatment. However, no control animals could be compared.

3. Autopsy and Histopathological Findings: There were no treatment related findings.

Conclusion: This was an incomplete test since only one dose of each test compound was employed. In addition, no control group was included for comparison. Compound G at the dose of 450 mg/kg caused some transient CNS symptoms such as tonic convulsion, Straub tail reaction and ataxic gait. However, the dose of compound G used for the study was exorbitantly high.

Following acute toxicity studies were submitted to IND amendment dated December 7, 1990, and were reviewed on 2/8/1991. These are reproduced below.

Acute Toxicity on Metabolite Products of Argipidine in Rats

Methods: The acute toxicity of MCI-9038 (a argipidine metabolite) after i.v. administration was studied in male SPF rats of Wistar - SLc strain. The drug was dissolved in 0.9% saline before injecting. Doses of 180, 268, 309, 355, 408, 470 and 540 mg/kg were used in this study. All animals were observed for mortality and toxic signs for 4 hours after the administration of the drug and daily thereafter for 14 days. At the end of the observation period, all animals were sacrificed and subjected to complete necropsy.

Results: Mortality occurred within 1 hr after administration of MCI-9038. Toxic signs were decreased spontaneous movement, mydriasis, palpebral ptosis, lacrimation, reddish lacrimation, anemia, bradypnea, clonic convulsion, tonic-clonic convulsion and increased limb tone. Histological examination revealed hemorrhage in the liver, kidneys and lungs in all dead animals. Surviving animals had no such histological findings. The LD₅₀ was 304 mg/kg and highest non-lethal dose was 268 mg/kg.

In acute toxicity studies, the highest tested doses of 15000 mg/kg oral and 81 mg/kg iv, in mice and rats resp were well tolerated. The minimum lethal ip doses in male and female mice were 241 and 347 mg/kg resp, these values by sc administration in male and female mice were 224 and 960 mg/kg resp. The minimum lethal ip (98 and 292 mg/kg) and sc (200 mg/kg in both sexes) doses in male and female rats were slightly lower than in mice. Time to death was mostly within 24 hrs. Clinical signs in both mice and rats were decreased spontaneous movement, balling, bradypnea, convulsion and dilation of the peripheral vessels. In dogs single iv dose of 200 mg/kg was lethal (time to death was 24-48 hrs due to hemorrhages). Deaths were preceded by vomiting, paralysis of hind limbs, tremors and coma, and in histopathology of dead animals necrosis of liver cells and renal tubular epithelium was noted. Paralysis of hind legs was noted in surviving animals who received 100-200 mg/kg. The iv acute toxicity study was also conducted in rats with M1, the major metabolite of argatroban. The minimum lethal doses of M1 were 309 mg/kg (time to death was 1 hr, due to hemorrhages in the liver, kidney and lungs).

Also, in acute toxicity studies, where argatroban was given continuously for 24 hrs (by an iv infusion) to rats (at doses of 0.7, 2.075, and 6.25 mg/kg/day), and dogs (at doses of 0.13-0.14, 0.40-0.44, and 1.15-1.22 mg/kg/day), it produced dose related increases in PT and aPTT (the exaggerated pharmacological effects) in both species. In addition, the drug produced severe irritation at the injection site from the vehicle (containing 2% tween 20) in rats, and clinical signs (peripheral vasodilation, edema, subdued behavior and/or dyspnea) in all treated dogs.

SUBACUTE/SUBCHRONIC/CHRONIC TOXICITY STUDIES:

Following toxicity studies were submitted to IND ~~initial~~ submission dated December 8, 1988, and were reviewed on 6/1/1989. These are reproduced below.

One-month Subacute Intravenous Toxicity Study in Rats

Test Period: March to April, 1979

Animals: Four-week old male and female rats of Wistar strain.

Methods: Groups of 10 males and 10 females each were treated i. v. through tail vein with bolus doses of 0 (10 ml/kg 0.96% NaCl), 3, 9 or 27 mg/kg argatroban once daily for 30 days (males) or 31 days (females). The selection of 3 mg/kg as the lowest dose was based on preliminary test while the highest dose was based on solubility of the drug. General behavior was observed everyday for 1 hour after the injection. Body weights were recorded everyday. Food consumption was recorded twice a week. Urine samples were collected on day 29 for urine analysis. Hematological and biochemical examinations were performed at the end of treatment.

Results:

1. General Observations: No abnormalities were observed in any of the test animals.
2. Body Weight: No significant effects were observed.
3. Food Consumption: No treatment-related effect.
4. Urinalysis: No significant differences were observed between the control and 27 mg/kg groups in urinalysis of pH, occult blood, ketone body, glucose, protein, urobilinogen and bilirubin. No analysis was conducted in either 3 or 9 mg/kg group.
5. Hematological Examination: There were significant increases of hematocrit (+3.9% from control) and monocytes (0.4 in treated, 0 in control) in female 27 mg/kg group.

6. Blood Chemistry: Significant increases of inorganic phosphate were seen in the male rats in the 9 mg/kg (+9.3%) and 27 mg/kg (+12%) groups.

7. Organ Weight: There were some sporadic significant differences in organ weights and the ratios of organ weight to body weight which seemed to be incidental.

8. Autopsy: No treatment-related changes were observed.

9. Histopathological Examination: Slight to moderate degrees of hepatic cell infiltration were found in some male rats in all treated groups. Slight proliferation of Kupffer's cells were found in most females in all treated groups.

Addendum to 1-Month Subacute Intravenous Toxicity Study in Rats:

Histopathological Examinations: Slight to moderate degrees of hepatic cell infiltrations (by what cells was not indicated) were found in livers of some male rats at 9 (6 of 10 animals) and 27 mg/kg/day (2 of 10 animals) vs none in controls (0 of 10 animals).

These studies indicate that no dose related increases in hepatic cell infiltration were noted in males, suggesting that the tolerated doses of argatroban in 1-month (iv) rat toxicity studies are 27 mg/kg/day.

Twenty-six Weeks Chronic Intravenous Toxicity Test in Rats

Study Start & Completion Dates: June 17, 1980 and Dec. 18, 1980.

Animals: Male (weighing 192-223 g) and female (weighing 158-184 g) rats of Sprague-Dawley (CD) strain.

Methods: Four groups of rats each consisting of 15 males and 15 females were treated intravenously through tail vein with 0 (10 ml/kg saline), 3, 9 and 27 mg/kg argatroban once a day for 26 weeks. General condition was observed daily. Body weight, food and water consumption were measured daily during the first week of treatment. Afterward, body weight was measured once a week, total food consumption was measured weekly, and two-day water consumption was measured. Blood specimens were collected from 5 males and 5 females each group on week 13 and from 10 males and 10 females each group on week 26 for hematological and biochemical examinations. Urine samples from individual animals were collected on weeks 13 and 26. Prior to dosing and after 4, 13 and 26 weeks of dosing, 5 males and 5 females from the control group and 27 mg/kg group were selected for ophthalmologic, auditory and ECG examinations. Five males and 5 females from each group were killed on week 13 after dosing and the remaining rats were killed on week 26 for histopathological examinations.

Results:

1. General symptoms: No treatment related symptoms were observed.
2. Body Weight: No treatment related changes in body weight were observed.
3. Food Consumption: There were no treatment related changes in food consumption.
4. Water Consumption: Water consumption in the male 27 mg/kg group increased by approximately 30% as compared to the control group from week 15 to the end of dosing.
5. Hematological Examination: After 13 weeks of treatment, the eosinophil counts and PTT in all argatroban-treated groups tended to vary, but there was no dose relation and significant changes were observed only in eosinophils in the female 9 mg/kg group (+101% from the control group) and PTT in the female 27 mg/kg group (-20%). These changes were not observed after 26 weeks of treatment. The only significant change in week 26 was a decrease (-34%) in the neutrophil count of females in the 9 mg/kg group.
6. Blood Chemistry: There were some sporadic but significant changes in the concentrations of serum GOT of males of 27 mg/kg group (+26%) on week 13 and serum GPT of males of 3 mg/kg group (-21%), serum GPT of males of 9 mg/kg (-19%), serum ALP of females of 27 mg/kg group (+43%), and serum TP of males of 3 mg/kg group (+ 4%) on week 26. There was no correlation between the changes and the dose or the duration of treatment.
7. Urinalysis: Increased volume of urine was observed in the males of 27 mg/kg group. Apparently, this increase was due to increased water intake. There were some significant changes in the concentrations of urinary excretion of sodium, potassium and chloride. However, the changes were slight, sporadic and had no correlation to the treatment.
8. Ophthalmologic, auditory and ECG Examinations: There were no treatment related effects.
9. Auditory Response: No abnormal changes in Preyer's reflex was found in the argatroban-treated animals.
10. ECG: There was no treatment related effects.
11. Organ Weights: There were slight but significant decreases in the relative weights of thyroid glands of males of 3 mg/kg group (-13%) in week 13, increases in the absolute weights of thyroid glands (+16%), absolute weights of adrenal glands (+21%) and relative weights of adrenal glands (+16%) of females in the 9 mg/kg group in week 26.

similar fashion. Additionally 5 rats/sex were also included in control and high dose groups which were used for 28-day recovery period. All animals were observed for clinical signs frequently on day 1 of the study and twice daily thereafter. Mortality check was performed once a day throughout the study period. Body weights and food consumptions ere recorded weekly. Ophthalmoscopic examinations were performed on all animals once pretest and at the end of treatment and recovery period. Just before sacrifice blood samples were collected for hematological (including PT and APTT) and serum chemistry tests. Urine samples were also collected for urinalysis. All surviving animals were sacrificed at the end of study/recovery period and subjected to complete necropsy. Only control and high dose group animals were examined histopathologically. Lungs and site of infusion from low and mid dose groups were also examined microscopically.

Results:

1. **Observed Effects:** No treatment related effects were seen.
2. **Mortality:** One rat from control group died on day 7 of the study due to infection.
3. **Body Weight/Food Consumption/Water Consumption:** No treatment related effects were seen.
4. **Hematology/Coagulation/Bone Marrow:** No treatment related effects were seen. Furthermore, it should be noted that no pharmacodynamic effects (PT and APTT) of the drug were evident in this study (see page 057 & 069, vol. 3).
5. **Blood Chemistry/Urinalysis:** No treatment related effects were seen.
6. **Vital Signs/Physical Examination/Ophthalmic Examination:** No treatment related effects were seen.
7. **Organ Weights:** No treatment related effects were seen.
8. **Gross Pathology:** No treatment related effects were seen.
9. **Histopathology:** No treatment related effects were seen. Perivascular hemorrhages, inflammation and thrombosis were seen in all rats at the infusion sites.

In this study the highest tested dose (0.20 mg/kg (bolus) + 1.25 mg/kg/hr for 28 days = 0.2 mg/kg bolus + 30 mg/kg/day for 28 days) was the "no effect dose".

Following toxicity studies were submitted under the current NDA.

1-Month Continuous Intravenous (iv) Toxicity Study of Argatroban in Rats: (Study # not stated)

Testing Laboratories: _____

Study Started: April 26, 1995

Study Completed: November 11, 1995

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

Animals: Male and female Sprague-Dawley rats (males 377-443 g, females 243-317 g, 12 weeks old).

Drug Batch No.: KEY01-01A1.

Methods: Three Groups (15/group) of male and female rats were given continuous intravenous infusions of argatroban (by placing indwelling catheters in the femoral veins), at 15, 30 and 60 mg/kg/day for 1-month, at a rate of 0.625, 1.25 and 2.5 ml/kg/hr (in a volume of 15, 30 and 60 ml/kg/day). The formulations contained 260 mg of the drug diluted with 250 ml of sterile 5% glucose solution. Actual doses given were 14.6, 28.5, and 58.2 mg/kg/day resp for males and 15, 29.7 and 59.4 mg/kg/day for females resp. Two control groups received, the 0.9% isotonic saline solution, and placebo (glucose solution) resp (60 ml/kg/day). Dose selection was based on the previous toxicity study (study # 95-00498-FR-00). Since in some animals infusion techniques did not comply with the protocol due to catheters withdrawal from the veins, or because animals went through 2 surgical procedures, these were excluded from the study. Therefore actual animals in this study ranged from 11 to 15. Mortality, clinical signs, body weights, food consumptions, ophthalmoscopic examinations, hematology and clinical chemistry/urine analysis tests were performed on animals. At the end of the 1-month study, all animals were sacrificed, and gross pathology and complete necropsy with histopathological examinations were carried.

Results:

1. Observed Effects: No treatment related effects were noted, except injuries due to infusion techniques and stress.
2. Mortality: In the placebo group, 10 animals (3 M + 7 F) died due to opportunistic bacterial infections from infusions. Also 6 animals (3 from saline and placebo, 2 and 1 from 30 and 60 mg/kg/day groups resp) were sacrificed due to deteriorating conditions, from similar infections.

3. Body Weight/Food Consumption: The initial and final (28 day) mean body weight of control male rats was 404.3 g and 490.6 g, and of female rats was 272.5 g and 332.6 g resp. The initial and final (28 day) mean food consumption of control male rats was 29.07 g/animal/day and 34.94 g/animal/day, and of female rats was 24.18 g/animal/day and 25.69 g/animal/day resp. No treatment related effects were noted.

4. Ophthalmological Examinations: No treatment related effects were noted.

5. Hematology: No significant drug related effects were noted.

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

7. Organ Weights: An increase in absolute (by 217%) and relative (by 224%) weights of spleen were observed in female rats at 30-60 mg/kg/day, compared to controls, however these were not associated with any histopathological changes.

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

9. Histopathology: Local reactions were observed at the injection site in both controls and treated animals. These included perivenous hemorrhage (at the vena cava site: in 75-100% of treated animals, vs 11-14% of controls), dilatation and intimal proliferation of the veins, phlebitis (in 25-43% of treated animals, vs 14-33% of controls). Perivenous tissue abscess at the injection site was more seen in controls (right side: 40-50%) than treated animals (none). All of these may be due to the catheterization techniques. Infectious and pneumonia, caused by infusion techniques (and in some, resulting in deaths) were found in all animals (controls: 5 and 10 of 10, treated: 10 of 13, 13 of 14, 11 of 11 animals at 15, 30 and 60 mg/kg/day resp). Histiocyte proliferation in the liver sinusoid was noted in all animals (controls: 5 of 11, 7 of 10, treated: 7 of 13, 11 of 14, 8 of 11 animals at 15, 30 and 60 mg/kg/day resp). However, sponsor states that animals receiving placebo which contained glucose, encouraged the bacterial growth more, and therefore more died in that group.

These studies indicate that many animals in this study, in the placebo group had intercurrent infections, however highest (60 mg/kg/day) doses of argatroban were not toxic in rats, when given continuously for 1-month.

Following toxicity studies were submitted to IND ~~initial~~ submission, dated December 8, 1988, and were reviewed on 6/1/1989. These are reproduced below.

Dog

One-Month Subacute Intravenous Toxicity Test in Dogs

Study Start and Completion Dates: May 25, 1979 and June 25, 1979.

Animals: Male (8.0 - 10.9 kg) and female (7.2-9.4 kg) beagle dogs at age of 9 months.

Methods: Groups of 3 males and 3 females each were treated i.v. through cephalic vein with 0 (10 ml/kg NaCl solution), 3, 9 or 27 mg/kg argatroban once daily for one month. The selection of low and high doses of argatroban was based on preliminary test and the solubility of the test drug, respectively. General behavior, health condition and feces were observed daily. Body weights and water consumption were recorded twice a week while food consumption was recorded once a week. Urine samples were collected 3-4 days before the beginning of treatment and on weeks 2 and 4 after the treatment. Examination of feces for occult blood, hematological examination and clinical blood chemistry test were performed on the same schedule of urinalysis. Electrocardiography and ophthalmoscopy were performed a few days before the treatment and on weeks 2 and 4 after the treatment. All animals were killed on the day following the final treatment for histopathological examinations.

Results:

1. General Conditions: There was no mortality during the treatment. All male and female dogs in the 27 mg/kg had licking of their lips for several minutes after dosing on about half of the treatment days. Occasional licking of lips were observed in all animals in the 9 mg/kg group. All dogs in the 27 mg/kg group vomited several times immediately after dosing on about half of the treatment days.
2. Body Weight Gain: There was no treatment related effect.
3. Food Consumption: There was no treatment related effect.
4. Water Consumption: There was no treatment related effect.
5. Urinalysis: No abnormalities in any dogs treated with argatroban were observed.
6. Fecal Examination: Slight occult blood was observed in all animals including controls but there was no increase as a result of argatroban treatment.
7. Hematological Examination: There were no treatment related effects.

8. Clinical Blood Chemistry: There were no treatment related effects.
9. Electrocardiography: No significant abnormalities were observed.
10. Gross Pathology: Hydropic degeneration of the ovary in one dog in the 9 mg/kg group and one jejunal petechia were observed at autopsy.
11. Organ Weight: There were no treatment related changes in wet organ weights and organ weight/body weight ratios in any of the organ studied.
12. Histopathology: No treatment related effects were observed.

Conclusion: The sponsor did not present any result of statistical analysis of the data. The selection of the highest dose of argatroban was based on its solubility rather than its maximal tolerated dose. The only change due to administration of argatroban was the slight, transient licking of lips in the 9 and 27 mg/kg groups. No significant toxicity of argatroban was found in the present study.

Six-month Chronic Intravenous Toxicity in Dogs

Study Start and Completion Dates: Jan. 18, 1985 and July 31, 1985

Animals: Six-month old male and female beagle dogs.

Methods: Four groups of 4 males and 4 females each were treated i. v. through cephalic vein with 0 (3.3 ml/kg 5.4% D-sorbitol), 1, 3 or 9 mg/kg/day argatroban once daily for six months. Clinical signs were observed once daily. Body weight and food consumption were measured once weekly for the first 3 months and fortnightly thereafter. Urinalysis of each animal was performed on day 0 before the test began and on weeks 13 and 25. Ophthalmology for each animal was performed on weeks 12 and 26. Blood samples were collected on the day before the beginning of treatment and on weeks 5, 14 and 26. Samples of femor bone-marrow were prepared after the animals were sacrificed and the granulocyte/erythroblast ratio (G/E) was measured. Histopathological examinations were performed at the end of treatment.

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Results:

1. **Clinical Signs:** Swelling of the mammary region was observed in one female dog in the 9 mg/kg group. There was a dose-dependent tendency of vomiting immediately after administration.
2. **Body Weight:** No significant changes were found.
3. **Food Consumption:** Since the entire amount of food provided was consumed by the males, the effect of argatroban on the food intake of male dog could not be determined. In general, there was a slight decrease in food intake in the females of argatroban-treated groups.
4. **Urinalysis:** There were no treatment related effects on the parameters of urinalysis.
5. **Ophthalmological Examination:** No treatment related effects.
6. **Hematological and Bone-marrow Examinations:** During week 5, there were significant decreases in the neutrophil ratio (-30%) of males of 1 mg/kg group, white blood cell count (-13.8%) and prothrombin time of females of 1 mg/kg group. There were significant increases in the mean corpuscular volume (+4.8%) of males of 9 mg/kg group, lymphocyte ratio (+58%) of males of 1 mg/kg group, platelet count (+115%) of females of 1 & 3 mg/kg groups and partial thromboplastin time (+37%) of females of 9 mg/kg group. On week 14, there were significant decreases in the red blood cell count (-16%), Hb concentration (-14%) and hematocrit value (-11%) of males in the 3 mg/kg group, and decrease in prothrombin time in all treated males. There were significant increases in platelet count of males (+53%) of males in the 3 mg/kg group, mean corpuscular volume (+5%) of males of 9 mg/kg group and platelet count (+25%) of females of 1 mg/kg group. On week 26, there were significant decreases in prothrombin times in all treated females (-14 to -22%), red blood cell count (-11%) of males of 3 mg/kg group and fibrinogen concentrations (about -22%) of males in the 1 & 9 mg/kg groups. There were significant increases in mean corpuscular hemoglobin volume (+6%) of males of 9 mg/kg group and platelet count (+28%) of females of 9 mg/kg group.
7. **Blood Chemistry:** There were significant decrease in ALB (-12%) of males of 3 mg/kg group on week 14 and Ca concentrations (-4%) of males of 9 mg/kg group on week 14 (-4%) and on week 26 (-4%) of 9 mg/kg group.
8. **Organ Weights:** Significant increases in the absolute (+15%) and relative weight (+17%) of liver of males of 3 mg/kg group and absolute weight of adrenal of females of 3 mg/kg (+32%) were found. There were decreases in the relative weight of spleen (-18%) in the females of 1 mg/kg group (-25%) and 3 mg/kg group (-18%).
9. **Gross Pathology:** No treatment related effects.
10. **Histological Examination:** No treatment related abnormality.

Conclusion: Treatment of dogs with argatroban (1, 3 or 9 mg/kg/day x 26 weeks, i.v.) produced no clear toxicity except a dose-related tendency to vomit. Due to the poor solubility of argatroban, the sponsor did not employ higher doses in the study. No target organ of toxicity was identified.

Following toxicity studies were submitted to IND _____ amendment dated September 11, 1992, and were reviewed on 12/15/1993. These are reproduced below.

28-Day I.V. Infusion Toxicity Study in Dogs
(Study # 90-246-1600)

Testing Laboratories: _____

Dates Study Started and Completed: April 30, 1991 and January 6, 1992.

GLP Requirements: A Statement of Compliance with GLP regulations and quality assurance unit was included.

Animals: Six months old Beagle dogs (males: 6.6 - 10.2 kg and females: 5.6 - 7.8 kg).

Drug Batch No.: M3-RD107 and M3-RD105.

Methods: Groups of dogs (4/sex/group) were given I.V. bolus dose (0.4 ml/kg) followed by a continuous I.V. infusion dose (2.5 ml/kg/hr) for 28 days. The low, mid and high dose levels were 0.02 mg/kg (bolus) + 0.125 mg/kg/hr, 0.07 mg/kg (bolus) + 0.417 mg/kg/hr and 0.20 mg/kg (bolus) + 1.25 mg/kg/hr respectively. The selection of highest tested dose was based on the maximum amount which could be given and solubility of the drug. The control group received the vehicle _____ in similar fashion. Additionally, 2 dog/sex were also included in control and high dose groups to be used for 28-day recovery period. All dogs were observed for clinical signs frequently on day 1 of the study and twice daily thereafter. Mortality check was done once a day throughout the study period. Body weights were recorded weekly and food intakes were monitored daily. Ophthalmoscopic examinations were performed on all dogs once pretest and at the end of treatment/recovery period. ECG recordings were obtained for all animals twice during pretest, and at the end of treatment period and at the end of recovery period. Blood samples were collected from overnight fasted dogs at pretest, and on days 7, 14 and 28 of the study and at the end of recovery period for hematological (including PT and APTT) and serum chemistry tests. Urine samples were also collected at the above mentioned time period for urinalysis. All surviving dogs were sacrificed at the end of study/recovery period and subjected to complete necropsy and histopathological examinations.

Results:

1. **Observed Effects:** No treatment related effects were seen except one high dose treated male (# 4155) had severe contusions at the femoral surgical site. This may be related to the changes in blood coagulation parameters due to the drug.
2. **Mortality:** None
3. **Body Weight/Food Consumption/Water Consumption:** No biological significant effects were seen which could be attributed to the treatment.
4. **Hematology/Coagulation/Bone Marrow:** No treatment related effects were seen except dose related increases in PT and APTT were seen in treated dogs.

	Measurement on Day 28 of the Study			
	Males		Females	
	<u>P.T. (sec)</u>	<u>APTT (sec)</u>	<u>P.T. (sec)</u>	<u>APTT (sec)</u>
Control	7.4 ± 0.36	9.8 ± 0.67	7.8 ± 0.26	10.4 ± 1.06
Low Dose	8.0 ± 0.35	11.0 ± 1.03	7.9 ± 0.28	12.7 ± 1.84
Mid Dose	9.2 ± 0.56	15.6 ± 2.64	8.8 ± 0.51	14.3 ± 1.16
High Dose	11.1 ± 0.68	18.0 ± 1.75	11.8 ± 1.18	18.7 ± 2.31

At the end of recovery period PT and APTT returned to normal levels.

5. **Blood Chemistry/Urinalysis:** No treatment related effects were seen.
6. **Vital Signs/Physical Examination/Ophthalmic Examination/ECG:** No treatment related effects were seen.
7. **Organ Weights:** Relative weights of spleen were increased by 33-35% in mid and high dose treated females. At the end of recovery period relative weights of spleen were comparable in dogs of control and high dose groups.
8. **Gross Pathology:** No treatment related effects were seen.
9. **Histopathology:** No treatment related effects were seen.

In this study, the highest tested dose (0.20 mg/kg (bolus) + 1.25 mg/kg/hr for 28 days = 0.2 mg/kg bolus + 30 mg/kg/day for 28 days) was the "no effect dose".