

Following toxicity studies were submitted under the current NDA 20,883.

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

Testing Laboratories:

Study Started: May 2, 1995

Study Completed: December 6, 1995

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

Animals: Male and female Beagle dogs (males 8.3-11.9 kg, females 8.3-10.1 kg, 7-11 and 9-13 months old resp).

Drug Batch No.: KEY01-01A1

Methods: Three groups (4/group) of male and female dogs 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, in a volume of 15, 30 and 60 ml/kg/day (at a rate of 0.625, 1.25 and 2.5 ml/kg/hr). Actual doses given were 14.9, 29, and 55.7 mg/kg/day resp for males and 15.2, 28.7 and 57 mg/kg/day for females resp. Two control groups received, the 0.9% isotonic saline solution, and placebo (containing glucose) resp (60 ml/kg/day). Dose selection was based on the previous toxicity study (study # 95-00506-FR-00). Dogs received following premedication for the surgical administration of catheters: subcutaneous atropine (0.05 mg/kg iv), 10-20 min before the anesthetic (zoletil 50, 20-25 mg/kg, iv), antibiotic (dalacine, 75 mg capsule, twice a day), from one day before, till 7 days after surgery, and profenid (non-steroidal anti-inflammatory drug, 2 mg/kg, im), on the day and 1 day after surgery. Since some dogs had swelling at the catheterization site, they received an oral anti-inflammatory proteolytic enzyme (extranase, 3 tablets daily), males received it for 3 days, females for 7 days. Additionally some dogs also received few applications of another proteolytic enzyme (alphachymotrypsine choay). Mortality, and clinical signs were observed daily. Body weights, food consumptions, electrocardiographic examinations, blood pressures, hematology and clinical chemistry tests were performed on animals. Plasma conc of the drug were measured after the 24th day (at 1, 3, 6 and 9 hrs after dosing). At the end of the 1-month study, all animals were sacrificed, and gross pathology and complete necropsy with histopathological examinations were carried out.

Results:

1. Observed Effects: Swelling of varying degree at the jugular site were observed in animals due to catheterization. However, no treatment related effects were noted.
 2. Mortality: One male in placebo group was sacrificed on day 23, due to accidental chest trauma.
 3. Body Weight/Food Consumption: No treatment related effects were noted.
 4. Electrocardiographic Examinations: No treatment related effects were noted.
 5. Hematology: No significant drug related effects were noted.
 6. Blood Chemistry: No treatment related changes in blood chemistry were observed.
 7. Organ Weights: No treatment related effects were noted.
 8. Gross Pathology: Changes due to surgery and infusion were noted in all animals. These included masses in the cervical and dorsal region and/or along the catheter, and skin wounds.
 9. Histopathology: Reactive inflammatory and vascular lesions were found in the lungs of all animals due to experimental procedures. Local reactions (intimal proliferations of infused veins associated with chronic irritative effect) were observed at the injection site in both controls and treated dogs. No other treatment related effects were noted.
- Toxicokinetics: These are shown in Table III (reproduced from volume 27, page 267 of the submission). In males the conc of the drug increased in a dose related manner between 30 and 60 mg/kg/day. In females similar conc were noted at 15 and 30 mg/kg/day.

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Table III.

The results of the mean plasma concentrations, expressed in ng/ml, observed after 24 days of treatment are listed in the table below:

Dose (mg/kg/day)	Sex	Time (hours)			
		1	3	6	9
15	Male	1685	1707	1600	1595
	Female	1349	1476	1317	1348
30	Male	3239	3124	3163	3287
	Female	1322	1410	1432	1362
60	Male	3951	4541	4380	4413
	Female	3606	3976	3740	3530

These studies indicate that 60 mg/kg/day doses of argatroban, did not produce any toxicity in dogs, when given continuously for 1-month.

Following toxicity studies were submitted in the current NDA.

A 1-month Bolus Intravenous (iv) Toxicity Study of Argatroban (containing 0.5% Impurities) in Rats (Study # 2110 TMR)

Testing Laboratories: _____

Study Started: September 29, 1993

Study Completed: January 18, 1994

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

Animals: Male and female Sprague-Dawley rats (males 232-245 g, females 175-205 g, 7 weeks old).

Drug Batch No.: ONZO1-07A2

Methods: This study examined the potential local reaction, after bolus iv administration of the drug and its impurities (formed during heat sterilization of the ampule) in rats. Two Groups (12/group) of male and female rats were given bolus intravenous argatroban (the drug was reesterilized by heating to produce maximum acceptable impurity of _____) in the lateral tail veins, at 0 and 5 mg/kg/day for 1-month, at a rate of 5 ml/kg/min

(formulated at 10 mg/20 ml of the drug). Control group received, the placebo. Dose selection was based on the volume (10 ml/kg) and the conc of the injectable solution (10 mg drug/20 ml), that could be administered. Mortality, clinical signs, body weights (on days -1, 1, 3, 7, 14, 21, 24, 28), food consumptions (on days 2, 6, 9, 13, 16, 20, 23, 27), 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.
2. Mortality: None.
3. Body Weight/Food Consumption: The initial and final (28 day) mean body weight of control male rats was 227.6 g and 413.7 g, and of female rats was 184.5 g and 263.7 g resp. The initial and final (27 day) mean food consumption of control male rats was 25.3 g/animal/day and 30.0 g/animal/day, and of female rats was 18.8 g/animal/day and 22.6 g/animal/day resp. In both male and female rats, a slight decrease in body weights (by 4% and 4.3% resp, % of difference from control) were observed, in females these were associated with decrease (8.4%) in food consumptions.
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: No treatment related effects were noted.
8. Gross Pathology: No treatment related effects were noted.
9. Histopathology: In spleen, the incidences of congestion were slightly higher in treated (2 of 12 males, and 6 of 12 females, vs controls (1 of 12 control males and females resp). Local reactions were observed at the injection site in both controls and treated animals (e.g. perivenous hemorrhage: in 4 of 12 treated males and females, vs 2 and 1 of 12 control males and females resp), these may be due to the pharmacological effects of the drug. No other treatment related effects were noted.

These studies indicate that 5 mg/kg/day doses of argatroban, containing maximum impurities of _____ did not produce any toxicity in rats, when given for 1-month. A group of rats receiving pure argatroban (without impurities) should have been included in this study to compare the effects of the pure vs impure drug.

A 1-Month Bolus Intravenous (iv) Toxicity Study of Argatroban (containing _____ Impurities) in Dogs. (Study # 2111 TMC)

Testing Laboratories: _____

Study Started: September 14, 1993

Study Completed: January 3, 1994

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

Animals: Male and female Beagle dogs (males 10.3-12 kg, females 9.3-11 kg, ~15 months old).

Drug Batch No.: ONZ01-07A2

Methods: This study examined the potential local reaction, after bolus iv administration of the drug and its impurities (formed during heat sterilization of the ampule) in dogs. Two groups (3/group) of male and female dogs were given bolus intravenous argatroban (the drug was reesterilized by heating to produce maximum acceptable impurity of _____) in the right and left cephalic and saphenous veins (by daily rotation of injection sites), at 0 and 2.5 mg/kg/day for 1-month, at a rate of 1.25 ml/kg/min (in a volume of 5 ml/kg). Control group received the placebo. Mortality, clinical signs, body weights, food consumptions, electrocardiographic, blood pressure, 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: In six treated animals, more colorations (reddish, bluish or purple) were found than controls (3 animals), during first 2 weeks which were reversible in 4 days. Two treated animals also had perivenous hematoma.

2. Mortality: None.

3. Body Weight/Food Consumption: No treatment related effects were noted.
4. Cardiovascular/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: No treatment related effects were noted.
8. Gross Pathology: At the injection site, redness of the subcutaneous connective tissue was observed, more in treated animals.
9. Histopathology: In treated animals at the injection site, post traumatic inflammatory reactions (such as increased incidences of hemorrhage, infiltration or fibrosis) were noted. These may be due to the antithrombotic (pharmacological) effects of the drug. In 1 of 3 female dogs, in one lobe of the liver, marked cholangitis and inflammatory infiltrate (composed of mononuclear cells, cholestasis, proliferation of bile ducts, cell necrosis and moderate pigmentation of Kupffer cells) was observed.

This study was deficient, because a group of rats receiving pure argatroban (without impurities) should have been included in this study to compare the effects of the pure vs impure drug.

REPRODUCTIVE TOXICITY STUDIES:

Following toxicity studies were submitted to IND _____ 1 amendment dated December 7, 1990 and were reviewed on 2/8/1991. These are reproduced below.

Segment I. Fertility and General Reproductive Performance Study in Rats (Report # M56-120)

Testing Laboratories: _____

Study Started: September 1981

Study Completed: January 1982

Date of Report: June 1982

GLP Requirements: Not mentioned

Animals: Male (6 week old) and female (12 weeks old) SPF Wistar rats.

Drug Batch No.: 800610

Methods: Groups of 28 male and 28 female rats were given MD-805 intravenously 0 (Saline), 3, 9 and 27 mg/kg/day. The male rats were treated from 60 days prior to mating and throughout the mating phase until they were sacrificed. Female rats were treated for 14 days prior to mating and throughout the mating phase. Additionally pregnant females received the drug for 7 more days after mating. The dose selection was based on solubility of the drug as well as on the results of acute and subacute toxicity studies. Parents were observed daily for mortality and toxic signs. Body weights, food and water consumptions were recorded weekly. The mating performance and fertility of both sexes were evaluated. All pregnant rats were sacrificed on day 20 of gestation. This study was not conducted according to FDA guidelines. Our guidelines require that females should have been treated 14 days before mating through gestation and lactation and one-half of dams should be sacrificed on day 13 of their respective pregnancies and the remaining dams should be allowed to litter normally. Therefore study only provides the effect of the drug on males fertility and general reproductive performance. Number of corpora lutea, number of implantation sites, number of live/dead fetuses, and numbers of pre- and post implantation losses were recorded. All fetuses were weighted, sexed and examined for external abnormalities. One-half of the fetuses were examined for skeletal malformations and variations, and the remaining half of the fetuses were examined for visceral abnormalities.

Results: At the highest dose (27 mg/kg/day), stained tail was seen more frequently than in the other groups. No significant effect on body weight gains and food consumptions were seen in treated rats. However, at high dose water consumptions were significantly increased (2-25%) in treated rats compared to control group during the latter part of the treatment period. The estrous cycle of the female rats revealed no differences between the control and treated groups. No significant differences in cooulation index, pre-coital time, or fertility index were seen between the control and treated groups. No significant differences were seen in the corpora lutea, total implantations, pre- or post implantation losses, embryonic deaths, number of live fetuses, fetus weight or sex ratio between the control and treated animals. No external abnormalities were found. Two females in the control group (A-3 and A-7) each had a fetus with abnormal origin of the left subclavian artery. Skeleton examinations revealed closure of the foramen transversarium of the 5th cervical vertebra in one or two fetuses of 7 females (3 in control, 1 in low dose group, 2 in mid dose group and 1 in high dose group). Three fetuses with 14th rib were seen in one female of mid dose group. One fetus with delayed ossification was seen in one female of the mid dose group and 1 in the high dose group. No abnormalities in F₀ males were evident.

In this study the males were treated for 60 days before mating and during mating, while females were treated 14 days before mating through day 7 of pregnancy. This study is not according to FDA Segment I guidelines, sponsor sacrificed all dams on the 20th day of gestation. Our guidelines require that females should have been treated 14 days before mating through gestation and lactation and one-half of dams should be sacrificed on day 13 of their respective pregnancies and the remaining dams should be allowed to litter normally. By not following the guidelines, sponsor could not study the effects of the drug on early stages of gestation. However, there were no abnormal effects on the fertility and mating performance of the treated male rats at doses upto and including 27 mg/kg/day of MD-805.

Addendum to Segment I Study:

Although the above segment I studies were not in accordance with the guidelines before, they comply with the present ICH guidelines.

Segment II. Teratology Study in Rats (Report # 55-458)

Testing Laboratories: _____

Study Started: May 1980

Study Completed: November 1980

GLP Requirements: This study was conducted according to Japanese GLP Standard.

Test Species: Pregnant SPF Wistar rats

No. of Animals: 40-43 pregnant rats/group

Drug Batch No.: 791101

Methods: The selection of the doses were based on the preliminary study in which i.v. doses of 0, 9 and 27 mg/kg/day were used. No significant effects on body weight gains, food and water consumptions were evident, but significant decreased number of implantations were seen in the treated dams (control = 11.6 ± 1.5 /dam, mid dose = 9.8 ± 3.8 /dam, and high dose = 9.2 ± 2.8 /dam) along with preimplant losses (control =

3.538%, mid dose = 18.182% and high dose = 18.485%). One F₁ fetus of dam # C-11 of high dose group had malformation of the eye (anophthalmia), another F₁ fetus of dam # C-8 had similar malformation of the eye. Based on these results and considering the solubility of the drug, dose levels of 0, 3, 9 and 27 mg/kg were selected for the main study.

In the main study, pregnant rats were given i.v. doses of 0, 3, 9 and 27 mg/kg from day 7 to 17 day of gestation. Control animals received the vehicle (saline) throughout the same period. The volume of administration was fixed at 10 ml/kg. Pregnant dams were observed daily for mortality and clinical signs. Body weight, food consumption and water consumption were recorded on days 0, 6, 8, 10, 12, 14, 16, 18 and 20 of gestation. Two-third pregnant rats were sacrificed on day 20 of gestation, and was examined for the number of corpora lutea, the number of implants, the number of dead or resorbed fetuses and number of live fetuses. The live fetuses were weighed and sexed. Approximately two thirds of the fetuses eviscerated and examined for skeletal major/minor abnormalities, the remaining fetuses were examined for visceral abnormalities and variations. The remaining 1/3 of the dams (11-15 /group) were allowed to deliver spontaneously. The number of live/dead pups were recorded, and the live pups were weighed and sexed. Culling was carried out to make 8 offspring (4 male and 4 female) per dam. The offspring were reared by the dams until day 21 of post partum. Following delivery, the dams were checked daily for clinical signs, body weight, food and water consumptions were recorded weekly. On day 21 of post partum all dams were sacrificed and necropsied, and examined as mentioned above. Postnatal body weight changes, food and water consumptions of the pups were recorded until the age of 8 weeks. During the nursing period the growth and differential of the pups were observed, and development parameters were assessed (fertility test, auditory test, learning ability test, ophthalmological examination, open field test). At week 10, 20 pairs of the animals per group were continuously mated for 14 days. F₁ dams were weighed on day 0 and 20 of gestation. Cesarean section was performed on the F₁ dams on day 20 of gestation, and fetuses were examined and preserved in acetone and Bouine's fluid.

Results: No significant effect on body weight or food consumptions were seen in treated rats. The number of corpora lutea, the number of implants, numbers of live/dead embryos, weights of fetuses and sex ratio did not show any significant difference between the treated groups and the control group. Pre implant losses were only significant at low dose (control = 13.177%, low dose = 29.532%, mid dose = 16.667% and high dose = 15.393%). External examinations were normal. Visceral examination of F₁ fetuses revealed microphthalmia in one fetus of dam # B-7 at 3 mg/kg dose group and anophthalmia in one fetus of dam # B-30 in the same dose group. In the 27 mg/kg dose group one dam (# D-3) had a fetus with anophthalmia and another dam (D-10) had a fetus with microphthalmia associated with severe dilation of renal pelvis. No skeletal abnormalities were observed.

Effect of MD-805 on Maternal and Fetal Parameters in Rats

<u>Parameters Measured</u>	<u>Control</u>	<u>Low Dose</u>	<u>Mid Dose</u>	<u>High Dose</u>
# Dam examined	21		22	20 21
# Corpora lutea/Dam	12.6+1.7	11.9+1.9	12.5+1.1	12.4+1.2
# Implants/Dam	10.9+2.7	8.5+3.5	10.5+3.0	10.4+2.6
# Pre implant loss (%)	13.177	29.532	16.667	15.393
# Post implant loss (1%)	4.860	2.244	7.835	6.123
# Total implant loss(1%)	16.340	31.444	21.657	20.457
# Early EmbryonicDeaths/ dam	0.0	0.0	0.0	0.0
# Late Empryonic Deaths/ dam	0.4	0.2	0.5	0.6
# Total Embryonic Deaths/ dam	0.4	0.2	0.5	0.6
# Live fetuses/dam	10.4+3.0	8.3+3.4	9.9+3.3	9.8+2.8
Fetal wt (g)				
male:	3.4+0.23	3.56+0.25	3.33+0.37	3.35+0.19
female:	3.16+0.21	3.24+0.15	3.22+0.22	2.15+0.15
Sex Ratio (M/F)	0.97	1.04	0.85	1.00

Dams allowed to deliver: No significant differences in the gestation period between the groups were noted. The number of fetuses/dam was somewhat lower in mid and high dose groups. There were no significant effects on postnatal development and differentiation (reflex reactivity test, auditory test, open field test, pole climbing test and ophthalmological test). At necropsy of F₁ generation at 10 weeks of age revealed a significant increase of brain weight in males (3.6%) and significant increase in adrenal glands weight in females (14.3%). There was no significant effect on fertility test and mating performance test of F₁-generation rats. External examination of F₂ fetuses revealed one case of anophthalmia in the control group (data not shown).

Thus no treatment related abnormalities were observed on external, visceral and skeletal examinations in any group except malformed eyeballs (anophthalmia/ microphthalmia) were seen in low and high dose group (combine incidence in preliminary and main studies: control = 0/503, low dose = 1/336, mid dose = 0/404 and high dose = 3/486). No teratogenic effects at dosage up to 27 mg/kg/day was observed. The postnatal development and the fertility of the offspring were comparable in all groups.

Segment II. Teratology Study in Rabbits (Report # D-11)

Testing Laboratories: _____

Study Started: June 8, 1984

Study Completed: March 8, 1985

GLP Requirements: This study was conducted according to GLP regulations.

Testing Species: New Zealand White pregnant rabbits.

No. of Animals: 17 pregnant rabbits/dose/group

Drug Batch No.: 43P101

Method: The highest i.v. dose selection was based on solubility of the drug. The drug was hardly soluble more than 5 mg/10 ml and technical limit for the repeated i.v. administration to rabbits was 4 ml/kg. In view of these limitation the highest dose selected was 2 mg/kg, and the other two remaining dose levels were 1 and 0.5 mg/kg. Thus pregnant rabbits were given i.v. injection of DK-7419 from day 6 through 18 of gestation. Pregnant rabbits were observed daily during pregnancy and their weights were recorded on 0, 6, 9, 12, 15, 19, 24 and 29 day of gestation. Food consumptions were also monitored at various time intervals. The dams were sacrificed at day 29 of gestation, and was examined for the number of corpora lutea, the number of implants, live fetuses, and reabsorbed/dead fetuses. The live fetuses were weighted and sexed. Their survival rate was observed 6 hours after putting them in an incubator at 28°C. All the fetuses were eviscerated and examined for skeletal/visceral anomalies.

Results: There were 3 deaths (2 in the control group and 1 in high dose group) during the study period. Additionally 6 dams (2 in control group, 3 in low dose group and 1 in high dose group) had abortion. Those dams found dead or had abortion revealed no gross abnormalities at necropsy. No significant effect on body weight gain/loss were evident during the study period. Dose dependent increases (45-194%) in food consumptions were seen in treated animals during 24-29 day of gestation. The number of corpora lutea, the number of implants, number of live fetuses, rate of fetal mortality (control = 16.4%, low dose = 13.8%, mid dose = 23.0% and high dose = 23.0%), sex ratio and mean body weight of live fetuses did not show any significant difference between the treated groups and the control group. In high dose group, only male live fetuses mean body weights were significantly higher (24.7%) than the control values. External anomalies such as omphalocele, dwarf, exencephaly, oligodactyly of the left forelimb and adactyly of the right limb were seen, and these findings were neither significant nor dose related. No visceral anomalies were seen in any group, and no treatment related skeletal abnormalities were evident in any group. Thus there was no evidence of a teratogenic potential in this study.

Segment II. Teratology Study in Rabbits (Report # D-20)

Testing Laboratorie _____

Study Started: January 22, 1987

Study Completed: April 15, 1987

GLP Requirements: this study was conducted according to GLP regulations.

Testing Species: New Zealand White pregnant rabbits.

No. of Animals: 17-18 pregnant rabbits/group

Drug Batch No.: 6QP102

In the previous study (Report # D-11), the drug was dissolved in water for i.v. administration, and the highest dose tested was 2.0 mg/kg due to its limited solubility in water. In the present study, a new formulation was used in which _____ was used as vehicle.

As a result a dose level of 10.8 mg/kg can easily be administered intravenously to rabbits. In this study only one dose level (10.8 mg/kg) was tested along with vehicle control group. Results were similar as mentioned above, furthermore no changes in male live fetus mean body weights, maternal food consumptions during 24-29 day of gestation, or fetal mortality rate were observed in this study. Thus there was no evidence of a teratogenic potential even at 10.8 mg/kg dose level.

Segment III. Perinatal and Postnatal Study in Rats
(Report # M58-163)

Testing Laboratories: _____

Study Started: July 25, 1983

Study Completed: May 31, 1984

GLP Requirements: This study was conducted according to Japanese GLP standard.

Test Species: Pregnant SPF Wistar rats

No. of Animals: 23 rats/group

Route of Administration: I.V.

Dose Levels: 0, 3, 9, 27 mg/kg (10 ml/kg body weight)

Drug Batch No.: P-ATB-52406

Methods: Pregnant rats were given i.v. doses of 0 (vehicle: saline), 3, 9, and 27 mg/kg from day 17 of gestation to day 21 after parturition. All dams were observed for clinical signs daily, body weights were recorded on 0, 3, 7, 10, 14, 17, 18, 19, 20 and 21 day of gestation, and daily on 0-21 day of post partum. Food and water consumptions were recorded on days 0, 4, 7, 10, 14, 17, 19 and 21 of gestation, and days 0, 4, 7, 14 and 21 day after parturition. The dams after completing the nursing were killed, and effects on dams and on the development (physical, behavioral, sensory development after birth, open field test, auditory test, learning ability test, estrous cycle) and reproductive performance of litters were examined. F₁ dams without treatment were evaluated in similar fashion F₂ pups were weighed and sexed and examined for external abnormalities. Approximately one-half of the fetuses were examined of skeletal abnormalities and the remaining fetuses were examined for visceral abnormalities.

Results: Throughout gestation and lactation period, no abnormalities were seen in clinical signs, body weight gains, food and water consumptions of F₀ dams. Length of gestation was comparable in all groups. No abnormalities were observed at autopsy of F₀ dams which would be attributed to treatment. No drug related effects were seen in the F₁ pups during postnatal period, development and reproductive performance, and no external abnormalities were observed in the F₂ fetuses. Thus no adverse effect were seen in rats following i.v. administration of up to 27 mg/kg/day of MD-805 during perinatal and postnatal period.

Segment III Perinatal and Postnatal Study in Rats

<u>Parameters</u>	<u>Dose (mg/kg)</u>			
	<u>0</u>	<u>3</u>	<u>9</u>	<u>27</u>
# Mated	23	23	23	23
# Pregnant	21	22	23	21
% Pregnant	91.3	95.6	100	91.3
Length of gestation (days)	22.2	22.2	22.2	22.1
No. of Implant/dam	7.3	7.6	8.3	8.5
No. of live fetuses/dam	6.8	7.4	8.0	8.0
Mean Fetal wt. (g)				
Male:				
day 0	5.49	5.76	5.53	5.45
day 4	8.90	9.49	9.00	9.05
Female:				
day 0	5.25	5.38	5.20	5.19
day 4	8.76	9.16	8.62	8.83
% Survived:				
day 4*	99.3	99.4	99.5	99.4
day 21**	99.3	100.0	98.9	99.4

* = # of fetuses alive at day 4/# of fetuses alive at birth.

GENOTOXICITY STUDIES:

Effects of Argatroban In Vivo Micronucleus Test in Mice
(Study # 10586 MAS)

Testing Laboratories: _____

Study Started: June 1, 1993

Study Completed: August 27, 1993

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

Test Strain: Adult male (30-35.5 g) and female (21.1-29.2 g) Swiss OF1/ICO mice, 6 weeks old.

No. of Animals: 5 animals/sex/group/sacrifice time.

Route of Administration: IV.

Doses Employed: 27 mg/kg body weight.

Basis of Dose Selection: Doses were selected based on the solubility of the drug. Argatroban was supersaturated at conc of 2.7 mg/ml.

Solvent Control: 0.9% physiological saline.

Positive Controls: Cyclophosphamide (CP), 5 mg/ml of water.

Drug Batch No: P-ATB-58201S

Criteria of Genotoxic Effect: If a statistically significant increase in the number of micro nucleated polychromatic erythrocytes (MPE) was observed, compared to the vehicle control, for at least one of the sampling time, and the increase doubled in the number, from the sponsor's historical data, the test was considered positive.

Methods: In preliminary toxicity studies, a group of mice (3/sex) received two, 27 mg/kg iv injections of argatroban, 24 hrs apart (in a volume of 10 ml/kg). For the main study, mice (5/sex) were given above doses of argatroban (24 hrs apart). A group of mice were similarly treated with the vehicle or CP (the positive control). Animals were sacrificed at 24, and 48 hours after second dosing, and bone marrow cells were prepared. Cells were stained with May-Gruenwald-Giemsa stain, and 2000 polychromatic erythrocytes per animal were examined for the presence of micronuclei. The statistical significance ($p < 0.05$) in the treated groups were determined, compared to the vehicle control.

Results: In the preliminary toxicity, argatroban given (once or twice), did not cause any toxicity in any of the treated mice, except piloerection. In the main study, It did not cause any mortalities or clinical signs, and also did not induce an increase in micro nucleated PCE, or the ratio of PCE to normochromatic erythrocytes (NCE), in either male or female mice bone marrow, at any collection time. In contrast, CP induced a significant increase in the micro nucleated polychromatic erythrocytes in both male and female mice, compared to the vehicle control.

Forward Gene Mutation Assay of Argatroban in Chinese Hamster Ovary Cells (HGPRT Locus) (Study # BGX 515/931162)

Testing Laboratory: _____

Study Started: Between May 13 and August 6, 1993.

Study Completed: September 22, 1993

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

Cells Employed: CHO-KI-BH₄ (Chinese Hamster Ovary) cells, obtained from _____

Concentration Employed: 30, 60, 180, 375, 750, 1500, 2250, 3000 µg/ml doses were used with or without metabolic activation for cytotoxicity studies. For the main mutagenicity studies, 500, 750, 1000, and 1500, 2000, 2500, 3000 µg/ml doses were selected.

Basis of Dose Selection: Dose selection was based on the solubility of the drug. The drug was soluble in DMSO at conc of 500 mg/ml, but precipitate was noted in the culture medium at this dose. Therefore 300 mg/ml, which gave final conc of 3000 µg/ml in the medium, was chosen for the preliminary toxicity studies. Concentrations for mutagenicity tests were also based on the cytotoxicity studies (using 30-3000 µg/ml), where cell survival was 76-114% in the absence of metabolic activation, and 92-131% in the presence of metabolic activation. 500-3000 µg/ml conc. were chosen for the main test.

Solvent Control: 1% (v/v) Dimethyl sulfoxide (DMSO).

Positive Controls: Ethyl methane sulfonate (EMS) 250 µg/ml, (the µg dose is not given) was used without metabolic activation, and 20-methyl cholanthrene 5 µg/ml were used with metabolic activation.

Source of Metabolic Activation: Rat liver S-9 fraction.

Drug Batch No: P.ATB-58201S (SL91.0279-00), 99.4% pure.

Criteria of Genotoxic Effect: The test is considered positive 1) if there was a statistical significant, and a dose dependent increase in mutant frequency, as well as reproducibility in the response, and these data were in the upper end of the historical control data (15 mutants/10⁶ cells).

Method: CHO Cells were exposed to the indicated concentrations of argatroban in the presence or absence of S-9 activation mixture, along with the positive and negative controls. After an expression period of 7 days, CHO cells were cultured in 6-TG selective medium for 7 days. At the end of the experiment, the mutant frequency and the viability of the cells was determined. Total of 4 studies were performed in duplicates, 2 with, and 2 without metabolic activation.

Results: In all 4 tests, with or without metabolic activation, no significant differences in mutant frequencies between the vehicle control and the treated samples (at all argatroban doses) were found, while the positive controls showed significant increases in the mutant frequency. The plating efficiencies were between 42-77% in all 4 tests. These findings suggest, that under the conditions described, argatroban is not mutagenic in this CHO/HGPRT forward mutation assay in the CHO cells, in the absence or presence of metabolic activation.

In Vitro DNA Repair Assay (or unscheduled DNA synthesis) With Argatroban, Using Rat Hepatocytes: (study # BGX 514/931120)

Testing Laboratories: _____

Study Started: Between May 18 and June 18, 1993.

Study Completed: September 8, 1993

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

Cells Employed: Rat Hepatocytes, isolated from male Hsd/Ola Sprague-Dawley rats.

Concentration Employed: 1.5 to 1500 µg/ml.

Basis of Dose Selection: The DNA repairing ability of the drug in this test is detected as unscheduled DNA synthesis (UDS). The dose selection was based on the solubility of the drug in DMSO. The drug was soluble in the culture medium at conc of 1500 $\mu\text{g/ml}$, and this dose produced a precipitate in the medium.

Solvent Control: Dimethyl sulfoxide (DMSO).

Positive Controls: 2-Acetylaminofluorene (AAF) dissolved in DMSO, was used at a conc of 20 $\mu\text{l}/2\text{ ml}$ culture.

Drug Batch No: SL91.0279-00, P.ATB-58201S, 99.4% pure.

Criteria of Genotoxic Effect: If the number of cells with a net grain counts exceeded ≥ 5 compared to control levels, or if a substantial and reproducible dose-related statistically significant increase in the net nuclear count was achieved (along with increase in gross nuclear count), compared to solvent control, the test was considered positive.

Methods: The hepatocyte cells (in triplicates) are seeded on to the coverslip, and are exposed to argatroban, in the presence of $^3\text{HTdR}$ (10 $\mu\text{Ci/ml}$), along with the vehicle, and positive controls. After ~41 hrs exposure (17 hrs and 24 hrs on days 1 and 2 resp), the cells are then subjected to autoradiographic procedure, and the slides are scored by counting, usually 50 nuclei, and only the hepatocytes with a normal morphology (not lysed), and without debris. The grain counts over nuclear areas were compared to grain counts over a single adjacent cytoplasm area of the same size, in the same cell (i.e. gross nuclear grain count minus the corresponding cytoplasmic grain counts). For every cell, the gross and net nuclear grain counts are determined, and comparisons are made between the drug vs control. Total of 2 separate tests were carried out.

Results: Argatroban showed a tendency towards increasing the net and gross number of nuclear grain counts, compared to controls at 4.75-1500 $\mu\text{g/ml}$, but no dose-related trend was noted, the data from 2 tests are shown in Table IV. However, much higher, and significant increases in the number of mean nuclear grain counts were observed with the positive controls. The percentage of viable cells, relative to controls were not indicated in this study.

Table IV. Effects of argatroban in 'In Vitro' unscheduled DNA synthesis (UDS) in rat hepatocytes.

Test 1

Concentration of Argatroban ($\mu\text{g/ml}$)	Mean Gross Nuclear Grain Count	Mean Net Nuclear Grain Count
Solvent control	30.4	-8.2
1.5	27.4	-8.2
4.74	30.0	-6.5
15	39.3	2.0
47.4	48.2**	13.6**
150	42.2*	4.1*
474	51.5**	13.0**
1500	48.7**	6.9**
Positive control 0.01, 0.032, 0.1, 0.32	64.4, 102.2, 120.2, 122.2	25.9, 63.6, 78.4, 76.2

Test 2

Concentration of Argatroban ($\mu\text{g/ml}$)	Mean Gross Nuclear Grain Count	Mean Net Nuclear Grain Count
Solvent control	27	-9.5
1.5	33.7	-6.5
4.74	51.1**	8.6**
15	54.2**	15.3**
47.4	62.5**	20.5**
150	59.1**	19.0**
474	65.6**	22.3**
1500	55.7**	14.1**
Positive control 0.01, 0.032, 0.1, 0.32	81.4, 88.2, 117.7, 109.6	29.2, 41.4, 63.2, 63.5

* $p < 0.01$, $p < 0.001$, significant differences were observed at these concentrations.

In Vitro DNA Repair Assay (or unscheduled DNA synthesis) With Argatroban, Using Rat Hepatocytes: (study # AHF 93-60)

Testing Laboratories: _____

Study Started: October 6, 1993

Study Completed: August 29, 1994

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

Cells Employed: Rat hepatocytes, isolated from male _____ rats.

Concentration Employed: 1.0 to 1000 µg/ml.

Basis of Dose Selection: The DNA repairing ability of the drug in this test is detected as unscheduled DNA synthesis (UDS). The dose selection was based on the solubility of the drug in DMSO. One mg/ml conc precipitated in the solution.

Solvent Control: Dimethyl sulfoxide (DMSO).

Positive Controls: 2-Amino fluorene (2-AF, 10^{-5} M) was used as a positive control, and nongenotoxic fluorene was used as a negative control.

Drug Batch No: P.ATB-58201S (SL91.0279-00), 99.4% pure.

Criteria of Genotoxic Effect: If at least 2 conc demonstrate the net nuclear grain counts significantly greater compared to control levels, or if there was a positive dose-related relationship for increased values up to toxic conc, or if at least one of the increased nuclear grain counts had a positive value, the test was considered positive.

Methods: The hepatocyte cells (in duplicates) are seeded on to the coverslip within 2 hrs, and are exposed to argatroban, in the presence of $^3\text{HTdR}$ (10 µCi/ml), along with the vehicle, and positive and negative controls. After 18-20 hrs exposure, the cells are then subjected to autoradiographic procedure, and the slides are scored by counting. Sixty nuclei, randomly selected were counted and cells exhibiting toxicity such as irregular shape or without surrounding cytoplasm were not counted. The grain counts over nuclear areas were compared to grain counts over a single adjacent cytoplasm area of the same size, in the same cell (i.e. gross nuclear grain count minus the corresponding cytoplasmic grain counts). For every cell, the net nuclear grain counts are determined, and comparisons are made between the drug vs control. Total of 2 separate tests were carried out.

Concentration Employed: 1, 3, 10, 30, 100, 300, and 1000 µg/ml.

Basis of Dose Selection: The DNA repairing ability of the drug in this test is detected as unscheduled DNA synthesis (UDS). The dose selection was based on the solubility of the drug in DMSO, 1 mg/ml conc precipitated in the solution.

Solvent Control: Dimethyl sulfoxide (DMSO).

Positive Controls: 4-Nitroquinoline 1 oxide (4-NQO, 0.1 µg/ml DMSO, and N-methyl-N-nitro - nitrosoguanidine (MNNG, 1.5 µg/ml water).

Drug Batch No: 810415.

Criteria of Genotoxic Effect: If significant differences are found in treated cells, compared to controls, and positive controls show significant differences, the test was considered positive.

Methods: The WI-38 cells (in duplicates) are seeded on to the coverslip, and are exposed to argatroban, in the presence of ³HTdR (10 µCi/ml), along with the vehicle, and positive and negative controls. After 2 hrs exposure, the cells are then subjected to autoradiographic procedure, and the slides are scored by counting. Two hundred nuclei, randomly selected were counted. The data were processed and the grain counts over nuclear were compared, between the drug vs control.

Results: Argatroban did not increase the grains per nucleus (0.32-0.37), compared to negative or solvent controls (1.20 and 0.70 resp), in fact the values in the treated cells were lower. However, a significant increases in grains/nucleus counts (3.3-5.3) were observed with the positive controls.

These studies suggest that argatroban was not genotoxic in 'in vitro' DNA repair assay in human fetal lung WI-38 cells, in this study.

Recombinational Repair Assay (Rec assay) With Argatroban: (study # 3223-103)

Testing Laboratories: _____

Study Started: February 1982

Study Completed: September 1982

GLP Requirements: Not given.

Cells Employed: Bacillus subtilis strain M45 (rec⁻, which lacks recombinational repair ability) and its wild type strain H17 (rec⁺). The M45 has rec 45 mutation which is derived from its parent strain H17 rec.

Concentration Employed: 6250-50000 µg/ml, solvent dimethyl sulfoxide (DMSO).

Basis of Dose Selection: Not given.

Solvent Control: Not included in the assay.

Positive Controls: Kanamycin (12.5-100 µg/ml), and mitomycin C (0.2-3.2 µg/ml), both dissolved in distilled water, were used as negative control and positive controls resp.

Drug Batch No: 810415.

Criteria of Genotoxic Effect: If the test compound had a clear rec effect (i.e. if M45 strain gives greater growth inhibition than H17), and the positive control (mitomycin C) similarly showed significant differences between the growth inhibition of 2 strains (i.e. M45 > H17), the test was considered positive. The positive Rec assay indicates that the compound has DNA-damaging properties and may potentially be a carcinogenic compound.

Methods: This assay examines the detection of DNA-damaging agents by repair-deficient bacteria B. Subtilis, M45 and H17 (rec⁻ and rec⁺ strains resp). The assay is based on the differential inhibition of growth of repair-deficient and repair-proficient bacterial pairs. In a petri dish (100 mm diameter), bacterial cell cultures of M45 or H17 strains are plated in an agar medium. Argatroban (50 µl solution) impregnated on a paper disc (8 mm diameter), is placed on to the agar (at 4°C for 3 hours) and allowed to diffuse into the plate. Incubations are carried out for 18 hrs at 37°C, and the diameter of the resulting growth inhibition area is measured.

Results: In both M45 and H17 strains, argatroban at a conc of 6250 µg/ml did not cause any growth inhibition, but at 12,500 µg/ml (or higher conc), M45 strain showed higher growth inhibition (9.0-15.2 mm diameter inhibition) than H17 (0-10.9 mm diameter zone inhibition), Table VI (reproduced from volume 33, page 113 of the submitted NDA). The positive control mitomycin C (a DNA-damaging agent), also caused a greater inhibitory effect with M45 (24-34.3 mm diameter inhibition) than with H17 strain (12.9-24.4 mm diameter inhibition). In contrast Kanamycin, a negative control (a protein synthesis inhibitor), inhibited growth of both H17 and M45 to a similar extent (H17 16-22.6, M45 16.9-22.9 mm diameter inhibition), because the lethal effect of the compound was not dependent on the presence of DNA repair capabilities.

Table VI.

Growth Inhibition of Rec+ and Rec- Strains of
Bacillus subtilis to MD-805

Compounds	Concentration ($\mu\text{g/ml}$)	Diameter of Zone of Inhibition (mm) ¹⁾	
		H17 (Rec+)	H45 (Rec-)
MD-805	6,250	-	-
	12,500	-	9.0 \pm 0.5
	25,000	-	13.8 \pm 0.4
	50,000	10.9 \pm 0.1	15.2 \pm 0.3
Kanamycin	12.5	16.0 \pm 0.3	16.9 \pm 0.3
	25	20.5 \pm 0.2	19.7 \pm 0.2
	50	22.6 \pm 0.4	22.9 \pm 0.4
	100	15.8 \pm 0.2	26.1 \pm 0.4
Nitomycin C	0.2	-	24.0 \pm 0.2
	0.4	12.9 \pm 0.2	27.5 \pm 0.1
	0.8	17.5 \pm 0.3	30.5 \pm 0.2
	1.6	20.1 \pm 0.1	34.3 \pm 0.2
	3.2	24.4 \pm 0.1	-

1) Mean of 10 plate \pm S.E.

These studies suggest that argatroban at conc of 6,250 $\mu\text{g/ml}$ does not have a DNA damaging effect, but at concs of 12,500 $\mu\text{g/ml}$ or higher, it may have a slight DNA damaging effect on B.subtilis in Rec assay. However, there were no solvent controls included in this assay, the basis of dose selection were not given, and no preliminary conc-finding experiments were performed to see what concs of the drug inhibited the growth of both tester strains. The concs of the drug used were higher (6.3 to 50 mg/ml) in this assay vs other in vitro mutagenic assays (maximum of 1-3 mg/ml). The maximum recommended doses of 5 mg/ml should have been tested in this assay. This effect of the drug was shown to be negative in DNA repair assay in human fetal lung WI-38 cells.

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

Mutagenicity Study of Argipidine (MD-805): Reversion Test
With Bacteria (Report # D-16)

Testing Laboratories: _____

Dates Studies Started and Completed: November to December 1978

Strains Employed: *Salmonella typhimurium* Strains TA 1536, TA 1537, TA 1538, TA 98 and TA 100, and *E. coli* WP2 UVrA.

Concentration Employed: 200, 500 and 1000 mcg/plate

Solvent Control: Dimethyl Sulfoxide (DMSO)

Positive Control: Benzo[a]pyrene (50 mcg/plate) and 2-aminoanthracene (20 mcg plate).

Source of Metabolic Activation: Rat liver microsomes (S-9 mix).

Results: Several fold increase in the number of revertant colonies above the solvent control value are considered as positive provided if the effect is also dose related. MD-805 was not mutagenic in any of the tester strains at doses ranging from 200-1000 mcg/plate, irrespective of treatment with metabolic activation system (S-9 mix). Increase in mutant colonies was noted in all the microbial strains employed in the presence of positive control (with or without S-9 mix).

Mutagenicity Test of DK-7419: Reverse Mutation Assay
(Report # D-19)

Testing Laboratories: _____

Dates Studies Started and Completed: January 31, 1985 and March 29, 1985

Strains Employed: *Salmonella typhimurium* strains TA 1535, TA 1537, TA 1538, TA 98, TA 100 and *E. coli* WP2 UVrA⁻.

Concentration Employed: 5-5000 mcg/plate

Drug Batch No.: P-ATB-52406S

Solvent Control: Dimethyl Sulfoxide (DMSO)

Positive Control: 9-aminoacridine (80 mcg/plate), 2-nitrofluorene (1-2 mcg plate), N-ethyl-N-nitro-N-nitrosoguanidine (2-5 mcg/plate), benzo (a) pyrene (5 mcg/plate), 2-aminoanthracene (0.5-80 mcg/plate).

Source of Metabolic Activation: Rat liver microsomes (S-9 mix).

Results: Criteria for positive result was not mentioned. However, the increase in the number of revertant colonies above the solvent control value was less than two-fold. Significant increases (3.2-157.5 fold increase over negative control value) in mutant colonies were noted in all the microbial stains employed in the presence of positive control (with or without S-9 mix). Thus DK-7419 was not mutagenic in any of the tester strains at doses ranging from 5-5000 mcg/plate, irrespective of treatment with metabolic activation system (S-9 mix).

Mutagenicity Test on DK-7419, Chromosome Aberration Test
(Report # D-18)

Testing Laboratories: _____

Dates Studies Started and Completed: December 17, 1984 and March 28, 1985

Strain Employed: Chinese hamster cultured cells (D-6 cells).

Concentration Employed: 10-2500 mcg/ml

Solvent Control: Dimethyl sulfoxide (DMSO)

Positive Control: Mitomycin C (0.05 mcg/ml) and 7, 12-dimethylbenz (a) anthracene (5 mcg/ml).

Drug Batch No.: P-ATB-52406S

Results: Chinese hamster cultured cells were treated with DK-7419 (10-2500 mcg/ml) in the presence and absence of metabolic activator (S-15 mix). At the end of the experiment 100 metaphases were examined per treatment group. Treatment with DK-7419 produce very low chromosomal aberration with gap (0.5-3.0%) and without gaps (0.0 - 2.5%) irrespective of the presence or absence of metabolic activation, and which was no different from the value for the control group (0.5 - 1.5%). The positive control produced about 42.5 - 53.5% chromosomal aberration at 24 hr in the absence of metabolic activator. The precipitate was seen in the incubation mix at 1000 mcg/ml and higher levels, however no cytotoxic effect was seen. Thus DK-7419 had no clastogenic activity in this in vitro cytogenetic test.

Addendum

Strain Employed is an error. Cells employed were fibroblast cultures derived from the lung of a fetal Chinese hamster (D-6 cells).

SPECIAL TOXICITY STUDIES:

Study to Determine Whether Saturated Solution of Argatroban When Given to Animals Precipitates in Plasma: (T34)

Testing Laboratories: _____

Study Started: Not given.

Study Completed: Not given.

GLP Requirement: Not given.

Animals: Male Sprague Dawley rats, (crj:CD, 5 weeks old).

Drug Batch No.: P-ATB-52406S, 99.7% pure.

Methods: The aim of this study was to determine whether saturated or supersaturated solutions of argatroban when given to animals, precipitates into the animal's blood. First the supersaturation of the drug in physiological saline was determined, which was 2.7 mg/ml saline, this was then added to blood plasma samples of 5 male rats (in vitro, at the plasma to argatroban ratio of 1.1:0.35). The mixture was filtered, and the presence of the precipitate on the filter (0.22 μ) was determined by stereomicroscopic examination.

Results: No argatroban precipitate was observed on the filter. These studies suggest that the argatroban, even at supersaturated conc in solution, does not precipitate out of solution in plasma.

Hemolytic Potential of Argatroban In Vitro in Rabbit Erythrocytes: (T35)

Testing Laboratories: _____

Study Started: October 1986

Study Completed: ~October 1986

GLP Requirement: Not given.

Animals: Male New Zealand white rabbits.

Drug Batch No.: Not given.

Methods: Rabbit erythrocyte suspensions (0.1 ml) were incubated with 1 ml of argatroban injection (containing 9 ml saline, the exact drug conc was not stated), or saline alone, for 24 hrs at 37°C and hemolysis was measured by recording absorbance at 540 nm.

Results: The drug did not induce hemolysis in rabbit erythrocytes.

Hemolytic Potential of Argatroban In Vitro in Rat and Dog Erythrocytes: (T37-T39, study # 2210BJR)

Testing Laboratories: _____

Study Started: Not given.

Study Completed: June 27, 1994, and -July 3, 1995

GLP Requirement: Not given.

Animals: Rats and dogs (details not given).

Drug Batch No.: 181, 182, and 183 used for rat studies.

Methods: Two formulations in rat erythrocytes were used here. In the first formulation, rat erythrocyte suspensions (1 ml) were incubated with 1 ml of argatroban (high micellar conc, 1 ml contained 5, 7.5 or 10 mg/ml of the drug) or saline. In the second formulation, rat erythrocyte suspensions (1.5 ml) were incubated with 0.05 to 0.25 ml of argatroban (low micellar conc, contained 5 or 10 mg/ml of the drug) or saline. Optical density and hemoglobin conc were determined in these studies. Also in 2 separate studies, the effect of 1 mg/ml of argatroban was evaluated in the rat and dog blood erythrocytes.

Results: The drug did not produce any hemolysis in rat erythrocytes, using low micellar formulation (at 10 mg/ml, the optical density was 0.8, vs 1.9-4.5 in placebo group, the hemoglobin was 0.8 vs undetectable in placebo). In the high micellar formulation, the drug slightly increased hemolysis with increases in conc (at 5-10 mg/ml, the optical density was 0.6-1.4%, vs 1.3-3.8 in placebo group, the hemoglobin was 1.2-35% vs 0.2-2.6% in placebo). No effects on hemolysis were seen with argatroban doses of 1 mg/ml in rat or dog erythrocytes. These studies suggest that at low micellar conc, mixed with erythrocytes, no hemolysis was observed, but mild hemolysis was noted with higher micellar conc mixed with erythrocytes.

Effect of Argatroban on Hemorheology: (T36)

Testing Laboratories: _____

Study Started: December 1981

Study Completed: January 1982

GLP Requirement: Not given.

Animals: Male Wistar rats.

Drug Batch No.: Not given.

Methods: Rat blood (3.6 ml, fresh or 3 hrs after collection at room temp) was used to determine the effect of the drug (500 μM) on blood viscosity (using rotating viscometer), and tolerance to mechanical destruction by hemolysis (by stirring at the maximal rate for 20 seconds, using drug conc of 1, 10 and 100 μM).

Results: The drug did not have an effect on the viscosity in the fresh blood. It caused a slight inhibition in viscosity, in blood at 3 hrs, due to softness of blood erythrocyte membranes, which is lost during 3 hrs of standing at room temp. At 100 μM , argatroban slightly inhibited mechanical hemolysis of red blood cells (%-inhibitory rate 12.1 vs 0 in controls), but no effects were noted at conc. (1-10 μM , %-inhibitory rates were 1.3 and 2.5 resp), which have thrombin inhibitory actions.

Local Adverse Effects of Argatroban, Given by IM and Perivenous Routes, in Rabbits: (T40)

Testing Laboratories: _____

Study Started: May 23, 1995

Study Completed: August 28, 1995

GLP Requirement: It is stated that the report is given in compliance with GLP.

Animals: New Zealand rabbits, 12-14 weeks old.

Drug Batch No.: KEY 01-01A1.

Methods: Four groups of (three/group) rabbits were used. Two groups were given one im injection (containing 1 mg/ml of the drug in 5% glucose) and two perivenous injections (24 hrs apart, 0.2 ml/injection). Two other groups were given 5 im injections (24 hrs apart, 1 mg/ml of the drug). Placebo (in 5% glucose) was given to rabbits on the opposite side of the ears. At 24 hrs and 7 days, the local irritation was examined. Rabbits in group 1 and 3 were sacrificed and necropsied at the end of 24 hrs and the other 2 groups after 7 days.

Results: At 24 hrs after administration, a slight local reaction was noted (score 1.3). Repeated administration increased the scores to 2.3, but the response was slight. After 7 days, minor muscle reactions noted were reversed. Histological examinations after 24 hrs (first or second injections) indicated minimal to moderate focal hemorrhages (may be due to antiplatelet activity of the drug) or inflammation. In 2 rabbits these were associated with minor muscle necrosis and minimal myositis. After 5 injections, the severity of these reactions increased slightly and was accompanied by muscle fibrosis, mineralization of giant cells or myofibrils, and oedema. After 7 days, there was a significant regression in all muscle reactions. These studies suggest a slight local reaction of the drug in rabbit muscle, following repeated im or perivascular injections.

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

1. Antigenicity Test on MD-805 by Systemic Anaphylactic Test in Guinea Pigs (Report # D-13).

Testing Laboratories: Department of Pharmacology, Mitsubishi Chemical Ind. Japan.

Study Started: Not given.

Study Completed: Not given

Animals: Male Hartley guinea pigs (approximately 300 g)

Methods: Antigenicity of MD-805 was examined with systemic anaphylaxis (SA) in guinea pigs. Five animals per group were used. One group of animals were sensitized by subcutaneous administration of MD-805 (2.5 mg/0.5 ml) emulsion containing Freund's complete adjuvant (FCA). The positive control group received 0.5 ml of horse serum and 0.5 ml of FCA in similar fashion. Systemic anaphylaxis was examined following i.v. injection of 5 mg/kg/ml of MD-805 or 1 ml/kg of horse serum at 3 weeks after the sensitization dose.

Results: Inflammation at the site of administration was seen in all animals, probably due to FCA administration. No anaphylactic symptoms were noted in MD-805 treated animals. Positive control group animals all had anaphylactic symptoms which resulted into deaths. Thus MD-805 may not have antigenicity in guinea pigs. This study was not conducted according to GLP regulations and the detail treatment of control animals was not provided.

2. Antigenicity of DK-7419 (Report # D-14)

Testing Laboratories: _____

Study Started: December 1981

Study Completed: April 1982

Drug Batch No.: 810415

Animals: Female Hartley guinea pigs (300-450 g)

Methods: Antigenicity of DK-7419 was examined with passive cutaneous anaphylactic (PCA) reaction in guinea pigs. Groups of guinea pigs (n=10/group) were sensitized by i.p. administration of 0.3 or 6 mg/kg of DK-7419 once every other day for 6 times or by i.m. (foot pad) administration of 5 mg of DK-7419 plus Freund's complete adjuvant (FCA) once a week for two weeks. The positive control group (n=5) received 1 mg/kg of BSA plus FCA once a week for two weeks. The negative control group (n=5) received saline plus FCA in similar fashion.

The PCA reaction was examined with serum collected from animals at weeks after the last sensitization dose. Serial dilutions of the serum was injected intradermally to normal guinea pigs for passive sensitization (n=5-10). These animals were challenged with the respective antigen mixed with Evan's blue intravenously.

Results: No PCA reaction was seen with DK-7419 or DK-7419/bovine serum albumin conjugate. All positive control group animals had PCA reaction (mean PCA titer = 2100). Thus DK-7419 did not elicit sensitization activity in passive cutaneous anaphylaxis test in guinea pigs. This study was not conducted accordingly to GLP regulations.

3. Studies on Antigenicity of Argipidine (MD-805) (Report # D-15).

Testing Laboratories: _____
Japan.

Study Started: September 25, 1985

Study Completed: March 26, 1986

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

Animals: Five weeks old Hartley guinea pigs (348-560 g), Sprague-Dawley rats (238-280 g), and BALB/C mice (16.0-17.7 g).

Drug Batch No.: P-ATB-52406S

Methods: Antigenicity of MD-805 was examined in guinea pigs and mice. Ten animals per group for systemic anaphylaxis test and 2-3 animals per group for passive cutaneous anaphylaxis test (PCA) were used. Groups of guinea pigs (10/group) were sensitized intradermally with foot pads injections of argipidine (2, 10 and 50 mg/kg), argipidine-ovalbumin conjugate (2mg/kg) or ovalbumin (positive control) emulsified in Freund's adjuvant three times at two weeks intervals. IN the first sensitization Freund's complete adjuvant (FCA) was injected while for second and third sensitizations incomplete Freund's adjuvant (IFA) was injected intradermally. Systemic anaphylaxis was examined following i.v. injection of 10 mg/kg of argipidine at 14 days after the last sensitization (the positive control group animals received 2mg/ml/kg of ovalbumin). The PCA reaction was examined with serum collected from animals at 12 days after the last sensitization dose. Serial dilutions of the serum was injected intradermally on the back of rats (recipients). For QCA test these rats were challenged with argipidine (10 mg/kg or argipidine-bovine serum albumin (2 mg/kg) mixed with ~~intravenously~~ intravenously.

Results: In guinea pigs, no anaphylactic symptom was identified in any of the tested groups, while positive control group animals 8/10 had anaphylactic symptoms and 2 out of these 8 died. Furthermore, no PCA reaction was seen with argipidine. Thus argipidine did not elicit sensitization activity in systemic anaphylaxis and passive cutaneous anaphylaxis tests in guinea pigs. No antibodies to argipidine were detected in sera obtained from argipidine-sensitized animals when PHA and PR were treated. Similarly, no IgE antibodies to argipidine were detected using rat PCA test in sera obtained from argipidine-sensitized mice, and no PCA reaction was seen in the rats passively sensitized with anti-argipidine serum after the i.v. administration of argipidine.

Thus data suggest that argipidine has no antigenicity potential in guinea pigs and mice.

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

Hemolytic Potential and Blood Compatibility
Testing With GN1600
(Study # 91-006-1600)

Testing Laboratories: _____

Dates Study Started and Completed: January 4, 1991 and October 8, 1991.

Hemolytic Potential

Half c.c. of dog or human blood was incubated with _____

_____ Hemolysis was evident at 2.5 mg/ml (i.e. 1.25 mg/ml [final concentration]) of GN1600. At lower concentration no hemolysis was seen. d

Blood Compatibility Test

Both carrier _____ and 1.4-2.5 mg/ml of GN1600 were compatible with human and dog serum and plasma.

LABELING:

The indicated labeling of argatroban generally conforms to the format under CFR 21, 201.50 to 201.57, dated April 1, 1996. However, following changes are suggested.

I. DESCRIPTION:

a. Under 'Metabolism, Excretion, and Protein Binding', the sponsor has proposed the following text:

II. PRECAUTIONS

[Redacted text]

Evaluation: The above format according to CFR 21, 201.57, dated April 1, 1996 is incorrect. This section should state whether the long term studies in animals have been performed to evaluate the carcinogenic potential of the drug, and if the drug is mutagenic or impairs fertility in either sex. The teratogenic effects (according to format) should be discussed under pregnancy category.

Recommended Version:

a. 'Carcinogenesis, Mutagenesis, Impairment of Fertility

No long term studies in animals have been performed to evaluate the carcinogenic potential of argatroban. Argatroban was not genotoxic in the Ames test, the Chinese hamster

[Redacted text]

Evaluation: According to the format, type of reproductive toxicity studies, and the maximal dose used in each animal species, in relation to the human dose should be stated. These were not given.

Recommended Version:

b. 'Pregnancy, Teratogenic Effects. Pregnancy Category B

Teratology studies have been performed in _____ rats _____

_____ human dose, based on surface area) and have revealed no evidence of impaired fertility or harm to the fetus due to argatroban.

III. Following need to be clarified:

a. Labeling does not indicate the maximum number of days, patients would stay on argatroban, this information should be provided.

b. Several errors were found in labeling (e.g. volume and page numbers given in the labeling, in several sections, do not reflect the studies), these should be corrected. For example on page 76 of labeling (Under 'Metabolism, Excretion, and Protein Binding'), the last sentence showing volume 1.21, p 324 indicates that it is the study of argatroban metabolites, but in fact it is a study of degradation products of argatroban. On page 78 of labeling (Under 'pharmacokinetics and pharmacodynamics'), the sentence which states that 'the disposition of argatroban is unaffected by renal dysfunction' (volume 1.13, p 081) is in fact an in vivo study of 2 isomers of argatroban in a rat model, the next one (vol 1.14, p 20) is an in vitro study of these isomers.

SUMMARY AND EVALUATIONS:

Argatroban is a synthetic direct thrombin inhibitor, which has selective inhibitory effects on the actions of thrombin. It is derived from L-arginine and in x-ray crystallography, it shows selective binding to catalytic site of thrombin. Heparin requires antithrombin III (AT III, an endogenous cofactor) to exert its thrombin inhibitory action and is therefore, an indirect thrombin inhibitor. Whereas, argatroban is a direct thrombin inhibitor and reversibly binds to the thrombin active site, does not require AT III, and does not effect other serine proteinases. Patients, who receive heparin for therapeutic purposes, up to 10% of them, experience heparin-induced thrombocytopenia (HIT), which is believed to be due to immune responses to heparin. Argatroban does not induce immune responses, therefore in these HIT patients, argatroban may be a useful anticoagulant agent.

Argatroban is indicated as an anticoagulant therapy in HIT patients. The recommended initial dose of argatroban for HIT patients without hepatic impairment, is 2 $\mu\text{g}/\text{kg}/\text{min}$, in HIT patients with hepatic impairment, it is 0.5 $\mu\text{g}/\text{kg}/\text{min}$. These doses can be adjusted (not to exceed 10 $\mu\text{g}/\text{kg}/\text{min}$), until the steady state aPTT is 1.5-3 times the initial baseline values.

The sponsor has provided preclinical in vitro and in vivo pharmacology studies, pharmacokinetics, which include absorption, distribution, metabolism and excretion (ADME) of the drug in rats, rabbits and dogs, acute toxicity studies in mice, rats and dogs (including 24-hrs continuous iv infusion studies in rats and dogs), subacute/subchronic/chronic toxicity studies in rats (1-month continuous iv infusion, 28-days iv bolus and infusion + 28-day recovery, 1-month bolus iv, 6-month bolus iv) and dogs (1-month continuous iv infusion, 28-days iv bolus and infusion + 28-day recovery, 1-month bolus iv, 6-month bolus iv). Segment I fertility studies in rats, Segment II teratology studies in rats and rabbits, Segment III peri and postnatal studies in rats, genotoxicity studies (Ames test, chromosome aberration test in Chinese hamster fetal lung fibroblasts, Rec assay, UDS test in rat hepatocytes and WI human fetal lung cells, in vivo micronucleus test in mice, Chinese hamster ovary cells gene mutation assay). Also special toxicity studies (antigenicity tests in guinea pigs and mice, subacute iv irritation studies in rats and dogs, and tests for production of hemolysis) are included.

The pharmacokinetics (PK) of argatroban indicate that after iv bolus dosing in rats, rabbits, and dogs, the peak plasma conc are reached within 2-5 min. The terminal half lives of argatroban were comparable in rats (80-85 min), rabbits (36 min), dogs (20-29 min), and humans (54 min). In dogs, the steady state drug levels were reached in 2 hrs (after 5 hr iv infusion), and the PK at the end of 5 hrs infusion were similar to those with bolus administration. In humans, the steady state drug conc are reached within 1-3 hrs, and were increased in proportion to the dose. Argatroban is rapidly distributed to tissues in rats after iv bolus administration, peak levels were attained at 5 min in most tissues, and very little (<1%) remained by 24 hours. Liver, kidney, and small intestine had 7.3, 6.2, and 1.2 fold higher conc resp, than the plasma. The mean in vitro protein binding of argatroban in rat, dog, and human serum, at 0.5 μ M was 44.4%, 62.1%, and 53.7% resp. In vivo (iv dosing), binding to serum proteins was ~50% in rats and dogs.

The drug is metabolized (M1-M4) mainly in the liver. The 4 major metabolites were present 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 major metabolite M1 of argatroban (formed by aromatization of the 3 methyl,4-tetrahydroquinoline ring of argatroban) had 0.025 times the thrombin inhibitory effects of the drug, and exerted 0.2 times the anticoagulant effects of argatroban. The drug and its metabolites are excreted mainly through the feces and urine. In rats 72-85% of the administered drug was excreted by fecal route, and 12-14% in urine, in the first 24 hrs. Similarly, in humans, argatroban is excreted mostly in the urine (22% in the urine within 12 hrs) and feces (65% within 6 days). In urine + feces, 96%, 65%, and 78% of administered drug was excreted within 24 hrs in rats, rabbits and dogs resp.

The interaction study in dogs indicated that the Combined administration of the drug with rt-PA by iv infusion with or without oral aspirin did not increase the toxicity, pharmacodynamics or pharmacokinetics of argatroban.

In single dose acute toxicity studies, the minimum lethal iv (bolus) doses in mice, rats and rabbits were 200, 124 and 150 mg/kg resp. Clinical signs of toxicity were loss of righting reflex, clonic convulsion (or head tremors in rabbits) and death from respiratory paralysis. 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 paralysis of hind limbs, tremors, and coma. In acute iv toxicity studies with the major metabolite (M1) of argatroban, the minimum lethal doses of M1 were 309 mg/kg in rats (time to death was 1 hr, due to hemorrhages in the liver, kidney and lungs).

In continuous acute toxicity studies, argatroban given to rats (up to 6.25 mg/kg/day) or dogs (up to 1.22 mg/kg/day) by continuous iv infusions for 24 hrs, was not lethal in animals. Clinical signs of toxicity in all treated dogs were peripheral vasodilation, edema, subdued behavior and/or dyspnea.

In a 28-day bolus + continuous iv infusion toxicity study (conducted in Canada), with a 28-day recovery period in rats, animals received initial iv bolus doses of 0.02, 0.07, or 0.2 mg/kg/day in low, mid and high dose groups resp on the first day of dosing, followed by daily infusion doses of 3, 10 and 30 mg/kg/day in above 3 groups resp. No drug related toxicity was seen in any organ, even at the highest dose (which was 30.2 mg/kg/day for 28 days). At the infusion site, perivascular hemorrhages, inflammation and thrombosis were seen in all animals.

In the 1-month bolus iv toxicity study of argatroban in rats (studies conducted in Japan), doses of 3, 9, and 27 mg/kg/day were used. Argatroban, at a dose of 9, and 27 mg/kg/day produced slight to moderate degree of hepatic cell infiltration (by what cells was not defined) in livers of some males, but it was not dose related. The 'tolerated doses' of argatroban were 27 mg/kg/day.

In the 26-weeks iv toxicity study of argatroban in rats (studies conducted in Japan), bolus doses of 3, 9, and 27 mg/kg/day were used. Argatroban, at a dose of 27 mg/kg/day produced higher incidences and degrees (slight vs very slight in controls) of renal lesions (degeneration of the proximal tubular epithelium, thickening of the proximal tubular basement membranes) in males. No effects on liver were observed. Kidney may be the target organ of toxicity in males here. The 'no toxic effect dose' of argatroban was 9 mg/kg/day in males, and 27 mg/kg/day in females.

In the 1-month continuous iv toxicity study of argatroban in rats (studies conducted in France), doses of 15, 30, and 60 mg/kg/day were used. Local reactions at the injection site (perivenous hemorrhage at the vena cava site) were noted in both controls and treated rats. However, highest doses were not toxic to rats.

In dogs, in a 28-day bolus + continuous iv infusion toxicity study (studies conducted in Canada), with a 28-day recovery period in dogs, animals received initial iv bolus doses of 0.02, 0.07 and 0.20 mg/kg on the first day of dosing, followed by infusion doses of 3 mg/kg/day (low dose), 10 mg/kg/day (mid dose), or 30 mg/kg/day (high dose) of argatroban. No drug related toxicity was seen in any organ, even at the highest dose (which was 0.2 mg/kg bolus + 30 mg/kg/day for 28 days).

In the 1-month iv toxicity study of argatroban in dogs (studies conducted in Japan), bolus doses of 3, 9, and 27 mg/kg/day were used. Argatroban, at a dose of 27 mg/kg/day produced clinical signs (increased frequency of licking and vomiting in all dogs). The 'tolerated doses' of argatroban were 27 mg/kg/day.

In the 6-month iv toxicity study of argatroban in dogs (studies conducted in Japan), bolus doses of 1, 3, and 9 mg/kg/day were used. No drug related toxicity was seen in any organ. The 'no effect dose' in dogs was 9 mg/kg/day.

In the 1-month continuous iv toxicity study of argatroban (studies conducted in France), doses of 15, 30, and 60 mg/kg/day were used. All animals in this study had inflammatory and vascular lesions in the lungs, due to experimental infusion techniques, as well as local reactions at the injection site (intimal proliferation of infused veins). However, highest doses were not toxic to dogs.

The 1-month bolus iv toxicity study of argatroban, containing degradents (sponsor designates it as "— impurities") was conducted (in France) in rats (at doses of 5 mg/kg/day), and dogs (at doses of 2.5 mg/kg/day). However, this study was inconclusive, as a group of animals should have also received intact undegraded (pure) argatroban, to compare the effects of pure vs degraded one.

In a segment I fertility study (conducted in Japan), in male and female rats, the animals were treated with iv bolus dose of argatroban, at 0, 3, 9, and 27 mg/kg/day. Females were given the drug for 14 days prior to mating, throughout mating and from days 0 to 7 postcoitus, males were given the drug for 60 days prior to mating, during the matings, until their necropsy. No drug related effects were seen at any drug dose on male or female fertility, or general maternal/paternal reproductive performance of rats or on the progression of pregnancy.

In a segment II teratology study in rats (conducted in Japan), animals were given iv bolus drug, at doses of 0, 3, 9, and 27 mg/kg/day from days 7 to day 17 of gestation. Two-third of pregnant rats were sacrificed on day 20, and the remaining one-third of dams were allowed to deliver spontaneously, so that F1 offsprings could be examined for reproductive performance at maturity. No treatment related abnormalities were observed. The postnatal development and the fertility of the offspring were not affected. None of the doses produced any teratogenic effects in rats.

Two, segment II teratology studies in rabbits were conducted (in Japan). In the first study animals were given iv bolus drug (dissolved in water), at doses of 0, 0.5, 1, and 2 mg/kg/day from days 6 to day 18 of gestation. In the second study rabbits were given new formulation (sorbitol/water) of the drug (iv bolus), at only one dose of 10.8 mg/kg/day, along with the vehicle. The drug at any dose did not cause any maternal/embryo/fetotoxicity or teratogenicity in either study.

In segment III study in rats (conducted in Japan), argatroban was given by an iv bolus, at doses of 0, 3, 9, and 27 mg/kg/day from day 17 of pregnancy to through day 21 of lactation period. No effects on maternal toxicity or in F1 generation animals were seen. Also no treatment related external abnormalities on F2 fetuses were observed. Argatroban, at 27 mg/kg/day had no effects on perinatal/postnatal period in rats.

No mutagenic potential of the drug was seen, when argatroban was tested in the following 5 different tests: Ames test, CHO gene forward mutation assay (HGPRT locus), in vitro UDS test in rat hepatocytes and human fetal lung cells, chromosome aberration test in Chinese hamster cultured fetal lung fibroblasts, and in vivo micronucleus test in mice.

Antigenicity of argatroban was evaluated by systemic anaphylaxis (SA) and passive cutaneous anaphylaxis (PCA) test in guinea pigs, and mice after iv injection. The drug was not anaphylactic, and did not cause any PCA reaction in guinea pigs or mice. These studies suggest that argatroban does not induce any antigenicity in guinea pigs and mice.

Argatroban did not induce hemolysis in rat, dog and human blood at doses up to 1 mg/ml, higher doses produced slight hemolysis.

The proposed dose for the marketing is 2 μ g/kg/min, (not to exceed 10 μ g/kg/min, or a maximum dose of 14.4 mg/kg/day) by a cont iv infusion, for patients with heparin-induced thrombocytopenia without hepatic impairment. Adequate subacute (iv bolus, and iv infusion for 1 month in rats and dogs) and chronic (iv bolus, 6 months in rats and dogs) studies of argatroban have been conducted to characterize its toxicity. From a preclinical standpoint, this application is recommended for approval.

RECOMMENDATIONS:

From a preclinical standpoint, approval of this application is recommended.

The sponsor may be asked to make the suggested changes in labeling, on pages 99-101.

/S/

3/23/98

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CC:

NDA

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HFD-181/CSO

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