CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

20-883

CHEMISTRY REVIEW(S)

JUN 2 2 2000

DIVISION OF GASTROINTESTINAL AND COAGULATION DRUG PRODUCTS
HFD-180
Review of Chemistry, Manufacturing, and Controls

NDA #: 20-883 CHEM.REVIEW #: 5 REVIEW DATE: 06/22/2000

SUBMISSION TYPE	DOCUMENT DATE	CDER DATE	ASSIGNED DATE
Amendment (FAX)	06/01/2000	FAX	FAX

NAME & ADDRESS OF APPLICANT:
Texas Biotechnology Corporation
7000 Fannin Street, Suite 1920
Houston, Texas 77030
USA

DRUG PRODUCT NAME

Proprietary:	NOVOSTAN®
Nonproprietary/USAN:	Argatroban
Code Name/#:	MD-805, DK7419, MCI-9038
Chem.Type/Ther.Class:	1P/Anticoagulant

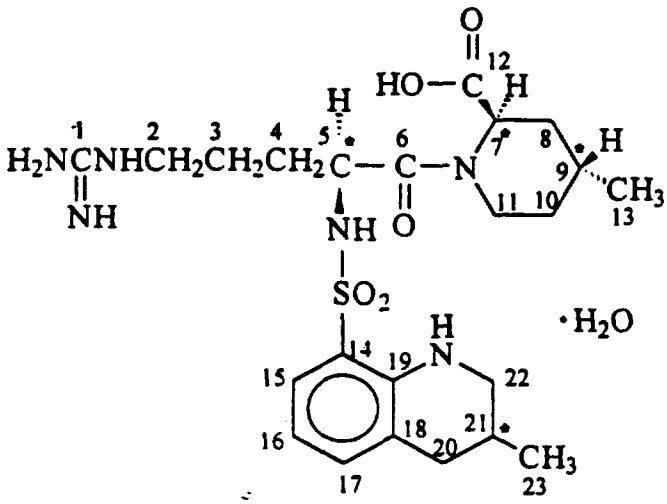
PHARMACOL.CATEGORY/INDICATION:
Antidiarrheal agent

DOSAGE FORM:	Injection
STRENGTHS	100 mg/ml
ROUTE OF ADMINISTRATION:	Intravenous
DISPENSED:	✓ Rx ___ OTC
SPECIAL PRODUCT	___ Yes ✓ No

CHEMICAL NAME, STRUCTURAL FORMULA, MOLECULAR FORMULA, MOL.WT:
See next page.

APPEARS THIS WAY
ON ORIGINAL

CHEMICAL NAME, STRUCTURAL FORMULA, MOLECULAR FORMULA, MOL.WT:



• = asymmetric carbon

Chemical Name:
1-[5-[(aminoiminomethyl) amino]-1-oxo-2[[1,2,3,4,-tetrahydro-3-methyl-8-quinolinyl)sulfonyl]amino]-4-methyl-2-piperidinecarboxylic acid, monohydrate
Molecular Formula: $C_{23}H_{36}N_6O_5S.H_2O$
Molecular Weight: 526.06

APPEARS THIS WAY
ON ORIGINAL

REMARKS/COMMENTS:
The firm submitted a document (FAX) dated June 06/01/00 which indicates that the final bulk solution weight will be range between [redacted]

The following query should be communicated to the NDA holder:
- The firm should remove any references to JP, Japanese Pharmacopeia and USP from the labeling sections of the drug product (carton, container and label insert).

Notes:
- OPDRA will review the new proposed trade name for the drug product (Acova).
- Based on the available real time stability data of 24 months, we recommend a tentative expiration date of 30 months at 25°C [redacted]
- The first sentence in the stability/compatibility section of the package insert should be deleted because it was repeated in the storage section.
- Methods validation have been completed (01/19/2000) and found satisfactory.

CONCLUSIONS & RECOMMENDATIONS:
The NDA may now be approved from the Chemistry, Manufacturing, and Controls point of view.

/S/ 06/22/00
Ali Al-Hakim, Ph.D.
Review Chemist, HFD-180

/S/ 6/22/00
Liang Zhou, Ph.D.
Chemistry Team Leader, HFD-180

cc:
Orig. NDA 20-883
HFD-180/Division File
HFD-180/AAl-Hakim
HFD-180/JDuBeau
HFD-180/JGibbs
R/D Init by: Lzhou

[redacted]

DuBeau
(180)

DIVISION OF GASTROINTESTINAL AND COAGULATION DRUG PRODUCTS
HFD-180
Review of Chemistry, Manufacturing, and Controls

MAY 22 2000

NDA #: 20-883 CHEM.REVIEW #: 4 REVIEW DATE: 05/17/2000

SUBMISSION TYPE	DOCUMENT DATE	CDER DATE	ASSIGNED DATE
Amendment (BC)	03/02/2000	03/03/2000	03/08/2000
Amendment (BZ)	04/20/2000	04/21/2000	04/28/2000
Amendment (BC)	04/26/2000	04/27/2000	05/09/2000
Amendment (AL)	05/03/2000	05/04/2000	05/12/2000

NAME & ADDRESS OF APPLICANT:
Texas Biotechnology Corporation
7000 Fannin Street, Suite 1920
Houston, Texas 77030
USA

DRUG PRODUCT NAME

Proprietary:	NOVOSTAN®
Nonproprietary/USAN:	Argatroban
Code Name/#:	MD-805, DK7419, MCI-9038
Chem.Type/Ther.Class:	1P/Anticoagulant

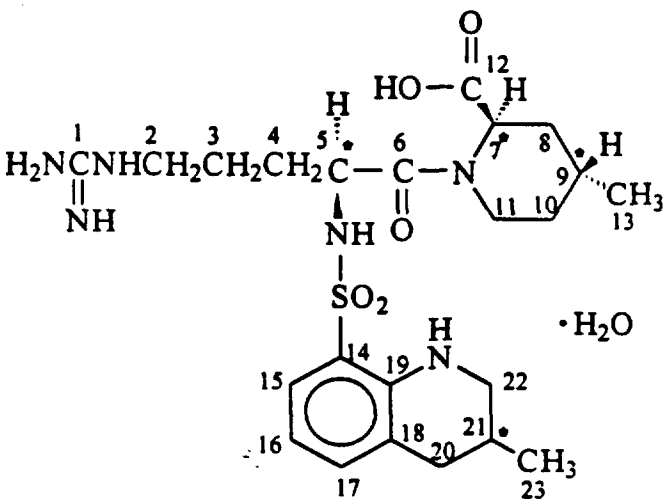
PHARMACOL.CATEGORY/INDICATION:
Antidiarrheal agent

DOSAGE FORM:	Injection
STRENGTHS	100 mg/ml
ROUTE OF ADMINISTRATION:	Intravenous
DISPENSED:	✓ Rx ___ OTC
SPECIAL PRODUCT	___ Yes ✓ No

CHEMICAL NAME, STRUCTURAL FORMULA, MOLECULAR FORMULA, MOL.WT:
See next page

APPEARS THIS WAY
ON ORIGINAL

CHEMICAL NAME, STRUCTURAL FORMULA, MOLECULAR FORMULA, MOL.WT:



• = asymmetric carbon

Chemical Name:
1-[5-[(aminoiminomethyl) amino]-1-oxo-2[[1,2,3,4,-tetrahydro-3-methyl-8-quinolinyl)sulfonyl]amino]-4-methyl-2-piperidinecarboxylic acid, monohydrate
Molecular Formula: $C_{23}H_{36}N_6O_5S.H_2O$
Molecular Weight: 526.06

APPEARS THIS WAY
ON ORIGINAL

REMARKS/COMMENTS:

Between March 3, 2000 and May 03, 2000, the NDA applicant submitted the following amendments:

- Amendment dated March 03, 2000. This document contains the updated stability data for 5 registration lots and 5 investigational lots. The data will be reviewed and evaluated.
- Amendment dated April 20, 2000. This document contains responses to the approvable letter dated February 18, 2000. Related CMC information will be reviewed and evaluated accordingly.
- Amendment dated April 26, 2000. This amendment contains information about the production scale batch size change from [redacted] batch size, as specified in the NDA, to [redacted] batch size.
- Amendment May 03, 2000. This amendment the proposed new name "Acova" which will be replacing the previous name " Novastan". The new trade name has been consulted to OPDRA.

CONCLUSIONS & RECOMMENDATIONS:

- Please remove any references to JP, Japanese Pharmacopeia and USP from the labeling sections of the drug product (carton, container and label insert).
- Please clarify the actual weight range of the proposed commercial batches. Variation in manufacturing process makes it difficult to produce consistent production scale batch sizes that weigh exactly [redacted]
- OPDRA will review the new proposed trade name for the drug product (Acova).
- Based on the available real time stability data of 24 months, we recommend a tentative expiration date of 30 months at 25°C [redacted]
- The first sentence in the satbility/compatability section of the package insert should be deleted because it was repeated in the storage section.
- Methods validation have been completed (01/19/2000) and found satisfactory.

/S/ 05/22/00
Ali Al-Hakim, Ph.D.
Review Chemist, HFD-180

/S/ 5/22/00
Liang Zhou, Ph.D.
Chemistry Team Leader, HFD-180

cc:
Orig. NDA 20-883
HFD-180/Division File
HFD-180/AAl-Hakim
HFD-180/JDuBeau
R/D Init by: LZhou

CSO | DUBEOLA

DIVISION OF GASTROINTESTINAL AND COAGULATION DRUG PRODUCTS
Review of Chemistry, Manufacturing, and Controls

NDA 20-883 CHEM.REVIEW: #3 REVIEW DATE: December 31, 1998

SUBMISSION TYPE	DOCUMENT DATE	CDER DATE	ASSIGNED DATE
Amendment	12/11/1998	12/14/1998	12/21/1998

NAME & ADDRESS OF APPLICANT:
Texas Biotechnology Corporation
7000 Fannin Street, Suite 1920
Houston, Texas 77030

JAN - 6 1999

DRUG PRODUCT NAME

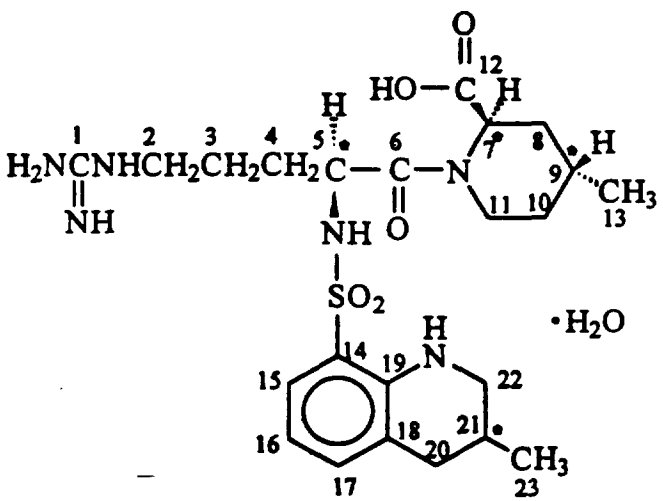
Proprietary:	**NOVOSTAN®**
Nonproprietary/USAN:	**Argatroban**
Code Name/#:	MCI-9038, MD-805, DO-7419, GN-1600, OM-7005 and argipdine
Chem.Type/Ther.Class:	**1P/Anticoagulant**

ANDA Suitability Petition/DESI/Patent Status: N/A

PHARMACOL.CATEGORY/INDICATION:
Anticoagulant Therapy in patients with Heparin-induced Thrombocytopenia.

DOSAGE FORM: Sterile Injection
STRENGTHS: 100 mg/ml Concentrate
ROUTE OF ADMINISTRATION: Intravenous
DISPENSED: ↓ Rx __ OTC

CHEMICAL NAME, STRUCTURAL FORMULA, MOLECULAR FORMULA, MOL.WT:
Chemical Structure



• = asymmetric carbon

Chemical Name:
1-[5-[(aminoiminomethyl) amino]-1-oxo-2[[1,2,3,4,-tetrahydro-3-methyl-8-quinolinyl)sulfonyl]amino]-4-methyl-2-piperidinecarboxylic acid, monohydrate
Molecular Formula: $C_{23}H_{36}N_6O_5S.H_2O$
Molecular Weight: 526.06

RELATED DOCUMENTS:

Type/ Number	Subject	Holder	Status	Review Date
[redacted]				

CONSULTS:

Biometrics: The statistical reviewer, based on the NDA stability data, granted 12 months expiration period (review dated 3/12/1998). This was also our recommended expiration dating after reviewing the stability data (12 months) submitted in the NDA (see chemistry review numbers 1 and 2). However, the firm presented in this amendment a real time stability data for 24 month and asked for an expiration dating of 30 month (see review notes below).

Microbiology: The microbiology reviewer recommended approval of the application (review date 10/20/1998)

REMARKS/COMMENTS:

The applicant of the NDA (Texas Biotechnology Corporation) has provided the above amendment, which contains responses to our Information Request letter, dated October 13, 1998. The applicant has also submitted additional stability data for the packaged drug product. Twenty-four Month (24) of real time stability have been provided and the firm requested an expiration period of 30 month.

CONCLUSIONS & RECOMMENDATIONS:

The amendment contains satisfactory responses to our information request letter dated may 8, 1998. With these satisfactory responses, there is no outstanding issues regarding the Chemistry, Manufacturing and Control of NDA 20-883.

Based on the stability data provided in this amendment (real time stability data for 24 months), an expiration dating of 30 month for the packaged drug product will be granted to the NDA holder.

/S/ 01/05/99
Ali Al-Hakim, Ph.D.
Review Chemist, HFD-180

/S/ 1/6/99
Eric P. Duffy, Ph.D.
Chemistry Team Leader, HFD-180

cc:
Orig. NDA 20-883
HFD-180/Division file
HFD-180/LTalarico
DISTRICT OFFICE
HFD-180/CSO/JDuBeau
HFD-820/JGibbs
HFD-180/AAl-Hakim
R/D Init: EDuffy/1-5-99

USO/Dudley

DIVISION OF GASTROINTESTINAL AND COAGULATION DRUG PRODUCTS
Review of Chemistry, Manufacturing, and Controls

NDA: 20-883 **CHEM.REVIEW:** #2 **REVIEW DATE:** September 21, 1998

SUBMISSION TYPE	DOCUMENT DATE	CDER DATE	ASSIGNED DATE
Amendment	July 8, 1998	July 9, 1998	July 16, 1998

NAME & ADDRESS OF APPLICANT:
Texas Biotechnology Corporation
7000 Fannin Street, Suite 1920
Houston, Texas 77030

OCT 13 1998

DRUG PRODUCT NAME

Proprietary:	**NOVOSTAN®**
Nonproprietary/USAN:	**Argatroban**
Code Name/#:	MCI-9038, MD-805, DO-7419, GN-1600, OM-7005 and argipdine
Chem.Type/Ther.Class:	**1P/Anticoagulant**

ANDA Suitability Petition/DESI/Patent Status: N/A

PHARMACOL.CATEGORY/INDICATION:
Anticoagulant Therapy in patients with Heparin-induced Thrombocytopenia.

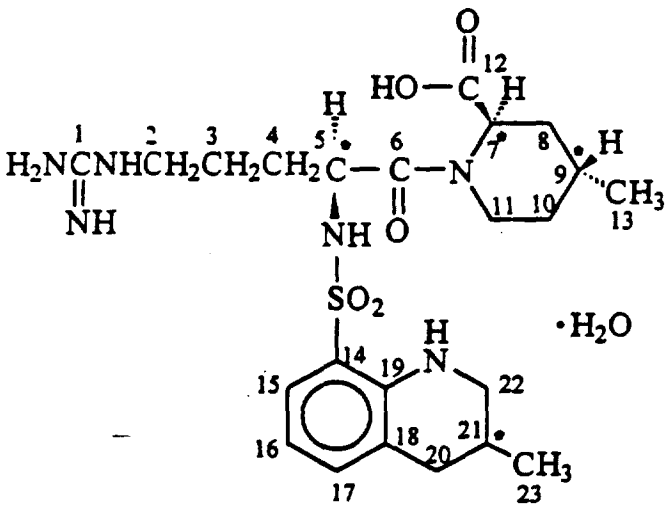
DOSAGE FORM: Sterile Injection

STRENGTHS: 100 mg/ml Concentrate

ROUTE OF ADMINISTRATION: Intravenous

DISPENSED: ✓ **Rx** _____ **OTC**

CHEMICAL NAME, STRUCTURAL FORMULA, MOLECULAR FORMULA, MOL.WT:
Chemical Structure



• = asymmetric carbon

Chemical Name:
1-[5-[(aminoiminomethyl) amino]-1-oxo-2[[1,2,3,4,-tetrahydro-3-methyl-8-quinolinyl)sulfonyl]amino]-4-methyl-2-piperidinecarboxylic acid, monohydrate

Molecular Formula: $C_{23}H_{36}N_6O_5S.H_2O$

Molecular Weight: 526.06

RELATED DOCUMENTS:

Type/ Number	Subject	Holder	Status	Review Date	Letter Date
[redacted]			Deficient*	5/8/1998	5/8/1998
			Adequate	02/01/94	
			Adequate	02/18/97	
			Adequate	11/18/96	
			Adequate	7-12-97	

* The DMF holder, [redacted] has submitted an amended DMF dated July 2, 1998 which contains responses to our Information Request letter. The responses will be reviewed in conjunction with this NDA.

CONSULTS:

Biometrics: Statistical stability was review completed on 3/12/1998. The reviewer, Dr. A.J. Sankoh of HFD-720, recommended 12 months expiry date for the drug product.

Microbiology: Microbiology review was completed on 10/29/98. The reviewer, Dr. Brenda Uratani of HFD-160, indicated that the application has a number of deficiencies.

REMARKS/COMMENTS: The applicant of the NDA (Texas Biotechnology Corporation) has provided the above amendment, which contains responses to our Information Request letter, dated May 8, 1998

CONCLUSIONS & RECOMMENDATIONS:

Most of the responses provided by the firm were satisfactory. However, the firm did not provide adequate replies for the following queries:

- Regarding justification for the impurities specifications. The proposed specifications for the impurities and degradation products based upon the data from the NDA studies showed that specifications limits are much higher than the reported ones. The firm should provide satisfactory response to this query, which we submitted in our previous Information request letter.

- With respect to the stability batches, the firm did not reply to our concern regarding the toxicology batches and whether they were also used in the stability studies.

- Concerning the sampling plan, details and description of the [redacted] sampling plan should be provided.

- Please note that statistical analysis of your stability data support an expiry of 12 months.

/S/ 10/9/98
Ali Al-Hakim, Ph.D.
Review Chemist, HFD-180

/S/ - 10/13/98
Eric P. Duffy, Ph.D.
Chemistry Team Leader, HFD-180

cc:
Orig. NDA 20-883
HFD-180/Division file
HFD-180/LTalarico
DISTRICT OFFICE
HFD-180/CSO/JDuBeau
HFD-820/JGibbs
HFD-180/EDuffy
HFD-180/AAl-Hakim
R/D Init: EDuffy/10-7-98
AA/dob F/T 10-9-98\Word: n: [redacted]

APR 1 4 1998

DIVISION OF GASTROINTESTINAL AND COAGUL[illegible] DRUG PRODUCTS
Review of Chemistry, Manufacturing, [illegible] Controls

NDA 20-683 CHEM.REVIEW: #1 REVIEW DATE: [illegible]ember 5, 1997

SUBMISSION TYPE	DOCUMENT DATE	CDER DATE	ASSIGNED DATE
Original	06/27/1997	06/27/1997	08/28/1997
Amendment	10/30/1997	10/31/1997	11/03/1997
Amendment	11/14/1997	11/17/1997	12/02/1997

NAME & ADDRESS OF APPLICANT:
Texas Biotechnology Corporation
7000 Fannin Street, Suite 1920
Houston, Texas 77030

DRUG PRODUCT NAME
Proprietary: NOVOSTAN
Nonproprietary/USAN: Argatroban
Code Name/#: MCI-9038, MD-805, [illegible]19, GN-1600, OM-7005 and argipdi
Chem Type/Ther Class: 1P/Anticoagulant

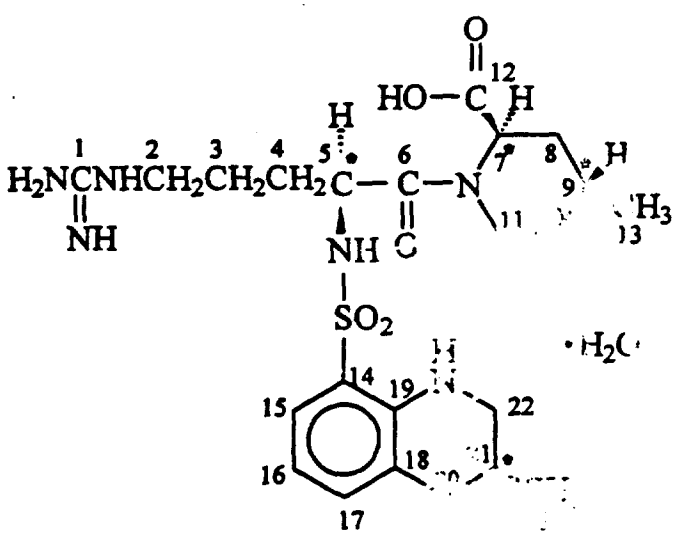
ANDA Suitability Petition/DESI/Patent Status:

PHARMACOL CATEGORY/INDICATION:
Anticoagulant Therapy in patients with Hepar[illegible]d Thrombocytopenia.

DOSAGE FORM: Sterile Injection
STRENGTHS: 100 mg/mL Concentrate
ROUTE OF ADMINISTRATION: Intravenous
DISPENSED: ✓ Rx ___ OTC

CHEM NAME, STRUCTURAL FORMULA, MOLECULAR FORMULA, MOL.WT:

Chemical Structure



• = asymmetric carbon

Chemical Name: 1-[5-[(aminoiminomethyl)amino]-1-oxo-2[[1,2,3,4,-tetrahydro-3-methyl-8-quinolinyl)sulfonyl]amino]-4-methyl-2-piperidinecarboxylic acid, monohydrate

Molecular Formula: $C_{23}H_{36}N_6O_5S.H_2O$

Molecular Weight: 526.06

RELATED DOCUMENTS:

Type/ Number	Subject	Holder	Status	Review Date	Letter Date
[redacted]			Deficient		
			Adequate	02/01/94	
			Adequate	02/18/97	
			Adequate	11/18/96	
			Adequate	7-12-97	

CONSULTS:

Biometrics: Statistical stability was review completed on 3/12/1998. The reviewer, Dr. A.J. Sankoh of HFD-720, recommended 12 months expiry date for the drug product.

Microbiology: Microbiology review was completed on 10/29/98. The reviewer, Dr. Brenda Uratani of HFD-160, indicated that the application has a number of deficiencies.

REMARKS/COMMENTS: The applicant of the NDA (Texas Biotechnology Corporation) should provide additional information regarding a number of queries relating to the drug product. These queries related to methods of manufacturing and packaging, tests and specifications, sampling, stability and labeling.

CONCLUSIONS & RECOMMENDATIONS: The application is lacking some additional information relating to methods of manufacturing and packaging, tests and specifications, sampling, stability and labeling. An information request letter will be submitted to the NDA applicant outlining these deficiencies and requesting additional information.
The application is approvable.

/S/ 4/13/98
Ali Al-Hakim, Ph.D.
Review Chemist, HFD-180

/S/ 4/14/98
Eric P. Duffy, Ph.D.
Chemistry Team Leader

cc:
Orig. NDA 20-883
HFD-180/Division file
HFD-180/LTalarico
DISTRICT OFFICE
HFD-180/CSO/JDuBeau
HFD-820/JGibbs
HFD-102/n [#1 only]
HFD-180/EDuffy/4-9-98
HFD-180/AAl-Hakim
R/D Init: EDuffy/4-9-98
AAA/dob F/T 4-10-98/WP:

APR - 6 2000

COMPLETION OF MV REVIEW

To: NDA 20-883

From: Ali Al-Hakim, HFD-180

Date: 04/06/2000

NDA No: *20-883*

Product: Novastan

Date of Approval: 01/19/00

The FDA laboratory in Laurel, MD has completed the review of the analytical methods validation. The methods are deemed suitable except the suitability calculations for the [redacted] method for determining the stereoisomers, type I and II when they were performed on the column prescribed by the MVP. The analyst reported that the Lichrosorb column used by the applicant did not resolve the two stereoisomers. The analyst recommended that a Symmetry column is more suitable alternative than the applicant's Lichrosorb column because it can spilt the stereoisomers.

Additional comments:
The above information has been noted.

/S/ , 04/06/00
Ali Al-Hakim, Ph.D. HFD-180

CC:
HFD-180/ NDA 20-833
HFD-180/CSO/J.DuBeau
HFD-180/L.Zhou
HFD-180/A.Al-Hakim /S/ 4/6/00

Dubeau

MAR 2 3 1998

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

Sponsor and Address: Texas Biotechnology Corporation
Houston, Texas.

Date of Review: March 23, 1998

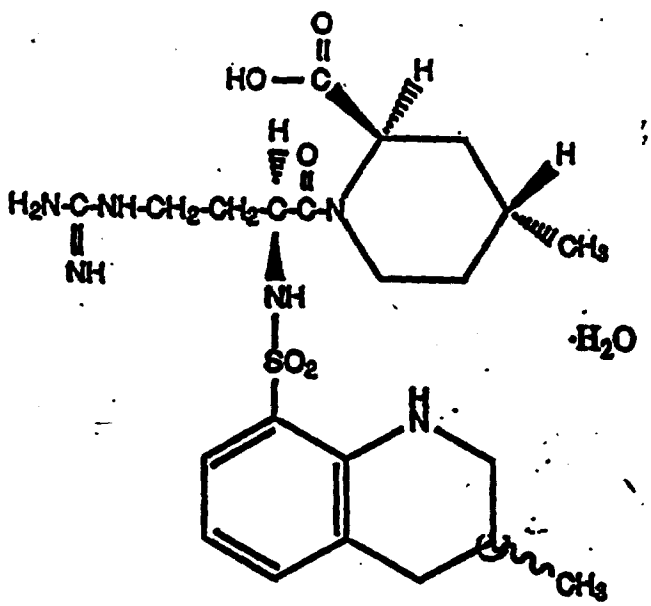
Date of Submission: August 11, 1997

Date of HFD-180 Receipt: August 19, 1997

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
Original Summary

DRUG: NOVASTAN/ARGATROBAN/ARGIPIDINE GN-1600/OM-7005/MD-805/ DK-7419 Injection.

(2R, 4R)-4-methyl-1S-(N^2-(RS)-3-methyl-1,2,3,4-tetrahydro-8-quinolyl)sulfonyl)-L-arginyl)-2-piperidinecarboxylic acid monohydrate.



MW: 526.65 Molecular Structure: $C_{23}H_{36}N_6O_5S.H_2O$

Formulation: A 2.5 ml injection vial contains 250 mg of argatroban, 750 mg D-sorbitol, JP, 1000 mg dehydrated alcohol, USP, and water for injection, USP.

Preparation for IV Administration: The drug must be diluted to a final concentration of 1 mg/ml, with 0.9% sodium chloride injection, USP, 5% dextrose injection, USP, or lactated Ringer's solution, prior to iv infusion.

Category: Direct thrombin inhibitor.

PROPOSED MARKETING INDICATION:

It is indicated as an anticoagulant therapy in patients with heparin-induced thrombocytopenia (HIT).

DOSE: The initial recommended dose of novastan for patients with heparin-induced thrombocytopenia (HIT) without hepatic impairment, is 2 μg/kg/min, and for patients with hepatic impairment, it is 0.5 μg/kg/min. These doses can be adjusted (not to exceed 10 μg/kg/min), until the steady state aPTT is 1.5-3 times the initial baseline values.

RELATED INDs/NDAs:

PRECLINICAL STUDIES AND TESTING LABORATORIES:

Type of Study	Study/Report #	Drug Batch #	Testing Lab.	Review Page #
Pharmacology				6-30
Absorption:				
Rat	Ref # 1	not stated		31-35
Rabbit	Ref # 2	not stated		31, 39
Dog	Ref # 2, 3, & 91-052-1600	not stated R9096AX		31-36
Distribution:				
Male rats	Ref # 1	not stated		37
Pregnant rats	Ref # 4	not stated		38, 43
Metabolism:				
Rat	Ref # 5 & 6	not stated		38, 39, 43, 44
Rabbit	not stated	not stated		39
Dog	not stated	not stated		39
Excretion:				
Rat	Ref # 1	not stated		39
Rabbit	Ref # 2	not stated		39
Dog	Ref # 2	not stated		39
Acute Toxicity with Argatroban: Bolus, i.v.				
Mice	M56-192	810415		45-49
Rats	M56-192,	810415		45-49
Dogs	M58-238	P-ATB-52608		45-49
24-hrs Cont, I.V.				
Rats	90-184-1600 (869/002)	M3-RD81		50-51
Dogs	90-183-1600 (869/001)	M3-RD81		52
Acute Toxicity with Degradation Products (G & H) of Argatroban: I.V.				
Rats	5M700G	G:SX396-3E H:SX396-2B3		53
Acute Toxicity with the Metabolite M1 of Argatroban: I.V.				
Rats	5M180G	840810		54

Subacute/Subchronic/Chronic Toxicity: I.V. with Argatroban.				
Rat				
1-month, bolus iv	Not stated	790314		55-56
6-months, bolus iv	Not stated	791101		56-58
28 days, bolus + cont iv	90-247-1600	M3-RD107/M3-RD91		58-59
Dog				
1-month, iv bolus	Not stated	790429		62-63
6-months, iv bolus	Not stated	P-ATB-52406S		63-64
28 days, bolus + cont iv	90-246-1600	M3-RD107/M3-RD105		65-66
Additional Toxicity Studies I.V. with Argatroban				
Rat 1-month, cont. iv	Not stated	KEY01-01A1		60-61
Dog 1-month, cont. iv	Not stated	KEY01-01A1		67-69
I.V. with Argatroban + 0.5%-impurities.				
Rat 1-month, iv bolus	2110 TMR	ONZ01-07A2		69-71
Dog 1-month, iv bolus	2111 TMC	ONZ01-07A2		71-72
Reproductive Toxicity:				
Segment I. Fertility and Reproductive Performance Rats, i.v.	M56-120	800610		72-74
Segment II. Teratology Rats, i.v.	55-458	791101		74-76
Rabbits, i.v.	D-11, D-20	43P101, 6QP102		77-78
Segment III. Perinatal/Postnatal Rats, i.v.	M58-163	P-ATB-52406		78-79
Mutagenicity:				
1. Mouse micronucleus test	10586 MAS	P-ATB-58201S		80-81
2. CHO gene mutation assay (HGPRT locus)	BGX 515/931162	P-ATB-58201S		81-82
3. In vitro UDS Test in rat hepatocytes	BGX 514/931120 AHF 93-60	P-ATB-58201S P-ATB-58201S		82-86
4. In vitro UDS Test in WI-38 human fetal lung cells	3223-103	810415		86-87
5. Recombinational repair Assay (or Rec Assay)	D-17	810415		87-89
6. Ames Test	D-16 D-19	P-ATB-52406S		90-91
7. Chromosome Aberration Test On Chinese Hamster Cultured Fetal Lung Fibroblasts	D-18	P-ATB-52406S		91

Special Toxicity Studies:				
1. 4-hr iv interaction study of argatroban, rt-PA and aspirin in dogs	91-051-1600	R9096AX		40-42
2. Precipitation of argatroban in plasma	Not given	P-ATB-52406S		92
3. Hemolytic potential of argatroban & its blood compatibility	2210BJR 91-006-1600	182, 183 ,184 12791-21, 12791-22		92-93, 98
4. Local im effects of argatroban in rabbits	Not given	KEY 01A1		94-95
5. Antigenicity in guinea pigs and mice	D-13 D-14 D-15 (ref #7)	Not stated 810415 P-ATB-52406S		95-97

Most of the studies submitted here have been previously submitted under IND ——— These include primary and secondary pharmacology studies, ADME studies in rats, rabbits and dogs, acute toxicity studies in mice, rats and dogs, also 24-hrs continuous iv infusion acute toxicity studies in rats and dogs, 1-month continuous iv infusion toxicity studies in rats and dogs, 28-day iv bolus ± continuous infusion toxicity studies in rats and dogs, 1-month bolus iv toxicities in rats and dogs, 6-month bolus iv toxicities in rats and dogs, segment I fertility studies in rats, Segment II teratology studies in rats and rabbits, Segment III peri and postnatal studies in rats, mutagenicity studies (Ames assay, chromosome aberration test in Chinese hamster cultured cells, recombinational repair assay (or Rec assay), unscheduled DNA synthesis (UDS) test in rat hepatocytes and WI human fetal lung cells, in vivo mouse micronucleus test, and Chinese hamster ovary cells gene mutation assay), antigenicity tests in guinea pigs and mice, local intramuscular irritation studies in rabbits, and tests for hemolytic potential of argatroban. Previous IND reviews for these studies, are incorporated in appropriate places.

PHARMACOLOGY:

Argatroban is a synthetic, potent, arginine-based thrombin inhibitor. In x-ray crystallography studies, argatroban binds selectively to catalytic site of thrombin in an inhibitor like fashion. Unlike heparin, which inhibits thrombin by enhancing the protease inhibitory action of antithrombin III (AT III, an endogenous cofactor), argatroban inhibits thrombin directly via an interaction with the active-site serine. Therefore, argatroban inhibits all thrombin-mediated effects, including platelet aggregation, fibrin formation, and activation of factor XIIa in the absence of AT-III. Although hirudin is also a direct thrombin inhibitor, argatroban differs from hirudin, because it is smaller in molecular size, it is selective, fast, and causes reversible inhibition of the catalytic site of thrombin, as well as it has greater potency for inhibiting clot bound thrombin. Therefore in patients, who experience heparin-induced thrombocytopenia (HIT), argatroban may be a useful anticoagulant agent. In the initial IND review, both primary and secondary pharmacology studies were submitted. In this NDA submission, additional primary and secondary pharmacology studies, along with pharmacological activity (including their antithrombotic activity) of the 21-R and 21-S stereoisomer of argatroban, are submitted.

I. Primary Pharmacology:

Following Pharmacology studies were submitted to IND [redacted] initial submission dated December 8, 1988, and were reviewed on 6/1/1989. These are reproduced in appropriate pharmacology subsections.

In Vitro Effects:

1. Thrombin Inhibitory Effect:

a. **Argatroban is strongly bound to the active site region of thrombin and inhibits the conversion of fibrinogen to fibrin induced by human thrombin. The concentration of argatroban to prolong thrombin time by a factor of 2 ($IC_{2.0}$) using human and bovine thrombin was 0.036 uM and 0.032 uM, respectively. Argatroban potently inhibited the breakdown of S-2238 (a chromogenic synthetic substrate) by thrombin, with inhibitory constants (K_i) of 0.039 uM and 0.019 uM using human thrombin and bovine thrombin, respectively.**

b. In another study, the comparative inhibitory effects of argatroban, heparin and hirudin on clot bound and solution phase thrombin (using S-2238, a thrombin specific chromogenic substrate) were examined. Solution phase thrombi were made by adding calcium chloride to citrated plasma (from normal healthy subjects) at 37°C, until a visible clot was formed (usually 30-60 min). Fibrin clots were made by incubating human fibrinogen with human thrombin, in the presence of calcium chloride for 30 min. Argatroban, hirudin, and heparin inhibited solution phase thrombin with IC_{50} values of 1.1 μM, 1.2 nM and 2.5 mU/ml resp. These 3 inhibited fibrin (clot) bound thrombin with IC_{50} values of 2.9 μM, 43.4 nM and 366.3 mU/ml resp. These studies suggest that hirudin and heparin were 36 and 147 times less active against fibrin (clot) bound thrombin, than thrombin in solution. Thus, argatroban, by its direct interaction with the thrombin active site, has a greater access to clot associated thrombin.

2. Selectivity of Thrombin Inhibitory Effects:

a. **In purified enzyme systems, Argatroban was considerably selective and stronger for thrombin (Ki=0.039 uM) as compared to other trypsin-like serine proteases (Ki=5-1700 uM). Unlike heparin, argatroban did not inhibit factor Xa. Argatroban did not show any inhibitory effect on factor VIIa and XIIa at 1.9 uM and 470 uM, respectively.**

b. In another study, using chromogen substrates (synthetic compounds with chromogenic leaving groups), the inhibitory constant (K_i) of argatroban was 5-38 nM. In this assay, the substrate interacts with the active site of the enzyme with higher affinity, than the natural substrate fibrinogen (K_m 10 μM). This method involves incubation of various concs of argatroban (4-250 μM) in microtiter plates with the enzyme (such as purified human plasma derived factor 1XA for comparing the K_i of argatroban vs factor 1XA), and its chromogenic substrate (for factor 1XA it is CBS3447, based on its K_m values in the literature). Absorbance changes are monitored using a microtiter plate reader, and 1/v is plotted against inhibitor concentration, from which a plot of K_i is derived. The K_i for other enzymes of the same family were much higher, trypsin had K_i of 50 μM, plasmin K_i of 50 μM, human factor Xa = K_i 210 μM, t-PA = K_i 21 μM, and kallikrein = K_i 150 μM. Others have reported K_i for bovine trypsin to be 17.2 μM, and for human factor IXa and for activated protein C, the K_i of >250 μM. In another study, argatroban had K_i for thrombin of 0.031 μM (31 nM), in comparison the K_i for plasmin (K_i 642 μM), human factor Xa (K_i 18.6 μM), single chain-t-PA (K_i 830 μM) and for factor VIIa (K_i 340 μM) were higher.

3. Platelet Aggregation Inhibitory Effects:

a. **Argatroban inhibited platelet aggregation induced by thrombin. The IC_{50} values of inhibition of rabbit and human platelet rich plasma (PRP) samples were 0.027 uM and 0.04 uM, respectively. Argatroban had little inhibitory effect on aggregation induced by collagen, arachidonic acid or ADP in rabbit PRP samples (IC_{50}=200-590 uM).**

b. In rat, rabbit and human washed platelets, the effects of argatroban on thrombin-induced platelet aggregation were examined. Argatroban inhibited platelet aggregation with the IC_{50} values of 6.8, 14.8 and 12.2 nM in rat, rabbit and human platelets resp. This antithrombotic effect of the drug was specific, since the IC_{50} values for collagen, ADP, and arachidonic acid, in rabbit platelet rich plasma, were 138-500 μM.

c. In canine platelets, argatroban inhibited thrombin (0.6 μM)-induced platelet aggregation in a dose related manner, with an IC_{50} of 0.2 μM. In contrast, 500-fold higher conc. of argatroban (IC_{50} 100 μM) were required to inhibit collagen-induced (5 μg/ml) platelet aggregation. Platelet aggregation was also examined in rabbit gel filtered platelets, following induction by thrombin or factor Xa (here thrombin was generated with factor Xa + prothrombin + calcium). Argatroban was 5-7 times more potent in inhibiting thrombin-induced (IC_{50} 0.016 μM) platelet aggregation, than factor Xa-induced platelet aggregation (IC_{50} 0.08-0.11 μM). In contrast the IC_{50} for heparin (in the presence of AT III) following factor Xa and thrombin-induced aggregation were 5.6 and 0.063 U/ml, suggesting that argatroban inhibits thrombin generating on the platelet surface more efficiently than heparin.

d. In rabbit washed platelets, argatroban inhibited platelet aggregation (induced by fibrin clots) with IC_{50} values of 22.8 nM. When fluid phase thrombin was used to induce platelet aggregation, argatroban inhibited platelet aggregation with IC_{50} values of 12.9 nM. With heparin, the IC_{50} value for platelet aggregation was 0.01 mU/ml induced by fluid phase thrombin, but 5000 times higher conc of heparin (IC_{50} of 50.4 mU/ml) was needed for clot-induced aggregation. These studies suggest that in platelet rich thrombi, argatroban has a superior effect over heparin.

e. When thrombin (0.07 U/ml)-induced platelet aggregates were used, argatroban (1.34 μM) inhibited platelet adherence to endothelial cells (aggregate adherence was 2.7 vs 38.1 with thrombin alone, using monolayers of umbilical vein endothelial cells).

f. Similarly, in the whole blood (not anticoagulated, from human volunteers), when argatroban (10 μg/ml) was added, prior to activation by recombinant (r)-tissue factor (as well as by other agonists e.g. ADP, arachidonic acid, collagen), argatroban inhibited r-tissue factor mediated platelet aggregation.

4. Anticoagulant Activity:

a. **In citrated human plasma samples, argatroban prolonged thrombin time (0.06 uM), activated partial thromboplastin time (0.6 uM) and prothrombim time (1 uM) to a factor of greater than 2 when compared to base line values. Prolongation of coagulation time by argatroban was dose dependent but it was less active than heparin due to its selectivity in antagonizing thrombin. Argatroban had a similar anticoagulation effect on tests using blood samples of rats, rabbits and dogs.**

b. In rat plasma, argatroban's potency was examined by measuring Thrombin time (TT), aPTT and factor Xa clotting time. The concentrations which increased the clotting time by 100% (EC_{100}) were determined for the drug and heparin. Argatroban had EC_{100} values of 53, 430 and 354 nM for TT, aPTT, and factor Xa resp. In contrast, heparin's EC_{100} values for these 3 parameters were 0.15, 0.093, and 0.18 units/ml resp, suggesting that argatroban increases TT preferentially, compared to other parameters. Similarly, in the rabbit plasma, argatroban had EC_{100} values of 63, 840 and 732 nM for TT, aPTT, and factor Xa resp. In contrast, heparin's EC_{100} values for these 3 parameters in rabbit plasma were similar (0.11 units/ml). These similar effects were observed in the dog plasma. These studies suggest that argatroban increases TT distinctly, compared to other parameters, whereas the effects of heparin on all 3 parameters were similar. In rabbits, argatroban (iv 5-40 μg/kg/min) increased aPTT in a dose related manner (at 5 μg/kg/min, aPTT was prolonged by 1.4 times of controls), with no change in the bleeding time. In contrast heparin at 100 U/kg, increased both aPTT (by 3.3 times), and the bleeding times (by 1.6 times).

5. Anticoagulation Effect in Antithrombin III Deficient Plasma.

Since argatroban selectively inhibited thrombin, its anticoagulant effect did not require the presence of antithrombin III. In contrast, heparin required antithrombin III for its anticoagulant effect. The above in vitro effects were confirmed in mouse model where the blood level of antithrombin III was reduced with anti-rat antithrombin III rabbit gamma-globulin.

6. Effect on Fibrin Cross-linking Reaction and Fibrin Dissolution Accelerating Effect.

Argatroban reduced the degree of fibrin cross-linking reaction in plasma clots where coagulation of citrated plasma was induced by addition of calcium chloride (IC_{50}=0.2 uM). Argatroban showed an accelerating effect on the dissolution of plasma clots formed by adding calcium chloride to citrated plasma containing plasmamogen activator. Heparin showed a similar but less potent effect. Argatroban itself had no direct inhibitory effect on activated factor XIIIa at 100 uM.

The above in vitro effect of argatroban was also demonstrated in rabbit study. When argatroban was administered concurrently with a thrombolytic agents (tissue plasminogen activator or urokinase) at doses which did not elicit a thrombolytic effect, the thrombi were dissolved. Heparin had no such thrombolysis-accelerating effect. Argatroban alone did not elicit a thrombolytic effect.

7. Effect on Thrombin Induced Vasoconstriction in Isolated Cerebral Arteries.

At concentration of 1-20 uM, argatroban dose-dependently suppressed thrombin-induced vasoconstriction in isolated cerebral arteries of dogs. At a concentration of 20 uM, argatroban showed no suppressing effect on vasoconstriction induced by serotonin, prostaglandin F2 alpha or KCl.

B. In Vivo Effectiveness:

1. Effect on Peripheral Arterial Occlusion Generated by Infusion of Lactic Acid into the Femoral Artery of Rats.

Pretreatment with subcutaneous injections of 10 or 30 mg/kg of argatroban produced significant suppression of the foot lesions (blackening and defluxion of the toes) induced by lactic acid in rats. Orally administered ticlopidine (a platelet inhibitor, 100 mg/kg) showed a partially suppresing effect in 2 of 8 animals. Subcutaneous administration of heparin at doses up to 900 U/kg showed no significant effect.

2. Effects on Antithrombotic Arterial Models: (These thrombi models are rich in platelets and other blood cells, but poor in fibrin strands). In these models, endothelial damage is produced either by electrical stimulation, chemical injury, laser induced thermal injury or an eversion of a vessel segment.

a. **Continuous intravenous administration of 1 or 3 ug/kg/min of argatroban suppressed the thrombus formation in rabbits induced by acetic acid treatment to the arterial wall. Intravenous administration of heparin at 300 U/kg but not at 100 U/kg showed a similar inhibition on the thrombus formation.**

Argatroban (10 ug/kg/min, i.v.) also suppressed the increases in both thromboxane B2 and fibrinopeptide A in blood of dogs where arterial thrombi were formed by acetic acid.

b. In an arterial thrombosis model (on the left carotid artery) of electrical stimulation in rats, when argatroban and heparin were given by continuous iv infusions, 45 min before electrical stimulation, argatroban (at 20 μg/kg/min, 111% increase in time to occlusion) gave similar response as heparin (at 25 μg/kg/min, 180% increase in time to occlusion) on weight basis. Heparin, at subthreshold dose (at 12.5 μg/kg/min) increased TT and aPTT to >150 sec (by 10 times), whereas argatroban only caused moderate changes (by 3 times) in these parameters.

c. In another study, efficacy of argatroban vs heparin was examined in prevention of delayed arterial thrombosis. Argatroban (100 μg/kg/min for 60 min) prevented arterial graft occlusion in 7 of 10 rabbits after 24 hrs, whereas heparin (50 units/kg, iv for 60 min) was effective in 1 of 10 rabbits at 24 hrs. Histopathology showed that graft segment thrombosis was significantly less extensive in argatroban vs heparin in rabbits. These studies suggest that argatroban is more efficient than heparin in reducing delayed arterial eversion graft thrombosis.

d. In a rabbit carotid artery model of thrombosis, occlusive thrombi were induced in rabbits by repeated vessel clamping, and by applying an electrical current. Administration of iv argatroban (60 min prior to thrombus induction, and continuing for 180 min) at 2.5 to 20 μg/kg/min had a dose related antithrombotic effect, such that at 20 μg/kg/min, 6 of 8 animals had normal blood flow. Heparin (40 μg/kg/min) was not effective, despite it increased aPTT values of >300 sec. These studies suggest that, argatroban has superior antithrombotic efficacy over heparin.

e. In a rabbit femoral arterial model of thrombosis when thrombi were stabilized for 5 min, before administration of argatroban (bolus + plus infusion doses for 180 min of 50 μg/kg + 10 μg/kg/min to 200 μg/kg + 40 μg/kg/min) or heparin (200 μg/kg + 40 μg/kg/min), argatroban, dose-dependently caused a significant increase in reperfusion index (area under the blood flow $AUC_{exptal}/AUC_{control}$) by 25.1-46.1%. At 90 and 180 min, the drug increased aPTT moderately by 2.9 times, and in 8 of the 9 animals, the drug was able to induce the reperfusion of occluded femoral arteries. In contrast, heparin was not effective in inducing clot lysis, despite its aPTT values of >300 sec. These studies suggest that, argatroban treatment alone is able to lyse the intra-arterial clot.

f. In the rabbit femoral arterial thrombosis model, argatroban (100 or 200 μg/kg/min for 60 min) prevented thrombosis in 9 of 11 rabbits, suggesting that the drug can prevent arterial occlusion, whereas heparin (200 units/kg, iv for 60 min) was ineffective in all 10 rabbits.

g. In a mongrel dog model of iliac artery injury by balloon inflation, argatroban (0.05 mg/kg) or heparin (30 U/kg) were infused into one side of iliac artery, and the other injured side was used as a control. In the controls, the degree of angioscopic % stenosis by thrombus at 60 min, was 44.4%-49.6, both argatroban and heparin reduced this to 2.3% and 5.7% resp. These effects on reduction in thrombus formation were observed by local treatment (without systemic administration).

h. In a laser lesion model of rat mesenteric venules, argatroban significantly inhibited thrombus formation at 0.1 mg/kg, which lasted for 4 hrs. Hirudin had similar effects at 0.05-0.5 mg/kg. In another study, both inhibitors (the drug, and PPACK) showed significant antithrombotic effects at 0.1-0.5 mg/kg doses in He-Ne laser induced thrombosis (in rat mesenteric micro vessels), which lasted for 50-60 min. These effects were more pronounced in arterioles (minimal effects noted at 0.1 mg/kg) than in venules (minimal effects noted at 0.25 mg/kg).

3. <u>Effects on Antithrombotic Venous Models</u>: (Unlike antithrombotic arterial models which are poor in fibrin strands, the venous models are rich in fibrin strands, and contain few erythrocytes).

a. In a venous thrombosis model in rats (endothelial damage was induced by repeated clamping of the jugular vein (with the hemostat), anticoagulant potency was examined based on the number of clampings. Argatroban and heparin both had an effect in a

dose related manner (10-15 clampings to cause vascular occlusion vs 4.4 clampings in controls), but argatroban (50-500 μg/kg) had a slightly better dose dependent response than heparin (125-1000 μg/kg). Hirudin had the strongest response (25-250 μg/kg).

b. In rats when argatroban and heparin (both at 1.25-40 μg/kg/min) were given by iv infusion, 45 min before thrombus formation and for 5 min during the study, both compounds prevented the thrombus formation in a dose-related manner, and significant decreases in the thrombus weights were noted at 1.25 μg/kg/min. Argatroban increased ecarin clotting time (ECT, ecarin is a highly purified protease, which activates prothrombin in the absence of any cofactors) in a dose related manner from 1.25-40 μg/kg/min. In contrast, ECT was not increased by heparin, at antithrombotic doses in the venous model (and only increased at 40 μg/kg/min in the arterial model). These studies suggest that ECT provides a predictive marker for antithrombotic activity of argatroban, whereas the classical coagulation markers are more conformed for indirect thrombin inhibitors (such as heparin).

c. Argatroban (0.5-1.5 mg/kg for 60 min) was given to rats, 20 min prior to starting the freezing injury (which causes endothelial damage in the jugular vein). At 1.5 mg/kg, the drug caused a significant decrease (by 89%) in the thrombus formation, without any bleeding. Ticlopidine (30 mg/kg, an antiplatelet agent) also had an antithrombotic effect (decreased thrombus formation by 53%).

d. In a rabbit venous thrombosis model, argatroban and heparin (by continuous iv infusion) prevented thrombus formation with an ED_{50} of 2.4 and 0.5 μg/kg/min resp. Both compounds showed similar effects on aPTT. Similarly in a rat venous thrombosis, both argatroban (ED_{50} of 1.5 μg/kg/min) and heparin (ED_{50} of 1.2 μg/kg/min) were effective in preventing thrombus formation.

e. In a hamster model of venous thrombosis (induced in the inferior vena cava by electrical stimulation and venous stasis), heparin at 210 U/kg, and argatroban (1 mg/kg) both given iv, decreased the thrombus weight (0.2 and 1.3 vs 11.1 mg in controls), however heparin increased the mesenteric bleeding in arterioles by 3-fold, argatroban did not affect the bleeding. Similarly in the hamster femoral vein platelet rich thrombosis model (by clamping the vessel wall), argatroban caused a 50% inhibition in thrombus formation at 2 mg/kg (which also correlated with prolongation of aPTT), hirudin at 1.4 mg/kg. Whereas 100 U/kg of heparin, which significantly prolonged aPTT (26 vs 177 sec in controls), inhibited thrombus formation only by 7% (p = NS).

4. Effects on Mixed Thrombi Generated by Arteriovenous Shunts: (In this model, thrombi constitute of erythrocytes and platelet rich components).

a. Continuous intravenous infusion of argatroban (2 or 6 mcg/kg/min) to rabbits inhibited the mixed thrombus (platelets and fibrin) formation induced by an arteriovenous shunt and a venous constriction.

b. In another rabbit arteriovenous shunt (mixed thrombosis) model, argatroban prevented thrombus formation with an ED_{50} of 4.5 μg/kg/min, ED_{50} with heparin was 2.8 μg/kg/min, but argatroban was ~2-fold less potent than heparin in increasing aPTT (at 10 μg/kg/ min, argatroban and heparin increased aPTT by 1.4 fold, and 2.7 fold resp). These studies suggest that, argatroban has superior antithrombotic effect over heparin, and has lower potential of hemorrhagic risk.

c. In an arteriovenous shunt rat model (by placing nylon micro filament between the carotid artery and the jugular vein), argatroban (continuous iv infusion) prevented thrombus formation with an ED_{50} of 6 μg/kg/min, ED_{50} with heparin was 3 μg/kg/min.

5. Effect in Disseiminated Intravascular Coagulation (DIC) Models.

Argatroban (0.063, 0.2, or 2 ug/kg/min, i.v.) dose-dependently suppressed the reduction in platelet count caused by i.v. administration of thrombin to rabbits. In rats, argatroban inhibited the fatal effect of intravenously administered thrombin. Thrombin occluded the microvessels of the lungs and produced respiratory arrest and death. Argatroban (5,10, and 20 mg/kg, i.v.) also reduced the number of thrombi in rats induced by an i.v. infusion of lactic acid.

Intravenous infusion of rabbits with argatroban (1.0 or 10 ug/kg/min) suppressed the "disseminated intravascular coagulation" like pathological changes (reduction in platelet count and fibrinogen concentration) induced by lactic acid and tissue thromboplastin. Intravenous administration of heparin (100 U/kg x 2) suppressed the reduction in fibrinogen concentration but its effect on platelet count was not equal to that produced by argatroban.

Intravenous infusion of dogs with argatroban (5 ug/kg/min) strongly suppressed the marked drop in fibrinogen and the increase in FDP induced by i.v. administration of endotoxins. Argatroban prevented deterioration of overall circulatory parameters.

6. Effects on Bleeding/Anticoagulation:

a. The effect of argatroban on bleeding time was studied in rabbits. The following table summarizes the results (Data were taken from ponsor's Table 7.22. Data are mean of 3 or 4 estimations).

Effect of Argatroban Administration on Bleeding Time

Dose	Bleeding time(min)	APTT (sec)	Plasma level (μM)
Vehicle	2.94±0.13	25.4±3.8	
5 μg/kg min	4.09±0.14	34.2±4.5	0.65±0.038
10 μg/kg min	4.97±0.18	42.7±1.6	1.69±0.140
20 μg/kg min	7.93±0.85	45.3±2.5	2.47±0.180

b. In anesthetized rats, argatroban increased tail transection bleeding time in a dose related manner, with an ED_{100} of 11 μg/kg/min, in contrast heparin ED_{100} was 2.2 μg/kg/min, suggesting that the hemorrhagic potential of argatroban is lower than heparin.

c. Duration of the anticoagulant activity of the drug after iv bolus in rats was examined. Argatroban (0.12 mg/kg, iv bolus in the caudal vein of anesthetized rats) increased thrombin time by 6.4 fold compared to controls, (53.3 vs 8.3 sec in controls) at 2 min. This increase was reduced to 1.6 fold at 10 min, suggesting that the anticoagulant effect, was of the short duration.

d. Argatroban, dose-dependently increased both TT and aPTT, with ED_{100} values of 0.1 and 1.2 mg/kg for TT and aPTT resp. The ED_{100} values for heparin were 0.16 and 0.14 mg/kg for TT and aPTT resp. These studies suggest that higher doses of the drug are required to increase aPTT than TT, and aPTT could be used as a marker for the anticoagulation activity of the drug.

e. Also, in another study in rats, combination of argatroban and heparin effects were examined on aPTT. Argatroban (5, 10, 20, and 40 μg/kg/min) or heparin (1.25, 2.5, and 5 μg/kg/min) were given in combination or separately to anesthetized rats by iv infusion over 90 min. Argatroban increased aPTT significantly at 20 μg/kg/min (32.8 vs 28.8 sec). Heparin increased this parameter significantly at 5 μg/kg/min (139.1 vs 26.2 sec). The combination of 2 did not further increase the aPTT, compared to heparin alone (heparin 5 μg/kg/min 139.1 sec, heparin + argatroban 20 μg/kg/min 153 sec).

f. The anticoagulation effects (PT, aPTT, and TT) of argatroban (1 mg/kg) were not altered by coadministration of aspirin (100 mg, po), indomethacin (3 mg/kg, po), tolbutamide (25 mg/kg, po) and clofibrate (100 mg/kg, po) in rats.

g. Similarly, the anticoagulant activity of argatroban (maximal conc of 1 μg/kg/min) was not lost, even after 7-days of continuous iv (by osmotic mini pumps) infusions in rats. Plasma thrombin times were increased by 30% after 24 hrs (51.7 vs 39.9 sec in controls), and by 60% after 7-days of treatment (61.5 vs 38.4 sec in controls). Argatroban had no effects on the leucocyte counts, platelets or red blood cell counts in rats, after 7 days treatment.

7. Effect on Cerebral Infarction Models.

Intraperitoneal administration of argatroban (5 or 10 mg/kg) improved the flow of blood in rat in microcirculatory cerebral disorder model as a result of its suppressing effect of argatroban on platelet aggregation.

8. Effects in Antithrombin III (AT-III) Deficient Rats and Mice:

In an AT-III deficient rat model (where rats were made AT-III deficient by injection of anti AT-III γ-globulin), argatroban (25 mg/kg, twice a day) was effective in preventing thrombus formation, while heparin (4 mg/kg, twice a day) was ineffective in these animals. Similarly in mice, heparin did not attenuate the thromboembolism, whereas argatroban was effective regardless of absence of AT-III levels.

9. Effects on Thrombolysis:

a. Argatroban (20 μg/kg/min for 90 min) was given with or without rt-PA (200 μg/kg/min iv bolus + 25 μg/kg/min infusion for 30 min + 8.3 μg/kg/min infusion for 60 min) to anesthetized rats and at the end of the study aPTT and TT were measured, along with plasma t-PA activity. Argatroban alone increased the aPTT by 1.4 fold and TT by 2.5 fold, but had no effect on t-PA activity. The combination of 2 had no effect on anticoagulant or fibrinolytic activity of t-PA (29.2 vs 36.2 with rt-PA alone). rt-PA slightly increased TT (44.1 vs 33.2 sec with vehicle) and aPTT (32.6 vs 27.2 sec with vehicle). These studies suggest that the drug does not affect the fibrinolytic process.

b. Thrombolysis induced by recombinant hirudin and argatroban in a rat model of arterial thrombosis was examined. Argatroban (bolus + plus infusion doses) at a dose (200 μg/kg + 40 μg/kg/min), which doubled the aPTT, caused a significant increase in the reperfusion index (area under the blood flow by 1186% vs 564% observed with the same dose of hirudin). In contrast, heparin (1000 μg/kg + 200 μg/kg/min) was ineffective. These studies suggest that argatroban is two times more potent than hirudin in this model.

c. Thrombolysis induced by recombinant tissue plasminogen activator (rt-PA) was improved by argatroban in a rat model of arterial thrombosis. Occlusive thrombi were induced in rats by applying an electrical current to abdominal aorta. The thrombi were stabilized for 60 min, before administration of rt-PA (bolus + plus infusion doses for 180 min of 62.5 μg/kg + 12.5 μg/kg/min to 1000 μg/kg + 200 μg/kg/min). Argatroban at a dose (200 μg/kg + 40 μg/kg/min), which doubled the aPTT, caused a significant shift in rt-PA dose response curve to the left, and increased reperfusion index (area under the blood flow $AUC_{exptal}/AUC_{control}$, 36.9% vs 25.6% with rt-PA alone). In contrast, heparin (500 μg/kg + 100 μg/kg/min) was not effective in shifting the rt-PA dose response curve, despite its 3.6 fold increase in aPTT values. These studies suggest that for maximal thrombolytic effect, argatroban treatment may lead to reduction of rt-PA dose, but neither agent alone caused the clot lysis in this model.

d. In a rabbit arterial stenosis thrombosis model, argatroban (100 μg/kg/min for 60 min) was given with rt-PA (0.45 mg/kg iv bolus at 15 min intervals until recanalization or maximum of 4 boluses) or with heparin (200 units/kg, iv over 60 min). Argatroban + rt-PA caused recanalization in 5 of 7 rabbits. Reflow was seen more rapidly with argatroban than with heparin (14 vs 35 min). These studies indicate that argatroban in relation to heparin enhances and sustains thrombolysis with rt-PA.

e. Thrombolysis was also examined in the rabbit model of arterial thrombosis generated by the endothelial cell injury in the rabbit carotid artery by acetic acid. t-PA (0.48 mg/kg), or uPA (90,000 IU/kg) alone did not dissolve the clots, but combination with argatroban (1.2 mg/kg, for 2 hrs) effectively dissolved the clots, suggesting that argatroban not only suppresses the fibrin formation but also accelerates the fibrin dissolution.

f. In femoral arterial thrombosis model in rabbits, clots were induced by insertion of a copper coil in the artery and by balloon injury. Two days later the clots were treated with intrathrombic delivery by PSPMT (pulse-spray pharmacomechanical thrombolysis). In this model, addition of iv argatroban did not increase the lysis but adjunctive intrathrombic argatroban significantly increased lysis at low and high doses of 0.3 and 3 mg (by 34-37%), compared with t-PA (3 mg) and heparin.

g. In a rabbit model of thrombosis (in a carotid artery), the effect of heparin and aspirin was compared to argatroban after thrombolysis with t-PA (0.5 mg/kg/90 min). Reperfusion was measured by proximal Doppler flow method. Free flow was determined in the segments at various times as prevention of reocclusion. Aspirin did not prevent reocclusion. Argatroban iv at 0.6, 1.25, and 2.5 mg/kg/hr maintained flow at 43, 53 and 68%, while heparin (160 U/kg) maintained it at 17%.

h. In a femoral arterial thrombosis model in rabbits, infusion of saruplase (3-12 mg/kg, for 60 min) caused a dose-related thrombolysis. At 3 mg/kg of saruplase, the reperfusion rate was 3 out of 6 vessels, and time to reperfusion was 42 min. When argatroban (1 mg/kg bolus followed by 3 mg/kg infusion) was given with saruplase, reperfusion rate was 6 of 6 vessels, and time to reperfusion was decreased to 26 min, and no reocclusion occurred at 120 min. Enhancement of thrombolysis was more effective with argatroban than with heparin.

i. In femoral arterial thrombosis model in mongrel dog, clots were induced by insertion of a thrombogenic copper coil in the artery. In this model, tPA (iv, 0.8 mg/kg) was given 60 min after occlusion and continued for 90 min. Vehicle or argatroban (iv, 10 μg/kg/min for 60 min) was given 10 min after occlusion. Argatroban prevented reocclusion and decreased the time to lysis in dogs, pretreated with the drug, but did not work if the drug was given after the clot formation.

j. In mongrel dogs, argatroban (10 μM) given in the femoral artery of the perfused hind leg of dog, totally inhibited the plasminogen activator release by thrombin, in a fibrinogen plate assay.

k. In a dog model, infra renal abdominal aorta was replaced with a knitted Dacron prosthesis. The effects of argatroban (1 μg/kg/min for 2 weeks), and clopidogril (12.5 mg/kg/day for 1 month) in graft potency were examined (by angioscopy and angiography), immediately, and at 1-4 weeks after the surgery. The mean maximum stenosis by angioscopy was 61% in controls, which was reduced to 40% and 9% in dogs, with argatroban and clopidogril resp. At the end of 4 weeks, the graft potency in above 3 groups were 50, 71 and 100% resp. These studies suggest that both argatroban and clopidogril are useful antithrombogenic agents in increasing the graft potency.

10. Effects on Sepsis, and Acute Myocardial Infarction:

a. In rats, endotoxin (1-10 mg/kg) injection causes decreases in plasma fibrinogen levels, platelet counts, and tPA activity in the lungs and increases non-plasmin fibrinolytic protease activity in the lungs and spleen. Argatroban (25 mg/kg) given iv, 10 min before endotoxin iv injection, prevented the decrease in platelet counts, and plasma fibrinogen levels, but did not alter the tPA (2-3 ng/ml in both controls and drug treated) and tissue fibrinolytic proteinase (0.7-0.8 cu/ml in both controls and drug treated) in the lungs.

b. In an acute myocardial infarction (AMI) model in dogs, an intra coronary thrombus was induced by mock rupture of atheromatous plaque consisting of cholesterol and collagen. In controls (AMI), 8 of 10 animals had intra occlusive thrombus formation, in argatroban treated (iv bolus 0.5 mg/kg, injected immediately after induction of the model), only 2 of 10 dogs had AMI. Heparin (200 U/kg) decreased AMI in 3 of 10 dogs. Thrombus weight was decreased from 7 mg in controls, to 2 and 3 mg resp with argatroban and heparin resp. These studies suggest that the drug is effective in reducing AMI in dogs.

11. Effects on Cerebral Ischemia:

a. The effects of the drug on the cerebral microcirculation were examined in 4-vessel occlusion model in rats. No-perfusion region was defined in these rats, by the infusion of India ink into rats. Argatroban (5 and 10 mg/kg ip) reduced the no-perfusion area (by inhibition of platelet aggregation) to 5.8-6.3% vs 14.6% in controls. In contrast urokinase did not alter the perfusion area.

b. Argatroban (0.1 and 0.3 mg/hr/rat) reversed a decrease in regional cerebral blood flow in the rat distal middle cerebral artery (DMCA) occlusion model, it reduced the size of cerebral infarction and caused a significant improvement in neurological deficits.

c. In another thrombotic middle cerebral artery (MCA) occlusion model in rats, argatroban (40 mg/kg twice, sc just after, and 3 hrs after MCA) inhibited the formation of micro thrombi up to 3 hrs, and reduced the size of ischemic cerebral lesions at 6 hrs, suggesting that the drug can reduce the formation of micro thrombi and ischemic lesions in the early stage.

d. In an ellagic acid (injection)-induced cerebral thrombo-embolism model in rats, argatroban at 3 mg/kg/h, decreased the mortality to 0%, compared to saline control animals (mortality 80%). Heparin (600 U/kg/h) reduced the mortality to 30%, but it caused marked bleeding in the wound. These studies suggest that argatroban is more useful than heparin in preventing ischemic damage on cerebral thrombosis.

e. Argatroban's effects in the middle cerebral artery (MCA, occluded by photochemical reaction) thrombosis in the rat were compared with clopidogrel (an ADP antagonist), and vapiprost (thromboxane antagonist). Both antagonists and argatroban (30 μg/kg/min), significantly prolonged the time for arterial occlusion (with argatroban 444.3 vs 288 sec in controls), whereas heparin was ineffective (298 vs 288 sec).

f. Similarly in cerebral (pial) vessels of rats, where occlusive thrombus was induced, using He-Ne laser technique, iv dose of argatroban (0.5 mg/kg) diminished the thrombus after 30 min in arterioles, and after 50 min in venules. In contrast continuous iv infusion of the drug at 2 mg/kg/h, prolonged the duration of antithrombotic effect up to 3 hrs.

g. The effects of argatroban in cerebral vasospasm were examined in a rabbit model of subarachnoid hemorrhage (SAH, by giving cisternal injection of 0.66 ml/kg of autologous arterial blood). Argatroban (1.5 or 3 mg/kg, iv for 3 hrs) was given either immediately, or 3 hrs after the formation of subarachnoid hemorrhage. Vasospasm was measured (by basilar artery diameter, by angiography) at 6 hrs, and on day 1, 3, and 5 after SAH. Argatroban prevented cerebral vasospasm in a group treated immediately with the drug, as well as on day 1 after treatment of 3 mg/kg doses in rabbits, and on day 3 in all other drug treated groups.

12. Effect on Blood Flow:

In an anesthetized femoral arterial model, rabbits received t-PA, aspirin and argatroban (1.25, 2.5, and 5 mg/kg/hr) or hirudin (2.5 and 5 mg/kg/hr), and blood flow was measured for 4 hrs. Both argatroban and hirudin at 2.5 and 5 mg/kg/hr resp, were effective in achieving full reperfusion in animals, argatroban at this dose increased aPTT by 4.9-fold and bleeding time by 3-fold, in contrast hirudin increased these by 3.3 and 3.5 fold resp.

13. Effects on Extracorporeal Circulation:

Fifteen dogs were subjected to extracorporeal circulation, 8 animals received 3 mg/kg of the drug, and the other 7 heparin (2 mg/kg). Both agents increased aPTT to a similar degree, but argatroban caused less hemolysis (0.17 vs 0.39 mg/dl/min for heparin). No effects on platelet aggregability were observed with the either drug. Argatroban was effective at flow rates of 92 ml/kg/min vs heparin at 86.4 ml/kg/min. The half life of the drug increased to 30 min in this model, vs 18.7 min by iv injection, however this was attributed to transient hepatic disorder due to extracorporeal circulation. These studies suggest that argatroban could be used as an anticoagulant in extracorporeal circulation.

14. Effects in Glomerulonephritis Model:

In a rapidly progressive glomerulonephritis rat model, argatroban (30 and 60 mg/kg oral for 39 days) inhibited urinary protein excretion on 14, 25 and 40th day. It inhibited mesangial proliferation by 50%, and showed changes in kidney histopathology by 60%, suggesting preventive effects of the drug in this model.

15. The Combined Effects of Argatroban With Thromboxane Synthetase Inhibition:

The combination of thrombin inhibition (with argatroban, 3.3 and 10 μg/kg/min) and thromboxane synthetase inhibition (with ozagrel, 10, and 30 μg/kg/min) on thrombosis in a rabbit femoral artery model were examined. Both the drugs were given by iv infusion to anesthetized rabbits. Ozagrel (30 μg/kg/min) prevented arterial thrombosis, but it prolonged bleeding time (131.7 vs ~80 sec with argatroban alone). When ozagrel (10 μg/kg/min, the conc that was not effective by itself) was given with argatroban (3.3 μg/kg/min), it prolonged the time to arterial occlusion, and did not affect the bleeding time. These studies suggest that the combination of argatroban with an antithromboxane inhibition, may be important in treatment of various thrombotic diseases.

II. Comparative In Vitro (inhibitor constants for thrombin) and In Vivo (anti-thrombotic activity) Actions of 2 Isomers (21-R, and 21-S isomers) vs Argatroban:

1. In Vitro Effects:

Chirality studies of argatroban indicates that it has 4 asymmetric carbons. One of the asymmetric carbons in argatroban at 21 positions (on the quinolyl group) has an R-configuration (stereoisomer type I) and an S-configuration (stereoisomer type II). Therefore, argatroban consists of a mixture of stereoisomer types I (R-isomer) and II (S-isomer) at a ratio of ~65:35. The aim of this study was to compare the relative activity of each isomer by determining the inhibitory constant values (k_i). Argatroban or its stereo isomers (at various conc) were incubated with fixed amounts of human α thrombin and the chromogenic substrate, S2238 (at 3 concentrations), and k_i values for argatroban and its isomers were calculated. The results indicate that 21-S isomer is ~two times more potent inhibitor of thrombin than the 21-R isomer. Argatroban, which contains 65%:35% of 21-R/21-S is less potent inhibitor than 21-S isomer (k_i 50.8 vs 37.3 nM), Table 1.

Table 1. Inhibitory constants (k_i) of argatroban and its S and R-stereo isomers.

nM ± SEM	Argatroban	21-S Isomer	21-R Isomer
k_i	50.8 ± 1.9	37.3 ± 2.6	81.8 ± 4.3

Rawson et al (J. Of Pharma Sci. 82: 672, 1993) have similarly shown that in human plasma, using an in vitro coagulation assay (with exogenous bovine thrombin), 21-S isomer was ~two times more potent in prolonging the clotting time, than the 21-R isomer (90 ng/ml vs 180 ng/ml). However, solubility of 21-S is limited (0.169 mg/ml in 10 mM sodium phosphate buffer with 10% sorbitol, pH 7.4 at 22^0C), compared to 21-R (soluble at 0.785 mg/ml). These studies indicate that argatroban has an intermediate activity against human α thrombin between the more active S isomer, and the less active R isomer.

2. In Vivo Effects:

Comparisons of the Antithrombotic Activity of Argatroban and its 2 Isomers at Position 21, in a Rat Model of Arterial Thrombosis:

The aim of this study was to compare the relative antithrombotic activity of argatroban with each of the isomers, using a rat model of carotid arterial thrombosis. All 3 compounds were given by continuous iv infusions (0.05 ml/min), starting 45 min before

thrombus induction, and continued throughout the study for 110 min. Also, activated partial thromboplastin time (aPTT) were determined at the end of the study. Argatroban caused a significant increase in the mean occlusion delay at 20 (in 3 of 8 animals or by 35 min) and 40 μg/kg/min (6 of 8 animals or by 50 min). The 21-S isomer had similar potency to argatroban, at 20 and 40 μg/kg/min, there was occlusion delay of 35 and 60 min resp (or at 40 μg/kg/min, 7 of 8 animals were without occlusion). The 21-R isomer was half as potent, compared to argatroban or the 21-S isomer. The effect of 21-R isomer at 40 μg/kg/min was similar to the effect of the two latter compounds at 20 μg/kg/min (there was occlusion delay of 25 and 30 min at 20 and 40 μg/kg/min vs 8 min in saline treated animals, or at 40 μg/kg/min, only 2 of 8 animals were without occlusion). However, these differences were not clearly distinct. All 3 compounds increased aPTT to similar levels (2.4, 2, and 2.2 fold with R, S isomers and argatroban resp at 40 μg/kg/min). These studies indicate that argatroban, (which contains only 35% of the S-isomer) has similar antithrombotic activity as the 21-S isomer in this rat model. However, the aPTT was not sensitive to reflect the differences in anticoagulant potency between these compounds, or the 2 isomers.

Therefore in vitro, argatroban has an intermediate activity against human α thrombin between the more active S, and the less active R isomer, but in vivo it has similar antithrombotic activity as the 21-S isomer.

III. Secondary Pharmacology:

A. Effect on the Central Nervous System.

The effects of argatroban on various tests of CNS are summarized in Table 1 (page 24). Doses of argatroban at 3 mg/kg or below given i.v. had no effects on the CNS.

B. Effect on Respiration, Cardiovascular System, and Renal Functions.

The results are summarized in Table 2 (pages 25 and 26) Argatroban at i.v. doses of 0.3 to 3.0 mg/kg had no effects.

C. Effect on the Autonomic Nervous and Digestive Systems.

The effects of argatroban on various tests of the autonomic nervous and digestive systems are summarized on Table 3 (page 28).

D. Effect on the Immune System.

Argatroban showed no effect on delayed-type allergic reaction, lymphocyte blast formation, and complement activation.

TABLE 1

(SPONSOR's Table 7.23) Effect on the Central Nervous System

Test item	Animals used	Administration route and dosage	Results	Reference
General behavior change	Wistar rats	1-30 mg/kg, i.v.	Slight decrease in exploratory behavior at 10 mg/kg. Slight decrease in alertness and muscle tone at 30 mg/kg.	
Spontaneous motor activity	ddY mice	10-100mg/kg, i.v.	Slight suppression at 100 mg/kg	
Hexobarbital sleeping time prolongation	ddY mice	10-100mg/kg, i.v.	Slight reduction at 10 mg/kg No effect at 30-100 mg/kg	
Muscle-relaxing action (suspension test)	ddY mice	10-100mg/kg, i.v.	Minimal effect at 30 mg/kg and above	
Coordinated movement suppression (rotating rod method)	ddY mice	10-100mg/kg, i.v.	Slight suppression at 30 mg/kg and above	E-21
Maximum electroshock convulsion	ddY mice	10-100mg/kg, i.v.	No effect	
Pentylene-tetrazol convulsion	ddY mice	10-100mg/kg, i.v.	Prolongation of incidence time at 30 mg/kg	
Strychine convulsion	ddY mice	30-100mg/kg, i.v.	No effect	
Normal body temperature	Japanese white rabbits	3-30mg/kg, i.v.	No effect	
Acute spontaneous electroencephalogram	Cats	1-10mg/kg, i.v. 150 µg/kg/min, i.v.	Causing slightly slow electroencephalogram at 10 mg/kg Causing slightly slow electroencephalogram	
Electroencephalogram arousal effect	Cats	1-10mg/kg, i.v.	Slight suppression tendency at 10 mg/kg	
Acetic acid-induced writhing	ddY mice	10-100mg/kg, iv.	No effect	

TABLE 2

(SPONSOR'S Table 7.24) Effect on Respiration, Cardiovascular System and Renal Functions

Test item	Animals used	Administration route and dosage	Results	Reference
[Blood pressure/ respiration/ heart rate/ electrocardiogram]				
Blood pressure	Anesthetized dogs (P)	0.3-30mg/kg, i.v.	Decrease at 10 mg/kg or above	
	Anesthetized open chest dogs (P)	0.3-30mg/kg, i.v.	Decrease at 10 mg/kg or above	
Respiration	Anesthetized dogs (P)	0.3-30mg/kg, i.v.	Increase at 10 mg/kg or above	E-21
Heart rate	Anesthetized dogs (P)	0.3-30mg/kg, i.v.	Increase at 10 mg/kg or above	
	Anesthetized open chest dogs (P)	0.3-30mg/kg, i.v.	Decrease at 10 mg/kg or above	
Electrocardiogram	Anesthetized dogs (P)	0.3-30mg/kg, i.v.	No effect	
Effect on blood pressure response	Anesthetized dogs (P)	10mg/kg, i.v.	No effect on blood pressure response by norepinepherine, histamine, isopreterenol, acetylcholine or arterial occlusion	
[Isolated heart]				
Contractive force	Left atrium of Hartley guinea pigs	1.9×10^{-7} – 1.9×10^{-3}M	Slight decrease at 1.9×10^{-7}M or above	
	Papillary muscle specimens from dogs	10-1000 µg,	Decrease at 300 µg or above	E-21
Heart rate	Sinoatrial node specimens from dogs	10-1000 µg,	Decrease at 100 µg or above	
Anterior septal artery	Papillary muscle specimens from dogs	10-1000 µg,	Increase at 100 µg or above (transient)	