

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: 020887

PHARMACOLOGY REVIEW(S)

Review and Evaluation of Pharmacology and Toxicology Data

Division of Medical Imaging and Radiopharmaceutical Drug Products

HFD-160

Reviewer: Adebayo, A. Laniyonu, Ph.D.

Review # 2

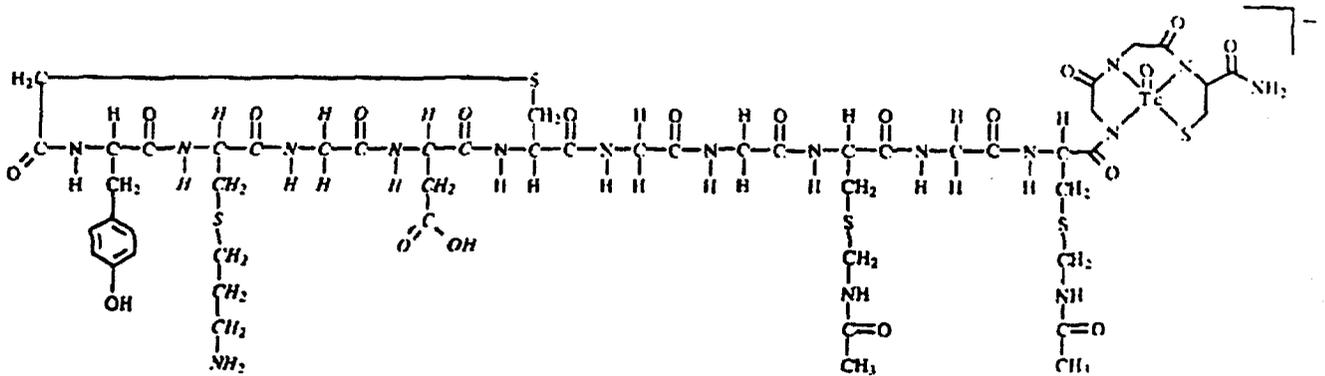
Electronic File Number:**NDA Number:** 20-887**Serial Number:****Receipt Date:** 03/16/98**Type of Submission:** Amendment to a pending application**Information to Sponsor:** Yes(x) No ()**Completion Date:** 06/12/98**Sponsor or Agent:** Diatide INC.
9 Delta Drive
Londonderry, NH 03053**Manufacture (if different) for drug substance**

The drug product as lyophilized powder in single use vial is manufactured by Dr. Rentscheler Biotechnologie GmbH D-88471 Laupheim, Germany.

Drug name: Acutect™; Kit for the preparation of Technetium Tc 99m Apcitide**Chemical Name:** Drug substance: [^{99m}Tc]Sodium hydrogen [N-(mercaptoacetyl)-D-tyrosyl-S-(3-aminopropyl)-L-cysteinylglycyl-L- α -aspartyl-L-cysteinylglycylglycyl-S-[(acetylamino)methyl]-L-cysteinylglycyl-S-[(acetylamino)methyl]-L-cysteinylglycylglycyl-L-cysteinamide, cyclic(1 \rightarrow 5-sulfidato(5-))-technetate(2-)**CAS Number:** [178959-14-3]

Structure:

(From Vol. 1:11, pp. 14)



Technetium Tc 99m Apcitide

Molecular Weight: 1525.5

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ON ORIGINAL

Relevant IND/NDA/DMF:

Drug Class: Radiopharmaceutical Imaging agent

Indication: Diagnostic radiopharmaceutical for the detection and localization of acute venous thrombosis.

Route of Administration: intravenous

Studies Reviewed within this submission:

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Studies not reviewed within this submission:

Disclaimer (Use of sponsor's material): Sponsor submitted information was utilized in the preparation of this review, and will be identified as quotes.

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Introduction/Drug History:

This submission is the sponsor's response to the comments in the agency's approvable letter of 02/20/98. The agency requested additional information, clarification of submitted data, and phase 4 commitments. The following is an evaluation of Diatide's responses to those requests impacting on nonclinical pharmacology/toxicology. The original FDA comment is included for clarity.

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**CONTAINED TRADE
SECRETS and/or
CONFIDENTIAL/
COMMERCIAL
INFORMATION**

NDA Issues:**Labeling Review:**

The sponsor requested that:

- 1) The binding site be designated GP11b/111a to conform with other product labelling (specifically ReoPro).
- 2) Deleting the statement "the peptide binds less avidly to the β_3 chain of the vitronectin receptor found on endothelial cells".
- 3) Substituting the statement "small peptides are generally not immunogenic for "small peptides are known to be immunogenic.

Reviewer's comments:

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The request that the binding site be designated GP11b/111a should be granted since GP11b/111a and $\alpha_2\beta_3$ are used interchangeably in the scientific literature.

The claim by the sponsor that "the study which was included in the original NDA supports the fact that apcitide does not inhibit the binding of vitronectin to its receptor at concentrations in excess of 1000 nM or that no inhibition was seen at levels as high as 10,000 nM is not supported by the available evidence. While my original review concurs that Diatide has demonstrated a reasonable degree of selectivity, the selectivity was not absolute and therefore the statement "the peptide binds less avidly to the β_3 chain of the vitronectin receptor found on endothelial cells" should not be deleted.

As to the immunogenic potential of apcitide, I agree with Diatide that the potential of small peptides to elicit immunogenic response is low. However, the statement concerning possible immunogenicity of apcitide was based on outcome of a preclinical animal model where there was a reduction in absolute and relative weight of spleen, and on clinical observation of hypotensive response in a patient. The statement should be modified to read

“Small peptides may be immunogenic”

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Investigator's Brochure/Informed Consent Review:

Reviewer's Signature:

/S/

Adobayo, A. Lanionu, Ph.D.

06/12/98

Date

/S/

Team Leader Concurrence:

Laraine L. Meyers, R.Ph., Ph.D.

6-12-98

Date

CC: list:

- HFD-160/Original IND
- HFD-160/Division File
- HFD-160/Lanionu/Meyers
- HFD-160/CSO/
- HFD-160/MO/
- HFD-160/CHEM/
- HFD-160/Biopharm

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Appendix:

Addendum:

1. Review and Evaluation of Pharmacology and Toxicology Data

Division of Medical Imaging and Radiopharmaceutical Drug Products

HFD-160

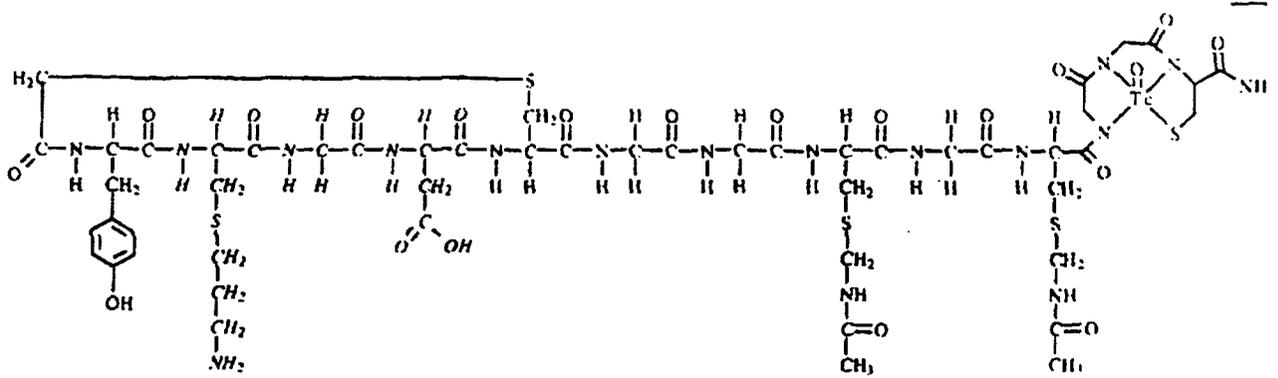
Reviewer: Adebayo, A. Lanionu, Ph.D.

Review #1

2. Electronic File Number:3. NDA Number: 20-8874. Serial Number: 000APPEARS THIS WAY
ON ORIGINALDate: 20 August 1997Type of Submission: N5. Information to Sponsor: Yes(x) No ()6. Completion Date: 12/23/977. Sponsor or Agent: Diatide, INC.
9 Delta Drive
Londonderry, NH 030538. Manufacturer (if different) for drug substance:The drug product as lyophilized powder in single use vial is manufactured by
Dr. Rentscheler Biotechnologie GmbH
D-88471 Laupheim, Germany.9. Drug name: AcuTect™; Kit for the preparation of Technetium Tc 99m Apcitide.10. Chemical Name: Drug substance: $[^{99m}\text{Tc}]$ Sodium hydrogen [N-(mercaptoacetyl)-D-tyrosyl-S-(3-aminopropyl)-L-cysteinylglycyl-L- α -aspartyl-L-cysteinylglycylglycyl-S-[(acetylamino)methyl]-L-cysteinylglycyl-S-[(acetylamino)methyl]-L-cysteinylglycylglycyl-L-cysteinamide, cyclic(1 \rightarrow 5-sulfidato(5-))-technetate(2-)11. CAS Number (if provided by sponsor): [178959-14-3]APPEARS THIS WAY
ON ORIGINAL

12. Structure

(From Vol.1.11, pp.14)

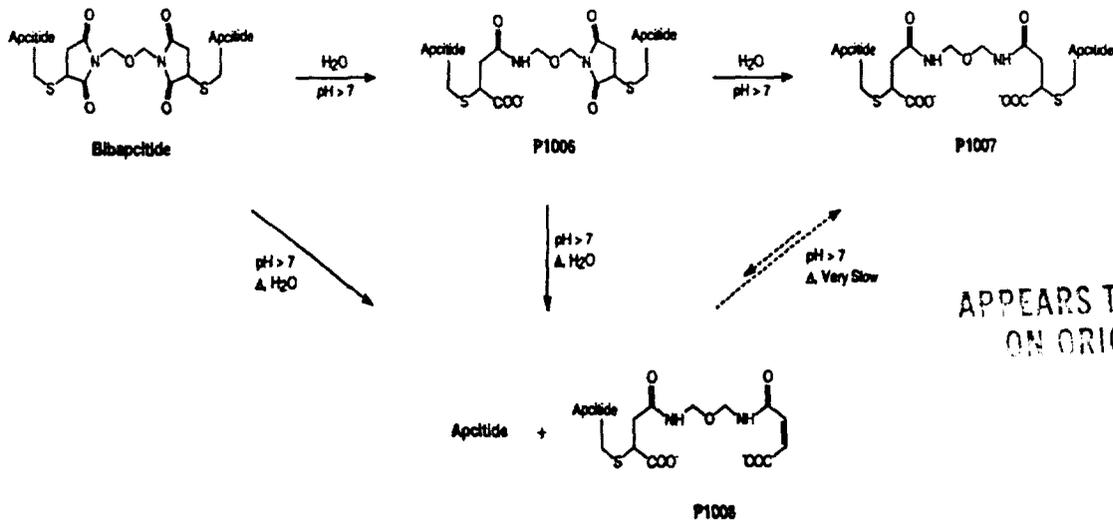


Technetium Tc 99m Apcitide

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Apcitide is a thirteen amino acid monomer with a primary amino acid sequence: (D-Tyr)-Apc-Gly-Asp-Cys-Gly-Gly-Cys(Acm)-Gly-Cys(Acm)-Gly-Gly-Cys. However, bibapcitide which is used in the formulation of apcitide is a symmetrical dimer of apcitide linked by a bis(succinimidomethyl)ether bridge through the C-terminal cysteine residues. During kit formulation and subsequent radiolabeling, the bibapcitide bridging group undergoes hydrolysis and reverse Michael addition to apcitide, and two related peptides, P1008 and P1007 in the approximate ratio of 20:15:65. The drug substance, technetium Tc 99 apcitide, is the technetium complex of apcitide. The original peptide, bibapcitide and an initial hydrolysis product, P1006, are present in the kit during the reconstitution process, but are absent in the Technetium Tc 99 apcitide injection.

(P280)



APPEARS THIS WAY ON ORIGINAL

Chemistry of Bibapcitide and Related Peptides. (From Vol. 1.11 pp.13)

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Diatide originally thought that bibapcitide (P280) was radiolabeled with technetium Tc-99m to give Tc-99m-P280. However, the reaction was instead found to produce technetium Tc-99m apcitide. Thus in _____ and _____ pharmacology and toxicology reports that refer to Tc-99m P280 or Kit for the preparation of Technetium Tc-99m P280, it should be understood to mean Technetium Tc-99m apcitide or Kit for the preparation of Technetium Tc 99m apcitide.

Reviewer's Comments: It should be noted that the Arg-Gly-Asp (RGD) sequence of the GP 11b/111a receptor is actually represented in apcitide by the sequence -Apc-Gly-Asp. The amino acid Apc, S-aminopropyl-L-cysteine acts as an arginine surrogate. Diatide claims that it replaces arginine in the receptor-binding sequence and that the mimetic sequence has been designed in a conformationally constrained cyclic sulfide structure that confers additional selectivity on the molecule. The concept of conformational constraint of the RGD sequence is similar to that seen in the RGD sequence of disintegrins (a family of small proteins mostly derived from snake venom) that confers high affinity binding. I agree with the theoretical concept. As to the nomenclature, Tc-99m P280, and Tc-99m apcitide, it is noted that the terms as used by Diatide are interchangeable. Moreover, Diatide originally applied to USAN for the generic name Sibapcitide for P280. Bibapcitide was given instead. Thus sibapcitide and bibapcitide are synonymous and taken by Diatide to refer to the peptide P280.

13 **Molecular Weight:** 1525.5 (average), 1523.3 (monoisotopic)

14 **Relevant IND/NDA/DMF:**

15 **Drug Class:** Radiopharmaceutical imaging agent

16 **Indication:** Diagnostic radiopharmaceutical for the detection and localization of acute venous thrombosis.

17 **Clinical Formulation (and components):**

Components	Quantity per vial (1mL) ¹
Bibapcitide Trifluoroacetate	100µg
Sodium α-D-Glucoheptonate Dihydrate	75 mg
Tin Chloride Dihydrate	89 µg
Hydrochloric Acid,	
Sodium Hydroxide	

The clinical formulation was used for pivotal safety and toxicological studies. The specific formulation used for the various studies will be identified during the course of this review. The earlier formulations had the following compositions. (Adapted from table 5.A.2., Vol. 1.11 pp.15)

Formulation #	Components	Quantity per vial (1ml)
<p align="center">Final commercial formulation</p>	<p>Bibapcitide Trifluoroacetate Tin (II) Chloride Dihydrate Sodium Glucoheptonate Dihydrate</p>	<p>100 µg 89µg 75mg</p>

18 Route of Administration: Intravenous

19 Proposed Clinical Dosing: Administered in a single dose of approximately 100µg of peptide radiolabeled with approximately 20 mCi of technetium-99m.

20. Studies Reviewed within this submission:

Study #, Vol #, pp.	Study Date	Study Title	Species	Formulation # Used For Study
Pharmacology Studies				
R2.79 Vol. 1.11, Pages 60-67	06/18/97	Binding of the Rhenium complex of Apcitide to Purified Integrins $\alpha_2\beta_3$ (Fibrinogen Receptor) and $\alpha_5\beta_3$ (Vitronectin Receptor)	N/A	Rhenium complex of apcitide and P280
R2.52. Vol. 1.11 pp.77-89	06/18/97	Binding of Technetium Tc99m Apcitide to Human Platelets In Vitro	Human Platelets	# 5, Lot No. 9603M01
R2.51 Vol. 1.11 pp. 90-97	06/26/97	Inhibition of Platelet Aggregation In Vitro by P280, P246, P1007 and P1008 Peptides and Technetium Tc 99m Apcitide Injection Prepared with Decayed Generator Eluate	Human Platelet	# 5 Lot No. 9603M01 Peptides P280, P1007, P246 and P1008
R2.97 Publication Vol. 1.11 pp. 98-107		Thrombus Imaging with a Technetium-99m-Labeled, Activated Platelet Receptor-Binding Peptide Journal of Nuclear Medicine 1996: 37: 775-781	Canine mongrel	# 1
Safety Pharmacology Studies				
R2.74 Vol. 1.11 pp. 108-177	06/20/97	Evaluation of the Potential effects of Technetium Tc 99m Apcitide Injection prepared with decayed Generator Eluate on Hemodynamics, Coagulation Parameters, and ex vivo Platelet Aggregation in Anesthetized Mongrel Dogs.	Canine, Mongrel	# 5 Lot No. 9703M01
R5.1 Vol.1.11 pp.178-196	06/19/97	Evaluation of the Antithrombotic Properties of Diatide Peptides P280 and P246	Canine Mongrel	# 1 P246
R2.72 Vol. 1.11, pp. 197-207	05/13/97	Evaluation of the Potential Interaction Between Technetium Tc 99m Apcitide injection Labeled with Decayed Generator Eluate and Aspirin or Heparin in Human Platelets In Vitro	Human Platelet	# 5 Lot No. 9603M01
Toxicology Studies				
R2.59, Vol.1.12 pp. 2-61	09/30/96	Single-Dose Intravenous Toxicity Study of Technetium Tc-99m Apcitide Injection prepared with Decayed generator Eluate in Mice with 48 hours and 14 Day Observation Periods.	Mouse (albino Swiss)	#5 Lot No. 9603M01
R2.73, Update 1 Vol. 1.12 pp. 62-143	01/07/97	Single-Dose Evaluation of Technetium Tc 99m Apcitide Injection With Decayed Eluate and Nonpeptide Formulation Excipients in Mice With 48 Hours and 14 Day Observation Periods	Mouse (albino Swiss)	# 5 Lot No. 9603M01
R2.76, Vol. 1.12, pp. 153-332	03/12/97	A Single-Dose Evaluation of the Acute Intravenous Toxicity of Technetium Tc 99m Apcitide Injection Prepared with Decayed Generator Eluate in Rabbits with 48-Hour and 14-day Observation Periods.	New Zealand White Rabbits	# 5 Lot No. 9603B04
R2.82 , Vol. 1.13 pp. 1-178	12/05/96	A 14 Day Repeated Dose Intravenous Toxicity Study of Technetium Tc 99m Apcitide Injection Prepared With Decayed Generator Eluate Administered To Rats.	Sprague Dawley Rats	# 5 Lot No. 9603B03
R2.83	01/19/93	A Single-Dose and 14-Day Repeated-Dose Evaluation of Intravenously Administered	Sprague Dawley	# 2 (Cont'd)

		Technetium Tc 99m Apcitide Injection (Original Formulation) Prepared with Decayed Generator Eluate in Rats	Rats	
R2.80, Update 1	12/03/96	A 14 Day Repeated Dose Intravenous Toxicity Study of Technetium Tc 99m Apcitide Injection Prepared With Decayed Generator Eluate Administered To Rabbits	New Zealand White Rabbits	# 5 Lot No. 9603B03
R2.61, Update 2	10/09/96	Evaluation of the Immunogenicity of Mock-labeled Technetium Tc 99m Apcitide Injection in Guinea Pigs	Guinea Pigs	# 5 Lot No. 9603M01
R2.60	10/02/96	Evaluation of the Potential for Producing Perivascular Irritation by Mock-Labeled Technetium Tc 99m Apcitide Injection in Rabbits	New Zealand White Rabbits	# 5 Lot No. 9603M01
R2.49	01/16/97	An Evaluation of the Compatibility of Mock-Labeled Technetium Tc 99m Apcitide Injection with Human Blood or Serum	Human Blood	# 5 Lot No. 9603M01
R2.63	05/27/97	Safety of Tin Chloride and Sodium Glucoheptonate - Non-Peptide Components of Technetium Tc 99m Apcitide Injection	Not Applicable	Not Applicable
R2.84	03/06/96	Evaluation of Mutagenicity of Technetium Tc 99m Apcitide Injection Prepared With Decayed Generator Eluate in the Salmonella/E. coli Mammalian Microsome Reverse mutation (Ames) Assay	Four Salmonella strains and one E. coli strain.	# 5 Lot No. 9603M01
R2.85	02/27/96	Mutagenicity Test of Technetium Tc 99m Apcitide Injection prepared With Decayed Generator Eluate in the L5178 TK± Mouse Lymphoma Forward Mutation Assay	Mouse lymphoma cell line with TK± locus	# 5 Lot No. 603M01
R2.86	03/27/96	Evaluation of the Clastogenicity of Technetium Tc 99m apcitide injection prepared With Decayed Generator Eluate in the Mouse Micronucleus Assay	Mouse	# 5 Lot No. 9603M01
R2.50	06/19/97	Distribution of Technetium Tc 99m Apcitide Between Human Blood Components In Vitro	Human blood samples	# 5 Lot No. 603M01
		Pharmacokinetics Studies		
R2.67	07/23/97	Pharmacokinetics and Biodistribution of Technetium Tc 99m Apcitide in Male and Female Rats	Sprague Dawley Rats	# 5 Lots No. 9603M01 and 9603B03
R2.53	05/20/97	An Evaluation of the Metabolism of Technetium Tc 99m Apcitide in the Sprague Dawley Rat	Sprague Dawley Rat	# 5 Lot No. 9603M01
R2.75	06/24/97	Distribution, Metabolism and Excretion of Technetium Tc 99m apcitide in Rats with Experimental Renal dysfunction	Sprague Dawley Rat	# 5 Lot No. 9603M01
R2.77	10/10/96	Distribution, Metabolism, and Excretion of [³ H]-Labeled Peptide Components of Technetium Tc 99m Apcitide Injection in Male Sprague Dawley Rat	Sprague Dawley Rat	[³ H]-labeled peptide
R2.96	05/01/97	Comparative metabolism of [³ H]-labeled Peptide Components of Technetium Tc 99m Apcitide Injection in Rat, Rabbit, and Human Liver and Kidney Slices In Vitro	Tissue Slices	³ H]-labeled peptides (Cont'd)

21. Studies not reviewed within this submission:

Study #, Vol #, pp.	Study Date	Study Title And Reasons For Not Reviewing	Species	Formulation # Used for Study
R2.78 Vol. 1.12 pp. 144-152	11/20/92	Single-Dose Evaluation of Intravenously Administered, Nonformulated Preparation of Technetium Tc 99m Apcitide Prepared With Decayed Generator Eluate in Mice with a 72 hour Observation Period. Content did not provide additional information beyond R2.59)	Mice (Swiss albino)	# 1
R2.99 Vol. 1.12, pp. 333-351	03/11/93	Modified USP Systemic Injection Test for Extraneous Toxic Contaminant in Nonformulated Preparation of Technetium TC99m Apcitide Injection Prepared With Decayed Generator Eluate. Non formulated preparation used	New Zealand White Rabbits	# 1
R2.87	09/28/92	Four-Day Repeated-Dose Evaluation of Intravenously Administered, Nonformulated Preparation Of Technetium Tc99m Apcitide Prepared with Decayed Generator Eluate in Rats. Studies R2.82 and R2.83 provided required information.	Sprague Dawley Rats	# 1
R2. 81	01/05/93	A Single-Dose And 14-Day Repeated Dose Evaluation of Intravenously Administered Technetium Tc 99m Apcitide Injection Prepared With Decayed Generator Eluate (Original Formulation In Rabbits (Similar in content and structure to other studies reviewed R2.80).	New Zealand White Rabbits	# 2
R2.88	10/13/92	Seven-Day Repeated-Dose Evaluation of Intravenously Administered, Nonformulated Preparation of Technetium Tc 99m Apcitide Prepared With Decayed Generator Eluate In Rabbits. (Nonformulated sibapcitide used for the study. Will not provide additional information to study R2.80	New Zealand White Rabbit	#1
R2.48	09/13/95	Mutagenicity Test of peptide Components in Reconstituted Kit for the Preparation of Technetium Tc 99m Apcitide in the Salmonella-E.coli/Mammalian microsome Reverse Mutation (Ames) Assay. Non formulated peptide with no kit excipient used for the study. It is noted that the study concluded that the peptide components are non-mutagenic. Study does not provide additional information to R2.84.		#1
R2.98	06/30/97	Validation of the Gel Filtration Chromatography and Reversed-Phase high- Performance Liquid chromatography Methods for Analysis of metabolism of Technetium Tc 99m Apcitide		Not applicable
R2.97	07/23/97	Biodistribution of Technetium Tc 99m Apcitide in The New Zealand White Rabbit	New Zealand Rabbits	# 1

22. **Disclaimer (Use of sponsor's material):** Sponsor submitted text was utilized in the preparation of this review. It will be identified as quotes.

23. **Introduction/Drug History:**

In the U.S., approximately 5 million patients experience one or more episodes of Deep Venous Thrombosis (DVT) per year with associated morbidity and mortality. Thus the clinical importance of developing a noninvasive, rapid and specific method of thrombus detection in early identification and treatment of intravascular thrombosis cannot be overemphasized. Duplex ultrasonography (DU) and contrast venogram are commonly used to detect DVT. However, there are a number of problems associated with clinical diagnosis. These include sub-optimal diagnostic accuracy (false negative and false positive results); diagnostic procedure-induced high incidence, and unacceptable level of patient discomfort and pain; inability of test procedures to distinguish between acute or active thrombosis from the residual of previous episodes of DVT, with attending clinical implications for pulmonary events. Moreover, diagnostic procedures such as duplex ultrasonography show anatomical variation in sensitivity and specificity. For example, DU is reported to have poor sensitivity in asymptomatic high risk patients, poor accuracy below the knee while maintaining excellent sensitivity and specificity in the thigh. Furthermore, the high incidence of side effects from thrombolytic therapy highlights the need for accurate diagnosis so as to prevent unnecessary therapy. The diagnostic drawbacks have led to the development of several thrombus-specific radionuclide-based probes. These include radiolabeled plasma protein, platelets and monoclonal antibodies. Diatide, INC. believes that an imaging agent that would bind to platelets being incorporated into an active thrombus, but which, if not bound to a thrombus, would clear rapidly from the blood, leading to improvement in target-to-background ratios would have great potential as an imaging agent for acute venous thrombosis. This led to the development of Technetium Tc 99m Apcitide which is the subject of this review.

The pharmacology of apcitide is linked with the mechanism whereby fibrinogen combines with the glycoprotein receptors present on platelets. The glycoprotein 11b/111a (GP11b/111a) receptor is a member of the integrins family of receptors. Upon activation, these receptors bind a number of naturally occurring proteins including fibrinogen. It is generally accepted that platelets become activated before GP11b/111a receptor undergoes the required conformational changes needed for binding fibrinogen. The pharmacophore of apcitide is a mimetic of the tripeptide argininyglycyl-aspartic acid (RGD in single-letter amino acid nomenclature) sequence present in fibrinogen which mediates the binding of fibrinogen to GP11b/111a receptor. Since platelet activation is required for thrombus formation, Diatide reasoned that a radiolabeled GP11b/111a-binding peptide could be used to detect and localize acute thrombi, in particular, deep venous thrombi.

Reviewer's Comments: From a pharmacological perspective, the fundamental claim of this submission, that apcitide offers potential utility in the detection of acute venous thrombosis hinges on the ability of apcitide to differentially bind to, and localize with activated platelets compared with non activated platelets presumably through the RGD sequence of GP11b/111a receptor. The theoretical concept appears reasonable, and the scientific rationale is appropriate for the disease. I will attempt to determine whether basic fundamental information connected with such a claim has been provided. This will be determined by seeking answers to the following questions.

However, it should be noted that based on the proposed mechanism of action, there are other pharmacological issues that are not easily addressed in a non clinical setting. These may affect the diagnostic utility of Technetium Tc 99m Apcitide. (1) Due to the fact that thrombus formation involves a cascade of events with platelet activation as one of the earlier steps, the time frame of the onset of acute or "active" thrombus formation and availability of the patient for diagnostic imaging becomes critical, the narrower the window of time, the higher the probability of accurate diagnosis. (2) Ability of apcitide to distinguish between acute thrombus formation, and trauma and inflammation conditions in which platelet activation is an integral component.

24. Previous clinical experience: None

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25. Pharmacology Studies:

Study # R2.79: Binding of Rhenium Complex of Apcitide to Purified Integrins $\alpha_2\beta_3$ (Fibrinogen Receptor) and $\alpha_5\beta_3$ Vitronectin Receptor.

The study is located in Volume 1.11 pages 60-75. This study was not in compliance with GLP.

This study was designed to evaluate the specificity and affinity of the Rhenium complex of apcitide, (Re-apcitide) for purified human GP11b/111a receptor (also designated $\alpha_2\beta_3$, fibrinogen receptor). The study compared Re-apcitide ability to inhibit the binding of fibrinogen to purified $\alpha_2\beta_3$ with its ability to inhibit the binding of vitronectin to $\alpha_5\beta_3$ receptor. Both $\alpha_2\beta_3$ and $\alpha_5\beta_3$ receptors belong to the heterodimeric family of integrins receptor with identical β_3 strand.

Fibrinogen (200ng/well) or vitronectin (300ng/well) receptors purified from Chinese hamster ovary cell lines transfected with human $\alpha_2\beta_3$ or $\alpha_5\beta_3$ were plated overnight in 0.05M carbonate-bicarbonate-buffer at pH 9.2. The wells were blocked with 5% nonfat milk in phosphate buffered saline containing 0.05% tween 20 (PSBT) for 60 minutes at 37°C, and washed with PBST. Re-apcitide (the non-radioactive rhenium complex of apcitide used as a surrogate for technetium Tc 99m apcitide), or sibapcitide (bibapcitide) or eristostatin (a snake venom-derived disintegrin known to bind to both $\alpha_2\beta_3$ and $\alpha_5\beta_3$ receptors) were added to each well at 0.01 to 4000 nM. The wells also contained either fibrinogen or vitronectin at 1.0 μ g. Following incubation and washing, polyclonal anti-fibrinogen or anti-vitronectin antibody was added for 60 minutes, thereafter, the wells were washed and secondary goat anti-rabbit antibody conjugated with alkaline phosphatase added. The plates were incubated for 60 minutes at 37°C and washed with PBST. Color development was followed at room temperature by the addition of nitrophenyl phosphate. The plates were read using plate reader at 405 nm.

Results: Both Re-apcitide and sibapcitide inhibited the binding of fibrinogen to purified human $\alpha_2\beta_3$ over a concentration range . The IC₅₀ for re-apcitide was 1.8nM. The binding of vitronectin to purified $\alpha_5\beta_3$ receptor was not inhibited by sibapcitide or by re-apcitide. Eristostatin inhibited the binding of both fibrinogen and vitronectin with their receptors.

Conclusion: The sponsor concluded that re-apcitide binds directly to the $\alpha_2\beta_3$ with high affinity and specificity.

Reviewer's comments: I agree with the conclusion that apcitide binds directly to the $\alpha_2\beta_3$ receptor. The nanomolar concentration required for inhibition is indicative of high affinity binding. The sponsor stated that "inhibition occurred over a concentration range of 0.5 nM to 80 nM, and that the inhibition of fibrinogen appeared maximal at approximately 80 nM since higher concentrations did not inhibit fibrinogen binding further". However, it was apparent from the graph that the % inhibition at 80nM ~ 50% was not different from that at 1.8nM clearly suggesting that either re-apcitide produced only a partial inhibition of fibrinogen binding and as believed by me, the concentration of fibrinogen and vitronectin (1.0 μ g) chosen for this experiment was far in excess of optimal concentration needed to demonstrate competitive interaction between fibrinogen and the peptides. The sponsor neither described how they arrived at the choice of fibrinogen and vitronectin receptor concentrations, nor the rationale for the choice of fibrinogen and vitronectin concentrations. These two factors may partially explain the incomplete inhibition of fibrinogen receptor observed with re-apcitide and sibapcitide. The less than 20% inhibition of vitronectin binding to $\alpha_5\beta_3$ demonstrated that re-apcitide has a greater specificity for $\alpha_2\beta_3$ than for $\alpha_5\beta_3$ receptor. It must be emphasized that what we are really describing is a *relative difference* in apparent maximal effect on these two receptors ~ 2.5 fold (at maximal inhibition). However, I *recognized* that the ~ 50 % inhibition of fibrinogen binding to its receptor by Re-apcitide was achieved at a concentration of 1.8 nM while the ~ 20% inhibition of vitronectin binding was achieved at a concentration of \geq 100nM. *This reviewer therefore concurs that Diatide has demonstrated a reasonable degree of selectivity.* Nevertheless, the possibility exists that in clinical diagnostic imaging, apcitide may also image the vitronectin receptor present on the endothelium. Minor comments on the technical details of this experiment included the fact that it was not clear from the report whether the study was carried out only once. Neither was the number of replicate samples given. These are crucial points for a biochemically based technique. It is noted that the study utilized a rhenium complex of apcitide as a nonradioactive surrogate for technetium Tc 99m apcitide instead of a decayed

generator eluate. However, I do not think that this change led to a significant difference in outcome. How variation of the amino acid sequence of RGD pharmacophore affected binding was not examined in this study. The reason for this omission may be connected with the fact that such studies have been published in high impact peer review journals by others. Plow et. al. Proc. Natl Acad. Sci. USA Volume 82, pages 8057-8061, 1985 and Parise et. al. Journal of Biological Chemistry Volume 262 pages 12597-12602 described a pharmacologically stringent and elegant approach to the structure-activity study of the RGD sequence. I will provide a synopsis of pertinent findings in my overall summary.

Study # R2.52: Binding of Technetium Tc 99m Apcitide to Human Platelets In Vitro. Study was performed by Diatide Inc., 9 Delta Drive, Londonderry, New Hampshire, USA. The study is located in Volume 1.11 pages 77-89. This study was not in compliance with GLP.

This study was designed to quantify the binding of Technetium Tc 99m apcitide to resting and activated human platelets in vitro.

Fibrinogen free "washed" human platelets or whole human blood from which platelet rich plasma (PRP) and platelet poor plasma (PPP) were obtained and used for these experiments. In the first series of experiments, PRP was prepared from citrated human blood by differential centrifugation at

The PRP obtained was centrifuged at for 15 minutes to obtain platelet pellets and PPP which was discarded. The pellets were sequentially washed and resuspended in modified tyrode buffer containing PGE₁ (to prevent platelet activation). PGE₁ was omitted from the final resuspension step to allow subsequent activation by ADP. Viability of washed platelets was assessed by examining aggregatory response to ADP (10µM) and comparing the response to that obtained with unwashed PRP.

Direct binding of Tc 99m -apcitide to washed platelets was assessed by the addition of Tc 99m apcitide injection in the absence, or presence of 40 µM ADP. There were four groups differentiated as follows:

- A Resting or non activated platelets (- ADP)
- B Non specific binding (-ADP, +excess unlabeled P280)
- C Activated platelets (+ADP)
- D Non specific (+ADP, + excess unlabeled 280)

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Table1(from Vol.1.11 pp. 84)

	Purpose	Ca ⁺⁺ /Mg ⁺⁺ 500 mM 1:1	Modified Tyrode	ADP 200µM	P280 (bibapcitide) 1 mg/mL	100nM Tc 99m Apcitide	Washed Platelet
A	Non activated platelet	5µL	70µL	0	0	25µL	125µL
B	Nonspecific	5µL	45µL	0	25µL	25µL	125µL
C	Activated Platelet	5µL	45µL	50µL	0	25µL	125µL
D	Nonspecific	5µL	20µL	50µL	25µL	25µL	125µL

Tc 99m apcitide bound to the platelet was separated from free Tc 99m apcitide by centrifugation, and counted in a well type gamma counter.

In another series of experiments, binding of Tc 99m apcitide to platelets was also assessed in the presence or absence of ADP in whole human blood from which PRP and PPP were obtained by slow and high speed centrifugation. In both studies, the concentration of Tc 99m apcitide was selected that was equivalent to a maximum human dose of 100 µg of total peptide distributed in 3 liters of plasma.

Results: Washed platelets showed aggregatory response to 10 µM ADP, and were thus deemed viable.

77% of the Tc-99m apcitide (100nM) bound to activated platelets was blocked by the addition of 100 µM P280

The addition of ADP (40 µM) significantly increased the specific binding of Tc99m apcitide to platelets from

Table 2 (From Vol.1.11 pp. 87)

(A) -ADP %total	(C) +ADP, 40 µM % total	+ADP/-ADP ratio
5.0%	10%	2.0
5.4%	13.4%	2.5
2.6%	7.0%	2.7
4.3 ± 0.87% n=3	10.1 ± 1.84% * n=3	2.4

Specific Binding of Technetium Tc99m Apcitide to washed Human Platelets

* significantly different (p<0.05).

Binding of Tc 99m apcitide to PRP isolated from whole blood was 6.7±0.5% and 21.0±3.2% in non activated and ADP-activated platelets respectively.

Conclusion: Technetium Tc 99m apcitide binds specifically to washed human platelets. 4% of applied radioactivity was bound to resting platelets. Activation of platelets by the addition of ADP increased the platelets binding 2.4 fold. In PRP from citrated whole human blood, 7% and 21% of original radioactivity appeared in the platelets pellet in the absence or presence of ADP respectively.

Reviewer's Comments: The study as described by the sponsor failed to provide sufficient details necessary to assess the adequacy of a biochemical study. No indication was provided as to the number of replicates for experimental protocol described in table 1. This information is crucial since (a) specific binding of technetium was low, averaging 4.3% in -ADP and 10.1% in +ADP groups. (b) results of only 3 experiments were provided. The number of experiments performed in binding studies using PPP and PRP from whole blood was not given. It was not clear as written whether experiments 1, 2 and 3 as listed in table 2 were from the blood of single or multiple volunteers. Although a minor omission, the standard deviation for values in table 2 were not provided.

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Despite these deficiencies, and my concern about the low level of specific binding to platelets, the trend of these results is that Technetium Tc 99m apcptide showed 2-3 fold increase in binding in activated washed platelets or PRP compared with non activated platelets or PPP. This result is indicative of the fact that the specificity of Technetium Tc 99m apcptide binding to activated platelets is not absolute, and that Technetium Tc 99m apcptide can bind to unstimulated platelets either via the same or other receptors or by non-specific adsorption. .

Study # R2.51: Inhibition of Platelets Aggregation In Vitro by P280, P246, P1007, P1008 Peptides and Technetium Tc 99m Apcptide Injection Prepared with Decayed Generator Eluate. Study was performed by Diatide Inc., 9 Delta Drive, Londonderry, New Hampshire, USA. The study is located in volume 1.11 pages 90-97. The study utilized kit for the preparation of Technetium Tc 99m apcptide, Lot No. 9603M01, Peptides P246: Lot Nos. ZM649, ERC-VI-167, P280: Lot Nos. FP2809402BR, FP2809301B, P1007: Lot No. P100796256, P1008: Lot No. P10089217 and Decayed Sodium Pertechnetate Tc 99m injection, Lot No. 9403M03. The study is located in Volume 1.11 pages 90-97. This study was not in compliance with GLP.

The purpose of the study was to determine the inhibition of human platelet aggregation by major components of Technetium Tc 99m Apcptide injection.

Agents that inhibit the binding of fibrinogen to the GP11b/111a receptor will inhibit platelet aggregation induced by global stimulants such as ADP. Thus in vitro inhibition of platelet aggregation by such agents can be utilized as a measure of affinity for the RGD sequence.

The reconstituted kit for the preparation of Technetium Tc 99m apcptide comprised mainly of peptides 280 (bibapcptide), P1006 and P1007 (see page 2 of this review). Following heating to yield Technetium Tc 99m apcptide injection, the peptide is present mostly as P1007, P1008 and P246 (apcptide). Typical amounts of each peptide in the injectate are shown in the table below (Vol.1.11 pp. 93).

P246	P1008	P1007	P1006	P280
21%	15%	64%	n.d. ¹	n.d. ¹

¹not determined

For the determination of inhibition of platelet aggregation (IPA) by these peptides, citrated blood obtained from adult volunteers was centrifuged at low speed to produce platelet rich plasma (PRP). Following the aspiration of PRP, the remaining blood was centrifuged at high speed to obtain platelet poor plasma (PPP) which was used to calibrate the aggregometer. Peptides were allowed to equilibrate with PRP for at least one minute before adding ADP. The maximum extent of aggregation by or within 3 minutes after adding ADP was measured and recorded. Percent inhibition of aggregation was calculated as follows:

$$100 \times \frac{[\% \text{ aggregation in presence of vehicle} - \% \text{ aggregation in the presence of peptide}]}{[\% \text{ aggregation in presence of vehicle}]}$$

Results:

Test Article	(N)	IC ₅₀ Range	Mean ± S.D.®
P280	15		63±21
P246	9		382±108
P1007	4		52±2
P1008	3		689±143
Tc Apcitide Injection	4		158±25#

(N) = number of times the assay was performed on each component

S.D. = standard deviation

® all IC₅₀s are based upon actual peptide content (e.g., the factor for trifluoroacetate salt taken into account)

#based upon 100 µg P280) peptide. Table was from Vol.1.11 pp.96

Conclusion: All the peptides inhibited ADP-induced platelet aggregation with a potency rank order P1007 = P280 > Tc apcitide injection > P246 > P1008. Diatide attributed the high potency of P1007 and P280 to the presence of intact peptide dimer .

Reviewer's Comments: As expected from the proposed mechanisms of action, these peptides inhibited platelet aggregation elicited by a global stimulant, ADP. The results demonstrated that the peptides share the pharmacophore that is capable of binding to fibrinogen receptor on platelets. According to the sponsor, apcitide (P246) is the only peptide in the injection solution that undergoes radiolabeling with Technetium Tc 99m, thus raising the possibility that unlabeled components of the formulation, P1007 and P1008 can compete with labeled apcitide for binding sites on platelets. The question then arises as to whether the levels of peptides in the formulation are high enough to inhibit the binding of Tc 99m apcitide, given the fact that for 100µg of Technetium Tc apcitide injection, apcitide, P1007 and P1008 are present in a ratio of 20: 65: 15. This question was not addressed by the sponsor in the study. However the unwanted effect of such an inhibition is a reduction in the quality of diagnostic images produced in clinical setting. Perhaps of greater concern is in vivo inhibition of platelet aggregation by the peptides. The clinical implications of such inhibition is obvious, especially in the population of patients that may require diagnostic use of acutect. This concern was addressed by the sponsor in the integrated summary section of the NDA (Vol. 1.11 pp. 32) " It is important to note that the concentrations at which platelet aggregation is inhibited by 50% are substantially higher than the maximum systemic plasma concentrations of any peptide component that could occur, assuming complete and uniform distribution in plasma immediately after injection. Using P1007 as an example, immediately following injection of Technetium Tc 99m apcitide injection (at the MHD) into a 50 kg patient, it can be calculated that the maximum systemic plasma concentration would be 7nM. Thus the systemic plasma concentration of the most abundant and potent antiaggregatory component would not exceed one seventh the IC₅₀ that was observed in vitro. Furthermore, the rapid elimination of the tritium-labeled peptide components of Technetium Tc 99m apcitide injection from the blood mitigates against actually attaining, and certainly not maintaining, the calculated maximal systemic plasma concentration". I agree with the sponsor. However, I am not sure of the potential of exposing the same patient to repeated diagnostic imaging within a short period of time. In such a scenario, the platelet inhibiting action is a potentially serious unwanted effect that must be given considerable attention.

It is noted that the standard deviation of IC₅₀ value for P1007 was small compared with others.

R2.97: Publication: Thrombus Imaging with a Technetium-99m-Labeled, Activated Platelet Receptor Binding Peptide. Journal of Nuclear Medicine 1996; 37: 775-781. The study that resulted in the publication was done by L.C. Knight, Ph.D. Temple University, Philadelphia, PA. The study is located in Volume 1.11 pages 98-107. This study was not in compliance with GLP. The study utilized non formulated technetium Tc 99m apcitide.

The study that resulted in this publication had many objectives. The only one that will be reviewed here is the ability of ^{99m}Tc-P280 to detect thrombi in vivo using a canine venous thrombosis model. Other subjects such as inhibition of ADP-induced platelet aggregation, and inhibition of the binding of fibrinogen or vitronectin to their receptors by P280 have been reviewed already (R2.51 & R2.79). The biodistribution studies will be reviewed in appropriate sections later on in the course of this review.

Mongrel dogs fasted overnight were used for this study. Following intravenous anesthesia with sodium pentobarbital, an 8mm dacron-entwined stainless steel embolization coil was placed in the right femoral vein at approximately the mid femur. On the following day, each animal was reanesthetized, i.v. saline drips were placed in each foreleg, and a urinary catheter was inserted. The animals were placed supine under a gamma camera set to acquire the 140 keV photopeak of technetium Tc-99m. In six dogs, ^{99m}Tc-P280 (185-370 MBq ^{99m}Tc and 0.2-0.4 mg peptide) was injected intravenously as a bolus into a foreleg line and flushed with saline. Positive control (3 dogs), and negative (2 dogs), received ^{99m}Tc-HMPAO-labeled autologous platelets (260 MBq) or ^{99m}Tc-glucoheptonate (290 MBq) respectively. Gamma imaging was started simultaneously with injection and continued at intervals throughout the experimental period. From the computer-stored images, average count per pixel were computed from region-of-interest (ROI) drawn manually around the areas of; the known thrombus (corresponding to the location of the coil in the radiograph), or over the contralateral femoral vein (blood background) or over both medial and lateral thigh (muscle background). Following image collection, the thrombus, saline-washed vessel samples and known fractions of injected doses were counted in a gamma well counter in the ^{99m}Tc channel.

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Results:

% injected dose per gram in selected tissue and ROI data 4hours in the canine venous thrombosis model (mean ±s.e. from table 1, Vol. 1.11 pp.101)

Compound	%ID/g Thrombus	%ID/g Blood	Thrombus/Blood	Thrombus/Muscle	Thrombus/vessel (ROI)	Thrombus/Muscle ROI
^{99m} Tc-P280	0.0059±0.0025	0.0012±0.0003	4.4±0.74	11.0±7.0	2.0±0.1	1.9±0.1
^{99m} Tc-glucohepto	0.0026±0.0002	0.0015±0.0007	2.2±0.8	4.3±2.4	1.5±0.0	1.6±0.1
^{99m} Tc-Platelets	0.18±0.08	0.037±0.006	5.4±3.2	230±100	1.8±0.6	5.0±2.2

As interpreted by Diatide INC., and quoted directly from the published paper, "An example of images acquired by with ^{99m}Tc-P280 are shown in figure 3 (photocopies of the images did not reproduce very well and therefore are not included in this review). The images were asymmetrical (more activity in the thrombosed vessel) as early as 13 minutes postinjection, with improving thrombus definition over the course of the study. As expected, ^{99m}Tc-glucoheptonate did not

image the thrombus. ^{99m}Tc-HMPAO-platelets gave excellent images of the venous thrombi". (^{99m}Tc-HMPAO; Technetium-99m -hexamethylpropyleneamineoxime)

Conclusion: ^{99m}Tc-P280 provided in vivo visualization of thrombi and good thrombus-to-blood and thrombus-to-muscle ratio in a canine venous thrombosis model.

Reviewer's Comments: Agree with the conclusion about in vivo visualization. It is also noted that ^{99m}Tc-HM-PAO-platelets provided a better visualization of the thrombi compared with ^{99m}Tc-P280. However Diatide Inc. claimed that "radiolabeled platelets have slow pharmacokinetics which distracts from their utility as clinical imaging agent". The discussion section of the manuscript also referred to a review article by Linda C. Knight, Ph.D. (*Seminars in Nuclear Medicine Vol. XX, Jan. 1990*). Dr. Knight was of the opinion that successful imaging of thrombi with platelets usually requires a delay of 24-72 hours. If thrombus images could be acquired with labeled apcitude in 4 hours, this is a definite advantage. Since labeled apcitude is rapidly cleared from the body (see pharmacokinetics review), this also constitutes another advantage when compared with the claimed slow pharmacokinetics of HMPAO-platelets.

26. Safety Pharmacology Studies

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Study # R2.74: Evaluation of the Potential effects of Technetium Tc 99m Apcitude Injection Labeled With Decayed Generator Eluate on hemodynamics, Ex Vivo Platelet Aggregation, and Coagulation Parameters in Anesthetized Mongrel Dogs.

The study is located in volume 1.11 pages 108-177 This study utilized kits for the preparation of technetium Tc 99m Apcitude (Lot No 9703M01 control No 970035), Decayed sodium Pertechnetate Tc99m injection (item code 10032 control #94049) This study was not in compliance with GLP.

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The study examined the effects of Technetium Tc 99m Apcitude injection at doses representing 1X, 30X, and 100X maximal human dose (MHD) (mass/dose) on cardiovascular responses in anesthetized Mongrel Dogs.

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Male Mongrel dogs were surgically prepared for artificial ventilation and instrumented following an overnight fast. Catheters were placed in both femoral veins (for test agents administration and blood sampling), and abdominal aorta via the right femoral artery (BP and HR). A

pressure transducer was placed into the lumen of the left ventricles (LVEDP, LV+dP/dt_{max} and -dP/dt_{max}). A Swan-Ganz catheter was introduced into the pulmonary artery for the determination of the cardiac output. There were four groups of at least 3 dogs each. *It is noted that group 2 was added near the completion of the main study.*

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Group 1: Vehicle, decayed generator eluate.

Group 2: 1X MHD (based on 2µg total peptide/kg) of technetium Tc 99m Apcitude Injection.

Group 3: 30X MHD.

Group 4: 100X MHD.

Following an equilibration period, basal parameters were obtained, thereafter, vehicle or test compound was administered intravenously and the lines were flushed with sterile saline.

Hemodynamic parameters were measured at intervals over the next three hours. Venous blood samples were collected before, and at intervals after the administration of vehicle or test article for the measurement of platelet aggregation, PT and aPTT.

Results: *The contractor stated that the results were not subjected to statistical analysis.* The sponsor reported that there were no changes in mean arterial pressure and no discernible trend was seen with LV systolic pressure changes. $LV=dP/Dt_{max}$ (an index of left ventricular contractility) was said to either remain unchanged or increase slightly from the pre-injection base line values in all three test groups. This index was said to decline with time in vehicle-treated groups. A 25% and 30% reduction in cardiac output was reported in the 100X MHD group at 2 and 3 hours post-injection. The sponsor questioned whether the reduction was test article related since the mean value of CO was reduced by 25% at 3 hours post-injection in vehicle-treated group. Both PT and aPTT were said to be unaffected. Platelet count was not affected at 1X and 30X, a 21% decline in count was observed in dogs in the 100X MHD group. This decline was not considered by the contractor to be noteworthy. The basis of this conclusion was not stated. There was no effect on platelet aggregation in control dogs or dogs receiving 1X MHD. Aggregatory responses to ADP declined in the group that received 30X and 100X MHD of Tc apcitide injection by 43% and 98% respectively. The aggregatory response was said to regain over half of the deficits observed immediately post-injection within one hour in both groups.

Conclusion: The sponsor stated that no effect on hemodynamic, coagulation or platelet count may be expected by doses of technetium Tc Apcitide injection as high as 100X MHD, the NOEL for these parameters. Tc 99m apcitide did not affect ex vivo platelet aggregation while at 100X MHD, ADP-induced ex vivo platelet aggregation was inhibited by 98% relative to baseline values. This inhibition waned with time.

Reviewer's comments: This obviously pivotal study was not subject to statistical analysis. However data from other studies tended to support the results on coagulation parameters and platelet aggregation. The results on the left ventricular contractility will have to be examined in greater detail, and subjected to statistical analysis in the light of the fact that discussion with the medical reviewer revealed that in one of the clinical trials, two of the patients showed a significant increase in systolic BP. In one of these patients, the increase in BP continued for 24 hours. I disagree with the conclusion that NOEL was 100X MHD for all parameters. The NOELs are

Ex vivo Inhibition of Platelet Aggregation	1X	APPEARS THIS WAY ON ORIGINAL
Platelet Count	100X	
PT and aPTT	100X	
Hemodynamic Parameters	30X	

Ex vivo inhibition of platelet aggregation appears to be the most sensitive parameter studied. It would have been helpful to know the maximum dose between 1X and 30X at which no effect occurs (NOEL).

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Study # R5.1 Update 2: Evaluation of the Antithrombotic Properties of Diatide Peptide(s) P280 and P246.

The study is located in volume 1.11 pages 178-196. The study was not in compliance with GLP.

The study was designed to test the hypothesis that peptides P280 and P246 exert antithrombotic effects. The design of the study also afforded the opportunity to assess hemodynamic and hematological safety profile of the peptides.

Two models of arterial thrombosis were used. The first model, Folt's coronary thrombosis model involving severe stenosis and deendothelialization of a major coronary artery in open-chest dogs. The second is a model of bilateral electrolytically-induced injury in the carotid artery of closed-chest dogs. The Folt's model is platelet-dependent and fibrin-independent. It is characterized by the cyclic flow reduction (CFR) produced by alternating gradual vessel occlusion by a platelet rich thrombus and abrupt restoration of flow caused by dislodgment of the thrombus. CFRs are completely abolished by agents with antiaggregatory action. Male mongrel dogs were surgically prepared for artificial ventilation, and instrumented to measure arterial blood pressure (ABP) from the carotid and fluid and drug delivery via jugular and femoral vein. The heart was exposed via a left thoracotomy followed by isolation of the left anterior descending (LAD) or left circumflex artery (LCX), and placement of a pulsed Doppler flow probe on the coronary artery proximal to the intended site of stenosis. Control ABP, pulsatile and mean Doppler flow, and limb lead 11 ECG were recorded on a Grass physiological recorder. Thereafter, the vessel inner surface is denuded and a rigid plastic constrictor placed on the artery. This maneuver led to the development of an occlusive thrombus over the following 3-7 minutes. After 1 hour of sustained CFRs, either P280 or P246 was infused i.v. at ascending doses at infusion rate varying from 0.1 to 2ml/min.

The second model, carotid artery model of electrically-induced thrombosis, was conducted in anesthetized closed chest dogs that underwent electrolytic injury of the intimal surface of the carotid artery. The carotid had a flow probe in place. The current was maintained for 3 hours and the time to arterial occlusion was noted as the time required for Doppler flow signal to reach zero and remain there. Control animals received either vehicle or nothing during electrical stimulation of both carotid arteries. For studies involving the peptides, vehicle was tested during stimulation of the first artery and the peptides were evaluated with the second. Additional tests including template bleeding time, ex vivo platelet aggregation, in vitro platelet aggregation, blood cell profile and coagulation parameters (prothrombin and partial thromboplastin times, PT and aPT) were also conducted.

Results: Folt's model: Using a scoring system attributed to Aikens that assigned a score graded from no effect to abolition of CFRs, both P280 and P246 were shown to dose-dependently reduce or abolish CFRs. Unlike in P280-treated dogs where CFR returned in about 30 minutes post infusion, CFR did not return in P246-treated dogs for at least 2 hours after stopping infusion. Ex vivo platelet aggregation was shown to be inhibited after the infusion of P280 or P246 in blood obtained from dogs used for the Folt's coronary thrombosis model experiments. In the carotid artery thrombosis model, it was stated by Diatide that "an occlusive thrombus formed in 104 ± 22 (SD) min after applying anodal current to the intima of the first (right carotid), during which vehicle for P280 was infused. The thrombi retrieved from "control" artery weighed 35 ± 20 (SD) mg. Thrombosis did not occur during 3 hours of continued application of electrolytic injury in

two of three dogs receiving 17µg /kg/min of P280. In the 3rd dog, the left carotid artery occluded 126 minutes after initiating anodal current. In all three dogs, the average time to thrombosis was 234±94 minutes (time 0=beginning of anodal stimulation)". No result was given for P246.

Ex vivo platelet aggregation was inhibited in platelets obtained from dogs used for the carotid injury experiments. Only P280 result was shown for the carotid artery thrombosis model.

The sponsors stated that template bleeding time (TBT) was elevated dose dependently by 3 peptides. Significant elevation in TBT was said to accompany fully effective doses in Folts model, yet the sponsors further stated that there are insufficient data to summarize statistically or graphically.

Both P280 and P246 inhibited ADP-induced platelet aggregation in human and canine platelets in vitro.

Although no data were provided, it was stated that no discernible hemodynamic effects or changes in WBC, RBC, platelet count or coagulation parameters were observed during or after administration of any of the peptides in any of the models.

Reviewer's comments: It appears that the write up for this study was part of a larger write up for another purpose. Pertinent information necessary for data analysis could not be found. The chemistry part of the introduction stated that the two dimensional structure of the three peptides study are shown on the next page " No structure was shown, and two not three peptides were under investigation. Moreover, this study suffered from a number of limitations. Starting with the technical aspects of the study, there was no true randomization in the protocol. Additionally, this reviewer could not understand why (1) for the Folts coronary thrombosis model, where flow dynamics appears to be of some importance, there was a 20 fold range in the flow rate used for peptides infusion. (2) Control animals for the carotid artery model of electrically induced thrombosis received "vehicle or nothing". (3) for the template bleeding time, the sponsor stated that "significant elevation in TBT accompanied fully effective doses in the Folts model". Whereas the next sentence following stated that "there are insufficient data to summarize statistically or graphically". The question that arises from these two contradictory statements is, how did they measure the significant elevation? In addition to the above, some of the histograms shown were not adequately annotated, for example it is difficult to deduce which bar represents P280 or P246 in figure seven. No time period was stated for the onset of inhibition of ex vivo platelet aggregation using the Folts model. Moreover, in the carotid artery model where it was stated that "within one hour of starting the infusion of P280, aggregation was inhibited by over 75%," it would have been helpful to know the minimal time interval required for the onset of inhibition of platelet aggregation effect following peptides infusion, and to compare this with the time required for acquiring thrombus images in canine. No data were presented for the effect of P246 in the carotid artery thrombosis model and for the ex vivo platelet aggregation effect. The write up emphasized the difference between P280 (bibapcitide) and P246 (apcitide) in the time interval needed for the resumption of CFR following cessation of peptides infusion in the Folts model, however the importance of such a difference was not commented on. It is impossible to estimate NOELs from this study.

Study # R2.72: Evaluation of the Potential Interaction Between Technetium Tc-99m Apcitide Injection Labeled with Decayed Generator Eluate and Aspirin or Heparin in Human Platelet In Vitro. Study was performed by Diatide Inc., 9 Delta Drive, Londonderry New Hampshire, USA. The study is located in volume 1.11 pages 197-207. The study utilized Technetium Tc 99m Apcitide Lot No. 9603M01 and Decayed sodium pertechnetate Tc 99m injection Lot No. 9403M03. This study was not in compliance with GLP.

Patients who are candidates for diagnostic imaging for DVT, may also have medical history that necessitate the use of non steroidal anti inflammatory agents such as aspirin or the anti coagulant, heparin. In view of the potential for either aspirin or heparin to be co-administered with Tc apcitide injection, the study examined potential interaction between the anti aggregatory effect of Technetium Tc 99m apcitide injection prepared with decayed generator eluate, and the anti aggregatory effect of the non steroidal anti inflammatory agent, aspirin. The study also examined potential interaction between the anticoagulant, heparin and Tc apcitide injection.

Blood samples obtained twice from two volunteers; before and 120 minutes after oral administration of 650mg acetylsalicylic acid were prepared for in vitro platelet aggregation study by slow centrifugation to produce a turbid platelet rich plasma (PRP). Platelet aggregation was measured turbidometrically as described for study # R2.51 IC₅₀s for the inhibitory effect of Tc apcitide injection on ADP- (10 µM) or on a thromboxane A₂-mimetic agonist, U44069- (10 µM) induced platelet aggregation was calculated. The effectiveness of aspirin inhibition of the cyclooxygenase pathway was verified by examining ADP-, U44069- or arachidonic acid-induced platelet aggregation before, and after aspirin treatment. The theoretical expectation is that arachidonic acid-induced platelet aggregation will be inhibited by prior administration of aspirin since it is dependent on cyclooxygenase action for conversion to cyclic endoperoxides and thromboxane A₂. Aspirin was not expected to affect ADP and U44069 (a stable analogue of PGF_{2α}). To examine potential interaction with heparin, 0.6 USP units/mL was added to PRP, thereafter platelet aggregation induced by ADP or by U44069 was examined.

Results: Orally administered aspirin (650 mg) was effective in attenuating arachidonic acid-induced platelet aggregation (78% pre-aspirin vs. 17% post-aspirin) without affecting aggregation elicited by ADP or U44069.

However, the IC₅₀ for the inhibition of ADP or U44069-induced platelet aggregation by Tc apcitide injection was not affected by aspirin. (Table 2, Vol 1.11 pp.206)

Agonist	IPA IC ₅₀ (nM) Pre-Aspirin	IPA IC ₅₀ (nM) Post Aspirin
ADP	153±18	175±14
U44069	145±49	157±46

The addition of heparin did not increase the antiaggregatory effect elicited by Tc-Apcitide injection in vitro. The IC₅₀ was increased by approximately 40%

Experiment No	IPA IC ₅₀ (nM) - Heparin	IPA IC ₅₀ (nM) + Heparin
1	210	280
2	120	180
Mean±SD	165±64	230±71

Conclusion: Neither aspirin nor heparin at clinically relevant concentrations, appear to enhance the inhibitory actions of Tc-Apcitide injection on human platelets.

Reviewer's comments: It is statistically incorrect to calculate SD for n=2 otherwise, I agree with the trend of the conclusion.

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27: Summary of Pharmacology

The specificity and affinity of a rhenium complex of apcitide for purified human GP11b/111a receptor was examined in Study #R2.79. The study compared re-apcitide ability to inhibit the binding of fibrinogen to purified $\alpha_2\beta_3$ with its ability to inhibit the binding of vitronectin to $\alpha_5\beta_3$ receptor. Re-apcitide inhibited the binding of fibrinogen by 50% at 1.8nM while it required ≥ 100 nM of re-apcitide to inhibit vitronectin binding by $\sim 20\%$. The sponsor concluded that *"This is a stringent demonstration of specificity, since vitronectin, like fibrinogen, contains the RGD sequence and because the vitronectin and GP11b/111a receptors share a common β strand"*.

This study specifically addressed question 2, and the results indicated that apcitide was able to distinguish between two members of a receptor family that shares a common β_3 strand. It is my opinion that the study demonstrated a reasonable degree of selectivity. In addition, question 1 was addressed indirectly by the study. The nanomolar concentration of apcitide required for inhibition of fibrinogen binding is indicative of its high affinity for $\alpha_2\beta_3$ receptor compared with the affinity of fibrinogen for the same receptor.

Study #R2.52 quantified the binding of Technetium Tc 99m apcitide to resting and activated human platelets in vitro. $\sim 80\%$ of the Tc -99m apcitide binding was said to be specific for the GP11b/111a receptor. Activation of platelets by ADP increased the binding of Tc 99m apcitide to platelets by approximately 2.4 fold. Binding of Tc 99m apcitide to platelet rich plasma isolated from whole blood was $6.7\pm 0.5\%$ and 21.0 ± 3.25 in non activated and ADP-activated samples respectively. It was concluded that technetium Tc 99m apcitide binds specifically to washed human platelets and that technetium Tc 99m apcitide bound 2.4 to 3.5 times more avidly to activated platelets than to resting platelets.

This study addressed question 4. The results demonstrated that Technetium Tc 99m apcitide showed ~ 3 fold increase in binding to activated washed platelets compared with non activated platelets. The mechanism of action as proposed by the sponsor requires that platelets become activated before binding can occur. No explanation was given by the sponsor for apcitide binding in the absence of activation. This reviewer holds the view that "binding" in the absence of activation reveals non-receptor mediated adsorptive processes, and it illustrates the practical difficulty of obtaining a "fibrinogen free" non-activated platelet. I am satisfied that question 4 has been answered.

In vitro inhibition of platelet aggregation by peptides P280, P1007, P1008 and technetium Tc 99m apcitide injection was examined in study # R2.51. Platelet rich plasma obtained from adult volunteers was used for the experiments. Percentage inhibition of ADP-induced platelet

aggregation by the peptides was estimated using standard pharmacological techniques. P280, Apcitide, P1007, P1008 and technetium Tc 99m apcitide injection inhibited aggregation with IC_{50} 's of 63 nM, 382 nM, 52 nM, 689 nM, and 158 nM respectively. The sponsors concluded that the results demonstrated that these peptides bind to the fibrinogen receptor on platelets.

This study indirectly addressed question 1. It demonstrated that apcitide and related peptides bind to the fibrinogen receptor present on platelets. Moreover, it showed that the dynamic activated platelet-peptide interaction resulted in a measurable physiological response. Moreover, the study raised an important issue of efficacy. Since P1007 and P1008 are present with apcitide in the final injectate, the question arises as to whether these components of the formulation are competing with the drug substance for its pharmacological site of action.

The scientific article entitled "Thrombus imaging with a technetium-99m-labeled activated platelet receptor binding peptide" (Journal of Nuclear Medicine 1996, 37: 775-781) examined the ability of ^{99m}Tc -P280 to detect thrombi in vivo using a canine venous thrombosis model. 24-hour old venous thrombi was produced in canine by insertion of dacron-entwined stainless steel embolization coil in the right femoral vein at approximately the mid femur. Technetium Tc 99m apcitide (185-370 MBq ^{99m}Tc and 0.2-0.4 mg peptide) was administered intravenously, and the animals imaged for 4 hours. Positive control and negative control animals received ^{99m}Tc -HMPAO-labeled autologous platelets (260 MBq) or ^{99m}Tc -glucoheptonate (290 MBq) respectively. Uptake of technetium Tc 99m apcitide by thrombi was quantified by excision and counting. It was concluded that ^{99m}Tc -P280 provided in vivo visualization of thrombi and good thrombus-to blood (4.4), and thrombus-to-muscle (11) ratios. It was also noted that HMPAO-labeled autologous platelets provided a better visualization of the thrombi compared with ^{99m}Tc -P280.

This study examined the ability of labeled apcitide to image 24-hour old thrombus. I agree that labeled apcitide provided in vivo visualization of the thrombi.

Study #R2.74 examined the effects of technetium Tc 99m apcitide injection at doses representing 1X, 30X, and 100X maximal human dose ($2\mu g$ total peptide /kg) on cardiovascular responses in anesthetized mongrel dogs. Mongrel dogs were surgically prepared for artificial ventilation and instrumented for measurement of changes in arterial pressure, heart rate and left ventricular pressure. The contractor stated that these results were not subjected to statistical analysis. The sponsor reported that there were no changes in mean arterial pressure and no discernible trend was seen with LV systolic pressure changes. $LV=dP/Dt_{max}$ (an index of left ventricular contractility) was said to either remain unchanged or increase slightly from the pre-injection base line values in all three test groups. No effects on coagulation parameters were observed. There was no effect on platelet aggregation in control dogs or dogs receiving 1X MHD. Aggregatory responses to ADP declined in the group that received 30X and 100X MHD of Tc apcitide injection by 43% and 98% respectively. The aggregatory response was said to regain over half of the deficits observed immediately post-injection within one hour in both groups. It was concluded that no effect on hemodynamic, coagulation or platelet count may be expected by doses of technetium Tc Apcitide injection as high as 100X MHD, the NOEL for these parameters.

This safety study did not demonstrate a NOEL between 1X and 30X for the antiaggregatory response. Thus no margin of safety was demonstrated.

Study # R5.1, was designed to test the hypothesis that peptides P280 (sibapcitide) and P246 (apcitide) exert antithrombotic effects and to examine the hemodynamic profile of these peptides. Two models of arterial thrombosis were used. (1) The Folt's coronary thrombosis model involving severe stenosis and deendothelialization of a major coronary artery in open-chest dogs. It is platelet-dependent and fibrin-independent, and is characterized by the cyclic flow reduction (CFR) produced by alternating gradual vessel occlusion by a platelet rich thrombus and abrupt restoration of flow caused by dislodgment of the thrombus. CFRs are completely abolished by agents with antiaggregatory action. (2) The; carotid artery model of electrically-induced thrombosis was conducted in anesthetized closed chest dogs that underwent electrolytic injury of the intimal surface of the carotid artery. Peptide doses of 10, 30 and 100µg/kg/min were employed. Additional tests including template bleeding time, ex vivo platelet aggregation, in vitro platelet aggregation, blood cell profile and coagulation parameters (prothrombin and partial thromboplastin times, PT and aPT) were also conducted. Both peptides exerted antithrombotic effects in both models of arterial thrombosis. Both P280 and P246 inhibited ADP-induced platelet aggregation.

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Impossible to estimate NOEL from this study. Moreover, in the carotid artery model where it was stated that "within one hour of starting the infusion of P280, aggregation was inhibited by over 75%," it would have been helpful to know the minimal time interval required for the onset of inhibition of platelet aggregation effect following peptides infusion, and to compare this with the time required for acquiring thrombus images in canine.

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Study # R2.72: Patients who are candidates for diagnostic imaging for DVT, may also have medical history that necessitate the use of non steroidal anti inflammatory agents such as aspirin or the anti coagulant, heparin. In view of the potential for either aspirin or heparin to be co-administered with Tc apcitide injection, the study examined potential interaction between the anti aggregatory effect of Technetium Tc 99m apcitide injection prepared with decayed generator eluate, and the anti aggregatory effect of the non steroidal anti inflammatory agent, aspirin. The study also examined potential interaction between the anticoagulant, heparin and Tc apcitide injection. Platelet aggregation was measured turbidometrically. IC₅₀'s for the inhibitory effect of Tc apcitide injection on ADP- (10 µM) or on a thromboxane A₂-mimetic agonist, U44069- (10 µM) induced platelet aggregation was calculated. The effectiveness of aspirin inhibition of the cyclooxygenase pathway was verified by examining ADP-, U44069- or arachidonic acid-induced platelet aggregation before, and after aspirin treatment. The IC₅₀ for the inhibition of ADP or U44069-induced platelet aggregation by Tc apcitide injection was not affected by aspirin. Addition of heparin did not increase the antiaggregatory effect elicited by Tc-Apcitide injection in vitro. The IC₅₀ was increased by approximately 40%. It was concluded that neither aspirin nor heparin at clinically relevant concentration, appear to enhance the inhibitory actions of Tc-apcitide injection on human platelets.

Safety study. I agree with the study conclusion.

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28: Pharmacokinetics/Toxicokinetics:

Study # R2.67: Pharmacokinetics and Biodistribution of Technetium Tc 99m Apcitide in Male and Female Rats. Study was conducted by Diatide Inc., 9 Delta Drive, Londonderry, New Hampshire 03053, USA. Final commercial formulation Lot No. 9603M01 used for this study. The study is located in volume 1.16 pages 36-56. This study was not in compliance with GLP.

This study examined the pharmacokinetics (PK) and biodistribution of technetium Tc 99m apcitide in rats.

Rats were anesthetized and instrumented for blood sampling via the jugular vein. Each rat then received 200 μ Ci Technetium Tc 99m apcitide which is equivalent to 2 μ g peptide /kg of the parent peptide sibapcitide, intravenously. Blood samples were obtained at intervals (1, 3, 5, 10, 15, 30, 60, 90, 120, 180, 240 minutes). Heparinized saline was used to replace the withdrawn blood samples. Samples were counted, and radioactivity expressed as a percentage of injected dose per gram. Data obtained were fitted to open compartment models using χ^2 and used to generate the data shown in the table. Urine was collected over the duration of the sampling period and counted. Fecal material was counted for radioactivity and considered as "contaminated by urine", and included in the urine count. Animals were euthanized following the conclusion of the experiment, and the following organs counted for radioactivity; liver, kidneys, spleen, urinary bladder, terminal blood and the entire gastrointestinal tract.

Results: Technetium Tc 99m was cleared rapidly from the blood with about 97% cleared within the first 30 minutes. Diatide used a biexponential fit for the report but stated that the data fitted a three exponential curve equally well. Since the area under the curve from 30-240 minutes accounted for less than 3 % of the injected radioactivity, the use of a biexponential curve is appropriate. Table 1 (from Vol. 1.16 pp.44) shows the pertinent pharmacokinetics parameters.

Most of these parameters were similar between sexes. However, the rate of elimination from blood was faster in female rats thus leading to differences in derived parameters that depended on the elimination rate constant such as AUC, V_{DSS} and MRT. The biological or clinical significance of such differences was stated to be uncertain. Urine accounted for the majority of injected radioactivity, although the excretion rate constant was not determined. Biodistribution data showed that overall recovery of radioactivity averaged 92.1 ± 8.7 % ID (Injected Dose) and 96.7 ± 6.8 % ID in male and female rats respectively. Four hours total urinary excretion was higher in female than in males, 91 vs 79% ID. Male carcass contained on average 8% ID compared to 0.7% ID for female rats.

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Parameters	Both Sexes	Males	Females
C_{bo} (% ID/g)	3.45±1.07	3.11±1.2	3.78±0.9
V_D (mL/kg)	138 ±39.6	126±46	148±32
$t_{1/2\alpha}$ (min)	1.81±0.81	1.78±0.95	1.85±0.74
$t_{1/2\beta}$ (min)	20.6±5.1	23.4±5.4	17.8±2.9
$AUC_{t_0 \rightarrow \infty}$ (%ID• min/g)	40.7±13.9	43.9±9.2	36.81±5.3
$AUC_{t_0 \rightarrow 240min}$ (%ID•min/g)	40.1±13.6	43.1±8.2	36.5±15.2
$V_{D_{ss}}$ (mL/kg)	315±66	280±68	351±44
MRT (min)	25.3±7.5	30.2±7.6	20.4±2.6
$K_{elim-half}$ (min)	7.8±3.4	9.6±4.0	6.0±1.2
Parameters	Both Sexes	Males	Females
Cl_{tot} (mL/min/kg)	4.66±1.46	3.77±1.07	5.81±1.02
%ID _{e-GI tract} (%ID) ⁴	3.5±1.74	2.99±2.34	4.03±0.72
%ID _{e-urine} (%ID)	84.9±10.0	78.9±9.7	91.0±6.3
%ID _{e-blood} (%ID)	98.6±0.8	97.8±0.8	99.2±0.1

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CONCLUSION: It was concluded that intravenously administered Technetium Tc 99m apcitide disappears rapidly from the blood in a biexponential manner with average distribution and elimination half life of 1.8 and 21 minutes respectively. The major route of elimination is via the urine. Some parameters such as $t_{1/2\beta}$, V_{DSS} , MRT and terminal uptake in certain tissues (GI tract and carcass) exhibited sex differences. The clinical significance of this difference was said to be uncertain.

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Reviewer's comments: I agree with the overall conclusion of this study. However, I disagree with some technical details such as regarding the counts from fecal matters as "urine contamination" and therefore lumping the two together. A minor percentage of injected apcitide may have been excreted through the feces. Moreover, I noted that urine was collected over absorbent paper thereby preventing the determination of urinary excretion rate. I agree with the sponsor that the clinical significance of the apparent gender differences in handling of injected apcitide is uncertain, especially in the light of the fact that elimination was almost completed during the 4 hour observation period. Nevertheless, this observation of faster elimination in females is intriguing given the fact that gender differences (including humans) in platelet reactivity is well documented. Males are reported to show enhanced platelet aggregatory activity

compared to females through testosterone-dependent mechanisms. If kinetics is different, one wonders whether dynamics of apcitide injection will also demonstrate subtle gender differences.

Study # R2.53 Update 1: An Evaluation of the metabolism of Technetium Tc 99m Apcitide in the Sprague-Dawley Rat. Study was conducted by Diatide Inc., 9 Delta Drive, Londonderry, New Hampshire 03053, USA. Final commercial formulation Lot No. 9603M01 used for this study. The study is located in volume 1.16 pages 57-94. This study was not in compliance with GLP.

The study utilized _____ to determine whether Technetium Tc 99m apcitide injection undergoes metabolism when injected intravenously.

Rats were anesthetized and instrumented for blood sampling and apcitide injection via both jugular veins. The bladder was cannulated for urine samples collection. Each rat then received weighted aliquots of Technetium Tc 99m apcitide to provide 20X MHD (40 µg peptide/kg, ≈ 20mCi) intravenously. Blood samples were obtained at intervals (15, 60, 120, minutes). Urine samples were collected over specified intervals covering 240 minutes. Plasma and urine samples were assayed by _____ to determine percentage of injected dose per gram or per collection interval, and for detecting the presence of metabolites.

Results & Conclusions: Urinary elimination was fitted into a single exponential model. Approximately 70% of ID was eliminated within the 4 hour observation period. The study identified 2 radiolabeled species in urine in a constant ratio of 1:3; intact Tc 99m apcitide and an unidentified TC 99m metabolite which was characterized as more hydrophilic and smaller in size compared to Tc 99m apcitide. This metabolite was not present in the plasma and was therefore concluded to be generated by the kidneys. The results suggested that the kidney is the major site of Tc 99m Apcitide metabolism.

Reviewer's comments: I agree with the study conclusion that a more hydrophilic metabolite appeared in the urine. However, I am equivocal about the conclusion that because this metabolite was not identified in the plasma, it was therefore generated solely by the kidney. I can not think of an enzyme metabolizing system that is unique to the kidney. An alternative explanation might be that this metabolite is rapidly excreted into the kidney, and therefore more difficult to detect in plasma. This scenario is plausible given the fact that the first blood sample was taken 15 minutes after injection. The first urine sample was collected 0-30 minutes after injection. It should be noted that in study R2. 76, 97% of injected Technetium Tc apcitide was cleared from the blood within 30 minutes.

Study # R2.75 Distribution, Metabolism and Excretion of Technetium Tc 99m Apcitide in Rats with Experimental Renal Dysfunction. Study was conducted by Diatide Inc., 9 Delta Drive, Londonderry, New Hampshire 03053, USA. Final commercial formulation Lot No. 9603M01 used for this study. The study is located in volume 1.16 pages 120-137. This study was not in compliance with GLP.

This study examined the distribution, metabolism and excretion of Technetium Tc 99m apcitide in rats with experimental renal dysfunction (ERD) produced by bilateral renal artery and vein ligation.

Bilateral renal vessels ligation was produced in rats (4 male and 3 female) by tying the renal arteries and veins under surgical anesthesia. Another group of rats had the bile duct cannulated for passive collection of bile in addition to renal vessel ligation. Four rats were used as sham operated controls while a rat had its bladder cannulated to provide analysis of urine metabolites. The jugular vein was cannulated in all rats for blood sampling and to administer supplemental anesthesia. Animals were dosed as illustrated in table 1 (vol. 1.16 pp.126)

Experimental Groups	Males	Females	Dose (X MHD)	Specific radioactivity (mCi/ μ g)
Historical normal controls ^a	6	3	1X	
Renal Artery Ligation	4	3	1X	
Sham-operated	2	2	1X	
Renal Artery Ligation and Bile Duct Cannulation	3 ^b	1	20X	1.0
Normal control With Bladder cannulation	1 ^b	0	20X	1.0

^aThese rats also served as the basis of study R2.67. ^bOne rat whose bile duct was cannulated following experimental renal dysfunction (group 4), and one normal rat with the urinary bladder cannulated (group 5) were used for bile and urine sampling to correlate the retention times of the primary metabolite found in bile and the primary metabolite found in urine. Rats in groups 1-3 were dosed at 2 μ g total peptide/kg. Rats in groups 4-5 were dosed at 40 μ g/kg. Samples from the plasma and bile were collected at stated intervals. Rats in groups 1,2, and 3 had tissue samples collected from them at the end of 4 hours.

Results: $T_{1/2\beta}$, MRT, $AUC_{0 \rightarrow \infty}$, $K_{elim-half}$ values from ERD rats were increased significantly ($p < 0.001$, 2 tail difference of means) compared to normal or sham operated controls. % ID disappearing from the blood in 4 hours was 46 ± 5.5 in ERD rats compared to 98.6 ± 0.8 and 99.0 ± 0.5 in controls and sham-operated rats. In ERD rats, the GI was stated to show an increase in uptake beyond that contributed by delayed disappearance from blood suggestive of increased hepatobiliary activity. Two metabolites (one hydrophilic, 70% of peak area; and one lipophilic; 10% of peak area) were detected in the bile of ERD rats in addition to Technetium Tc 99m apcitide. The hydrophilic metabolite seen in bile had an identical relative retention time to the major metabolite observed in urine, and the minor metabolite observed in plasma of rats with ERD. The similarity in relative retention time was taken by the sponsor to indicate "mutual identity". The lipophilic metabolite was not identified in the plasma of ERD rats. The lack of identification was attributed to low relative abundance.

Conclusion: It was concluded that renal dysfunction significantly affected systemic clearance of Technetium Tc 99m apcitide. The liver metabolized technetium Tc99m apcitide to two species: a major hydrophilic metabolite that appeared to be identical to urinary metabolite seen in normal rats, and a minor lipophilic metabolite. That hepatobiliary excretion route becomes important during ERD with the rate of systemic elimination of technetium Tc 99m apcitide approximately one-tenth of the rate in rats with normal renal function.

Reviewer's comments: Agree with the conclusions. It seems that hepatobiliary excretion does take over when renal elimination is impaired. Moreover, the results argues against the sponsor's claim in previous study that the kidney is the site of the hydrophilic metabolite. A major criticism of this study is that the pharmacokinetics profile was monitored for a four hour period

at the end of which only about 50% of the injected radioactivity had disappeared from the blood in ERD rats compared with 98% for normal rats during the same interval. Thus it was impossible to determine from the study comparable time to achieve 98% elimination in ERD rats.

Study #R2.77: Distribution, Metabolism, and Excretion of [³H]-Labeled Peptide Components of Technetium Tc 99m Apcitide Injection in Male Sprague Dawley Rats. Study was conducted by

. The study is located in volume 1.16 pages 157-322. This study was in compliance with GLP.

The stated objective of this study was to assess the distribution, metabolism, and excretion of the peptide components of Technetium Tc 99m apcitide injection in rats.

[³H]-bibapcitide (8μCi/μg) was labeled with a non exchangeable tritium label in order to preserve the radiolabeling on apcitide, P1007 and P1008 following the conversion of bibapcitide to the peptide components of Technetium Tc 99m apcitide injection. The study had five groups (A-E) of 3-5 animals each. All the groups received approximately the same dose of peptide, 40.6 ± 0.2μg/kg (~80μCi/rat) intravenously. The major differences between groups were the sampling time and parameters analyzed (Vol.1.16 pp.168). Samples collected included plasma, urine, major organs and tissues: kidneys, liver, spleen, lungs, heart, bone, gastrointestinal tract and content. The samples were prepared for scintillation counting and for analysis. Pharmacokinetics parameters were estimated with a software program.

RESULTS: Biodistribution data showed that none of the organs examined showed uptake of more than 1% ID by 4 hours post-injection. Most of the injected dose was contained in the carcass 11 % or was excreted in the urine 84%. The GI content was 1%. By 24 hours, 15% of injected dose remained in the carcass and organs or eliminated in feces. The sponsors stated that "because urine capture was incomplete, excretion in the urine was taken to be the difference between injected dose and the total recovered radioactivity in the carcass and excised organs". No metabolite was detected in plasma, and with the exception of [³H]-P1007, the peptides were not detectable beyond 15 minutes post injection. The injected [³H]-peptide components were not identifiable in urine at any time point. Up to seven [³H]-labeled metabolites were found in the urine and the sponsor concluded that they were generated by metabolism in the kidney. Pharmacokinetics results were not significantly different from those obtained in study # R2.53, and will therefore not be discussed further.

CONCLUSIONS: The sponsor concluded that the distribution, metabolism and excretion of [³H]-peptide components of technetium Tc99m apcitide injection were very similar to those of technetium Tc 99m apcitide.

Reviewer's comments: While it is noted that the total number of metabolites identified is incomplete because of numerous technical reasons, I agree with the overall conclusion of the study that these peptides are rapidly eliminated from the blood and that the kidney appears to be a site of peptide metabolism. The rapid elimination of the metabolites from the blood may also be responsible for lack of detection in the plasma.

Study # R2.96: Comparative Metabolism of [³H]-labeled Peptide Components of Technetium-Tc 99m Apcitide Injection in Rat, Rabbit, and human Liver and Kidney Slices In Vitro.

The study is located in volume 1.16 pages 323-462. This study was in compliance with GLP.

The study compared the metabolism profile of [³H]-peptide components of Technetium Tc 99m Apcitide injection by rat, rabbit and human kidney and liver slices in vitro

[³H]-bibapcitide (8μCi/μg) was labeled with a non exchangeable tritium label in order to preserve the radiolabeling on apcitide, P1007 and P1008 following the conversion of bibapcitide to the peptide components of Technetium Tc 99m apcitide injection. Prior to use, the tritium - labeled peptide was mixed with an equivalent standard clinical preparation of Technetium Tc 99m apcitide injection. Tissues slices prepared from rat, rabbit and human liver and kidney were incubated in triplicates in biochemically appropriate media containing [³H]-peptide components at 3 dose levels 100, 316, and 1000 ng total peptide/mL, for 0.5, 2 and 4 hours. The experiments included appropriate controls such as media, metabolic negative, and metabolic positive controls. The positive control utilized the ability to metabolize 7-ethoxycoumarin (7-EC) to specific metabolites as a test of tissue viability. Following the completion of the experiments, media containing the [³H] components as well as tissue slices were analyzed methodology. Retention time relative to [³H]-P1007 (Rt = 1.0) and percent peak areas for each tissue and time point were estimated.

Results: All tissues remained viable as evidenced by their ability to metabolize 7-EC through P450-dependent mechanisms. A single, more hydrophilic metabolite (14% of applied radioactivity) was generated by the human liver while the human kidney produced multiple hydrophilic metabolites totaling 23.9% of applied radioactivity (AR). The rabbit liver and kidney produced one (5.1% AR), and two (4.6 and 10.7% AR) metabolites respectively. The rat liver generated one major (16.4% AR) and two minor (3.2% AR total) metabolites respectively. The rat kidney showed extensive metabolism with 47% and 67% of AR metabolized to a major, more hydrophilic metabolite by 30 minutes and 4 hours respectively. Due to the fact that the chromatograms varied in the absolute retention time of [³H]-P1007, mutual identity of metabolites across species was not possible.

Conclusion: The sponsor concluded that the parent peptides are metabolized to more hydrophilic metabolites with proportions varying from for human, rabbit and rat liver and human and rabbit kidney to 76% for rat kidney. The results suggested that some of these metabolites may be similar.

Reviewer's comments: I agree with the overall conclusion. It is also noted that in vitro, the human kidney may not play a role as prominent as the rat kidney in the metabolism of these peptides. It was not possible to reach a decision on whether the metabolites were the same across species, since they were identified based on relative retention time and on their hydrophilic or hydrophobic nature.

Study # R2.50, Update 1: Distribution of Technetium Tc 99m Apcitide Between Blood Components In vitro. Study was conducted by Diatide Inc., 9 Delta Drive, Londonderry, New Hampshire 03053, USA. Final commercial formulation Lot No. 9603M01 used for this study. The study is located in volume 1.16 pages 13-35. This study was not in compliance with GLP.

This study examined the distribution characteristic of Tc 99m apcitide in human blood.

Citrated human blood samples were used for the experiments. Standard biochemical techniques including differential centrifugation, blood cell fractionation using plasma and plasma protein fractionation using were employed to obtain required samples. Distribution of Tc 99m apcitide (with or without ADP stimulation) in the various fractions was investigated.

Results: 95±8% of applied radioactivity was recovered with the highest recovery (86%) obtained from plasma. 7% of radioactivity was associated with platelets. In the presence of ADP, the amount of radioactivity recovered from platelet rich plasma (PRP) increased three fold to 21 %. Percentage of Technetium TC 99m apcitide associated with WBC, luekocytes, or RBC after density gradient separation using neutrophil isolation medium were less than !%.

CONCLUSION: The sponsors concluded that:

- a high percentage of apcitide was contained in plasma with less than 1% of applied radioactivity associated with PMN, WBC or RBC and platelets.
- platelet binding accounted for nearly all measurable cell associated binding.
- addition of ADP to platelet increased radioactivity associated with platelet three fold.
- at concentration range of 0.1 to 10nM, Tc Apcitide association with plasma protein was less than 2%..

Reviewer's Comments: Agree with the conclusions.

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