CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

20-484

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

Clinical Pharmacology and Biopharmaceutics Review

NDA: 20-484

Submission Date: 7/1/1999, 2/9/2000, 2/22/2000

Trade Name: innohep®

Active Ingredient: Tinzaparin Sodium

APR 11 2000

Sponsor: Dupont Pharmaceuticals Company

Reviewer: Suliman I. Al-Fayoumi, Ph.D.

Type of Submission: Original NDA-NME (1S)

Synopsis

The pharmacokinetics (PK) of IV and SC tinzaparin, measured as anticoagulant activity (anti-Xa and anti-IIa activities), have been characterized in healthy subjects as well as in the relevant patient population. The sponsor demonstrated bioequivalence between the To-Be-Marketed and Clinical Trial formulations. The effects of various covariates such as age, weight and renal function, have been examined on tinzaparin PK in the relevant patient population using population PK analysis.

Recommendation

NDA 20-484 for tinzaparin sodium (innohep®) was submitted on 7/1/1999. The Human Pharmacokinetics and Bioavailability section of the application has been reviewed by the Office of Clinical Pharmacology and Biopharmaceutics (OCPB/Division of Pharmaceutical Evaluation II). From the view point of OCPB, the submission is acceptable.

/S/ 4/11/00

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/S/ 4/11/00

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cc: HFD-180: NDA 20-484 (1x); DIV FILE (1x); KOLIVER (1x); SDODDAPANENI (1x); SALFAYOUMI (1x); HFD-870 SHUANG (1x); CDR: ATTN Zom Zadeng

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I. Introduction

Tinzaparin is a low molecular weight heparin (LMH) that is produced by controlled enzymatic depolymerization of unfractionated porcine heparin using heparinase. Tinzaparin inhibits reactions that lead to the clotting of blood and formation of fibrin clots both *in vitro* and *in vivo*. It acts as potent co-inhibitor of several activated coagulation factors, in particular factors Xa and IIa (thrombin).

Tinzaparin sodium is currently approved in over 20 countries for treatment of deep vein thrombosis (DVT), and perioperative thromboembolism prophylaxis in orthopedic (hip/knee replacement) or general surgery settings. Three other LMHs, enoxaparin sodium (NDA 20-164), dalteparin sodium (NDA 20-287) and [redacted] are currently marketed in the US.

NDA 20-484 for tinzaparin sodium (innohep®) was submitted to the Agency on 7/1/1999. The sponsor's proposed indications include the treatment of acute symptomatic DVT with and without pulmonary embolism when administered in conjunction with warfarin sodium. [redacted]

For the treatment of DVT, a tinzaparin dose of 175 anti-Xa IU/kg of body weight is proposed, given SC once daily for at least 6 days and until the patient is adequately anticoagulated with warfarin. [redacted]

Due to difficulty in measuring the chemical entity in blood, kinetics of LMHs are characterized by measurement of two commonly used biomarkers, anti-factor Xa (anti-Xa) and anti-thrombin (anti-IIa) activities.

The sponsor has submitted 11 pharmacokinetic (PK)/pharmacodynamic (PD) studies in support of this submission, including two additional population PK analyses, which were aimed at assessing the PK of anti-Xa activity of tinzaparin in the targeted patient population as well as, evaluating the influence of several covariates such as body weight, age and renal function on tinzaparin clearance. A Question-Based Review of the NDA yielded the following information:

II. Analytical Assay

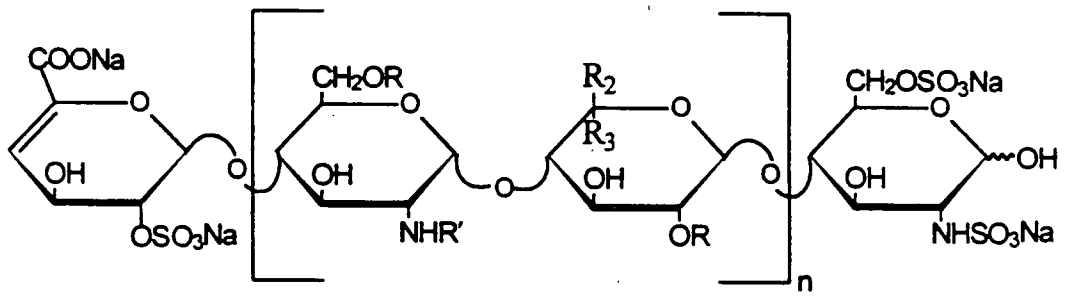
Is the analytical assay methodology adequately validated?

Since tinzaparin plasma concentrations can't be directly measured, several pharmacodynamic biomarkers are used to determine the activity of tinzaparin in blood for

the assessment of tinzaparin pharmacokinetics; the most relevant of which are anti-Xa and anti-IIa activities (determined using amidolytic (chromogenic) methods).
The amidolytic methods are based on an enzymatic reaction in which the target enzyme interacts with a specific substrate. This substrate is an oligopeptide, which contains a specific site for binding to a given enzyme, with an attached chromophore end group: paranitroaniline. The binding of the substrate with the target enzyme (factor Xa or factor IIa) releases the chromophore group, which can be quantified spectrophotometrically at 405 nm.
The development of tinzaparin has spanned the evolution of chromogenic assay methodologies for LMH. Hence, out of all the PK/PD studies submitted in the NDA application, only two studies included full analytical methods validation reports (studies DMP 702-001 & DMP 702-918). PK studies with tinzaparin have come from 7 different laboratories using 8 methods for anti-Xa activity and 4 methods for anti-IIa activity. Some of the limitations posed by the use of invalidated chromogenic assays include: 1) unspecified limits of quantitation, 2) activity greater than the upper limit of quantitation were extrapolated from the standard curve, 3) large baseline variability. Appendix A contains sponsor's clarification regarding this reviewer's query on the aforementioned aspects. In addition, table 1.4 in Appendix B contains a summary of the available analytical methods validation data for all the submitted PK studies.

III. Formulation

What is the composition of the SC tinzaparin formulation?



Tinzaparin sodium

n= 1 to 25, R = H or SO_3Na, R'= H or SO_3Na or $COCH_3$
R_2 = H and R_3 = COONa or R_2 = COONa and R_3= H

The average molecular weight for tinzaparin ranges between 5,500 and 7,500 daltons.
The molecular weight distribution is:
<2,000 daltons < 10%
2,000 to 8,000 daltons 60% to 72%
8,000 daltons 22% to 36%

In early supportive PK studies, innohep® SC injections consisted of aqueous tinzaparin solutions preserved with benzyl alcohol (10 mg/ml) and were produced aseptically In more recent PK studies, several changes were made to the formulation including; variation in benzyl alcohol content from 0 to 13.5 mg/ml (see study DMP 702-918), the addition of sodium metabisulfite and the use of in the formulation process. However, none of these variations has been shown to alter bioavailability of the formulation.
A list of the tinzaparin batches used in the PK studies is included in table 1.3 in Appendix B.

IV. Bioavailbility

How large a fraction of tinzaparin is systemically available after SC administration?

The absolute bioavailability (following administration of 4,500 anti-Xa IU SC and IV formulations) is 86.7% based on anti-Xa activity (see study DMP 702-918).

V. Bioequivalence

Is the To-Be-Marketed formulation equivalent to the Clinical Trial formulation?

The to-be-marketed tinzaparin containing < 10% of the molecules in the sub-2k D[1] fraction appeared to be bioequivalent to a specially-produced tinzaparin-like LMH with a fraction of sub-2k D molecules of approximately 16%, which corresponds to the highest fraction used in clinical trials (15.2%) (see study DMP 702-001).

VI. Dose Proportionality

Is there dose proportionality for the anti-Xa activity?

The administration of 4,500 anti-Xa IU and 12,250 anti-Xa IU doses of tinzaparin resulted in increases in anti-Xa activity that were greater than dose proportional (based on $AUC_{0-\infty}$) (see study DMP 702-918).

VII. Pharmacokinetics

The sponsor has characterized the single dose PK of tinzaparin (see study DMP 702-918). In addition, a study is currently ongoing to characterize the multiple dose PK of tinzaparin 175 anti-Xa IU/kg, SC in healthy subjects (Study DMP 702-945).

a) Absorption

Plasma levels of anti-Xa activity increase in the first 2 to 3 hours following SC injection of tinzaparin and reach a maximum within 4 to 5 hours. Maximum concentrations (C_{max}) of 0.25 and 0.87 IU/mL are achieved following a single SC fixed dose of 4,500 IU

[1] **The sub-2k D molecules is considered to be the primary pharmacologically-inactive fraction of LMH.**

(approximately 64.3 IU/kg) and weight-adjusted dose of 175 IU/kg of tinzaparin respectively.

b) Distribution

Tinzaparin has a volume of distribution of 3.1 to 5.0 L.

c) Elimination

The elimination half-life following SC administration of 4,500 IU tinzaparin is approximately 3.4 hours based on anti-Xa activity. Following a single SC administration of 175 IU/kg of tinzaparin, the elimination half-life based on anti-Xa activity is approximately 3.9 hours. Clearance following IV administration of 4,500 IU tinzaparin is approximately 1.7 L/hr. The primary route of elimination is renal.

VIII. Overdose

In case of overdosage, can protamine sulfate be used to effectively neutralize tinzaparin?

Overdosage of tinzaparin may lead to bleeding complications. Protamine sulfate can be administered to neutralize excess tinzaparin. Two studies were conducted to examine the neutralizing effect of protamine sulfate (IV infusion of 1 mg/100 anti-Xa IU) on tinzaparin PK. As with other LMHs, protamine did almost completely neutralize anti-IIa activity, but not anti-Xa activity (maximum about 60%) (see studies DMP 702-922 & 702-924).

IX. Interaction with Aspirin

A single drug-drug interaction study was conducted to examine the effect of concomitant administration of aspirin on tinzaparin PK. However, the results were inconclusive due to inadequate study design (see study DMP 702-923). Upon discussing the issue with Dr. Lilia Talarico, the GI & Coagulation division director, it was decided that, based on current experience with other LMHs, a repeat of the study was not necessary.

X. Special Populations

a) Elderly population

Is there a need for dose adjustment in geriatric patients?

Population PK analysis was utilized to determine the effect of age on tinzaparin PK (see Pop PK Analysis report). Tinzaparin clearance does not seem to depend on age. However, tinzaparin should be used with care in these patients, since renal function generally declines with age.

b) Pediatric population

Is there a need for dose adjustment in pediatric patients?

No adequate and well-controlled clinical or PK studies have been conducted in the pediatric population. The sponsor has included the following statement in the package insert under PRECAUTIONS, "Safety and effectiveness of INNOHEP in patients below the age of 18 years have not been established".

c) Renal Impairment patients

Is there a need for dose adjustment in renal impairment patients?

A study was conducted to determine tinzaparin PK in chronic renal failure (RF) patients on haemodialysis. Compared to healthy subjects, the major PK parameters (CL, V_d and AUC) were similar, except for half-life (5.2 hrs in RF patients compared to 1.6 hrs in healthy subjects). In addition, the population PK analysis in the target patient population investigated the effect of renal function on tinzaparin PK (see study DMP 702-925 & Pop PK Analysis report). Evaluation of individual clearance estimates showed that in severe renal failure (CLcr < 30 ml/min), clearance of tinzaparin was reduced by 24% compared to normal. The sponsor indicated in the proposed package insert under the SPECIAL POPULATIONS section as follows; "Patients with severe renal impairment should be dosed with caution".

d) Hepatic Impairment Patients

Is there a need for dose adjustment in hepatic impairment patients?

No studies have been conducted to assess the effect of hepatic impairment on tinzaparin PK, as the hepatic route is not a major route of elimination of LMHs.

e) Pregnancy

Does tinzaparin cross the placenta into the fetus during pregnancy

Two studies were conducted to determine whether tinzaparin crosses the placenta into the fetus during pregnancy. A clear discrepancy was observed between the results of the two studies, which might be related to the different routes of administration employed in those studies (see studies DMP 702-926 & 702-927). Overall, results of the two studies were inconclusive.

f) Obesity

Is there a need for dose adjustment in obese patients?

Population PK analysis was utilized to determine the effect of body weight on tinzaparin PK (see Pop PK Analysis report). Obese patients (BMI > 30 kg/m^2) had tinzaparin clearance that was 22% lower than normal. Dosing based on body weight is generally sufficient to normalize the differences in tinzaparin PK among patients of varying weights. However, the sponsor indicates in the package insert under SPECIAL POPULATIONS section that "Clinical trial experience is limited in patients with a BMI >40 kg/m^2".

g) Nursing Women

Is there a need for dose adjustment in nursing women?

No studies have examined excretion of tinzaparin in human milk. Hence, caution should be exercised when tinzaparin is administered to nursing women.

h) Pharmacokinetics in Patients

Is tinzaparin PK different in the target patient population from that of healthy subjects?

Population PK analysis was utilized to determine tinzaparin PK in the target patient population (see Pop PK Analysis report). Estimates of the average PK parameters for tinzaparin were similar to those obtained previously in Phase I studies: CL = 0.0193 L/h/kg (CI = 0.013-0.026), V_c = 0.143 L/kg (CI = 0.105-0.181) and $t_{1/2}$ = 5.1 hrs (CI = 3.5-6.7).

IX. Proposed Package Insert (Pages 10-13, CPB-related only)

X. Comments (not to be sent to the sponsor)

- Large inter-study variability was evident in the reported PK parameters.
- Several Phase I studies (DMP 702-920, 702-921, 702-923) were of little value to the current review, as they held little or no relevant PK or PD data.
- Study DMP 702-945 is currently ongoing to evaluate the multiple dose PK of tinzaparin in healthy subjects, and the data will be submitted to the Agency upon completion.
- Per this reviewer's discussion with the Pharmacometrics reviewer, the reviewer's comments included in the Population PK consult are not to be forwarded to the sponsor.

4 page(s) of revised draft labeling has been redacted from this portion of the review.

NDA: 20-484/ Study 702-001 **Study Date:** Dec 1998-Jan 1999

Type of Study: Bioequivalence

Study DMP 702-001 is entitled,

"A PHASE I, SINGLE DOSE, CROSSOVER, BIOEQUIVALENCE STUDY COMPARING TO BE MARKETED TINZAPARIN AND TINZAPARIN-LIKE LOW MOLECULAR WEIGHT HEPARIN IN HEALTHY VOLUNTEERS".

Background

Tinzaparin was developed jointly by two pharmaceutical companies, Leo Pharmaceutical [redacted]. Many early clinical trials were conducted by [redacted] However, the company discontinued its anticoagulation business in 1995; thereafte[redacted] became the sole tinzaparin supplier.

Low molecular weight heparins such as tinzaparin, are polydisperse polysaccharides which include biologically inactive species. The fraction of heparin sub-2k D molecules is generally regarded to be without physiological activity. Due to different volumes of ethanol during the fractionating stage of the manufacturing process of tinzaparin, the fraction of molecules below 2000 D varied between 2.5% and 15.2% in the preparations used in the clinical development of the drug. The to-be-marketed tinzaparin will have less than 10% of the sub-2k D fraction. The remaining molecular distribution (2000-8000 D and > 8000 D) is unchanged.

Objectives

- To demonstrate bioequivalence between tinzaparin formulations containing varying fractions (<10%-16%) of sub-2k D molecules, thought to be the primary pharmacologically-inactive fraction of LMH.

The present study will compare the bioequivalence of the to-be-marketed tinzaparin containing < 10% of the molecules in the sub-2k D fraction to a specially-produced tinzaparin-like low molecular weight heparin with a fraction of sub-2k D molecules of approximately 16%, which corresponds to the highest fraction used in clinical trials (15.2%). Bioequivalence will be assessed based on anti-Xa activity, which is accepted as a pharmacodynamic measure to characterize low molecular weight heparins.

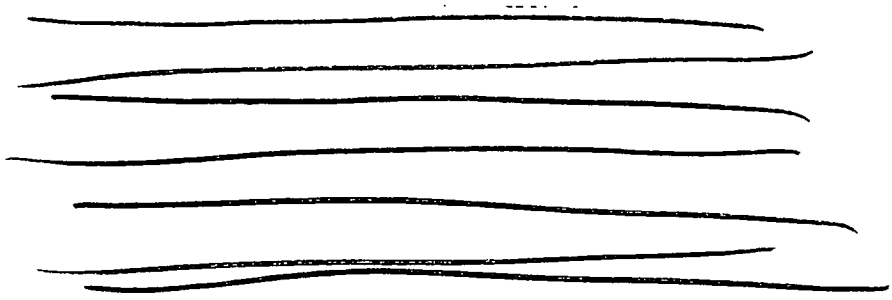
Primary Review Issue

- **Is the sub-2k D fraction in tinzaparin (10-16 %) critical for anti-Xa activity of tinzaparin?**

Study Design

Single dose, open-label, randomized, two-period, crossover study in healthy subjects

Subjects	30 subjects: 22 men and 8 women, (age 41 ± 13 yrs)
Duration of enrollment	2 months
Key Inclusion Criteria	Healthy male and female subjects, over 18 yrs of age Subjects should have a body weight within 15% of the appropriate weight range Female subjects should be non-lactating and of non-childbearing potential Have no significant diseases or clinically significant abnormal lab values
Key Exclusion Criteria	History of allergy or hypersensitivity to heparin or any of its components Evidence of history of diseases associated with bleeding risk or bleeding disorder Any disease or condition that might compromise the cardiovascular, hematological, renal, hepatic, pulmonary, endocrine, central nervous or gastrointestinal systems Any medication that could induce or inhibit hepatic microsomal enzymes within one month of the start of the study Consumption of any medication (including OTC drugs) within 2 weeks of the study start
Study Procedure	Subjects are randomly allocated into one of the two treatment sequences: • **Reference Product**: The to-be marketed tinzaparin formulation (20,000 IU anti-Xa/ml) • **Test Product**: Clinical trial tinzaparin formulation (20,000 IU anti-Xa/ml)
Interperiod Washout	(7-14) days

Sampling	Serum samples collected at the following time points (for both treatments): **Pre-dose** (at 15 min prior to drug administration) **Post-dose**: at 0 min, 15, 30, 45, 60, and 90 min and at 2, 3, 4, 6, 8, 12, 16, 24 and 30 hrs
Anti-Xa Assay Validation	
Safety	Subjects monitored for adverse events. No subject discontinuations were reported in this study.

Pharmacodynamic Data

Two pharmacokinetic / pharmacodynamic parameters were used to determine the activity of tinzaparin in biological samples for the assessment of tinzaparin pharmacodynamics: anti-Xa and anti-IIa activities (using amidolytic methods).
The following pharmacokinetic parameters were determined using non-compartmental analysis: C_{max}, t_{max}, $t_{1/2\lambda z}$, AUC_{0-t} and $AUC_{0-\infty}$.

Statistical Analysis

Assuming a coefficient of variation of 17% and a difference from the reference of no more than 5%, a sample size of at least 20 subjects was determined to provide at least 90% power to establish bioequivalence between the reference and test formulations at a significance level of
0.05.

Prior to testing for bioequivalence, the presence of carry-over effect was assessed using an analysis of variance (ANOVA) model for a two-period crossover. Effects for treatment, sequence, period and subject nested within sequence were included in the model.
Summary statistics (n, mean, SD, median, minimum and maximum) were provided for each pharmacokinetic parameter except for t_{max}, for which median, minimum and maximum values were determined.
Bioequivalence of the test and reference formulations was established if the 90% confidence intervals on the difference in the mean log-transformed C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ fell between 80% and 125%.

Results

1. Anti-Xa Activity

Table 1. Mean (± S.D.) pharmacodynamic parameters of the anti-Xa activity (n = 30)

Treatment	C_{max}	$AUC_{0\rightarrow last}$	$AUC_{0\rightarrow\infty}$
Test	0.887 (0.141)	9.769 (1.628)	10.322 (1.558)
Reference	0.869 (0.236)	8.643 (1.537)	9.552 (1.587)

Table 2. Schuirmann confidence intervals for anti-Xa activity PD parameters

Parameter	Treatment Comparison	Estimate %	Confidence Interval
Ln A_{max}	Test vs. Reference	103.5	(97.4-110.0)
Ln $AUC_{0\rightarrow last}$	Test vs. Reference	113.2	(108.5-118.1)
Ln $AUC_{0\rightarrow\infty}$	Test vs. Reference	108.6	(104.8-112.5)

1. Anti-IIa Activity

Table 3. Mean (± S.D.) pharmacodynamic parameters of the anti-IIa activity (n = 30)

Treatment	C_{max}	$AUC_{0\rightarrow last}$	$AUC_{0\rightarrow\infty}$
Test	0.330 (0.073)	2.901 (0.582)	3.531 (0.669)
Reference	0.301 (0.074)	2.507 (0.538)	3.114 (0.652)

Table 4. Schuirmann confidence intervals for anti-IIa activity PD parameters

Parameter	Treatment Comparison	Estimate %	Confidence Interval
Ln C_{max}	Test vs. Reference	90.8	(84.1-98.1)
Ln $AUC_{0\rightarrow last}$	Test vs. Reference	86.2	(81.3-91.3)
Ln $AUC_{0\rightarrow\infty}$	Test vs. Reference	87.3	(82.2-92.7)

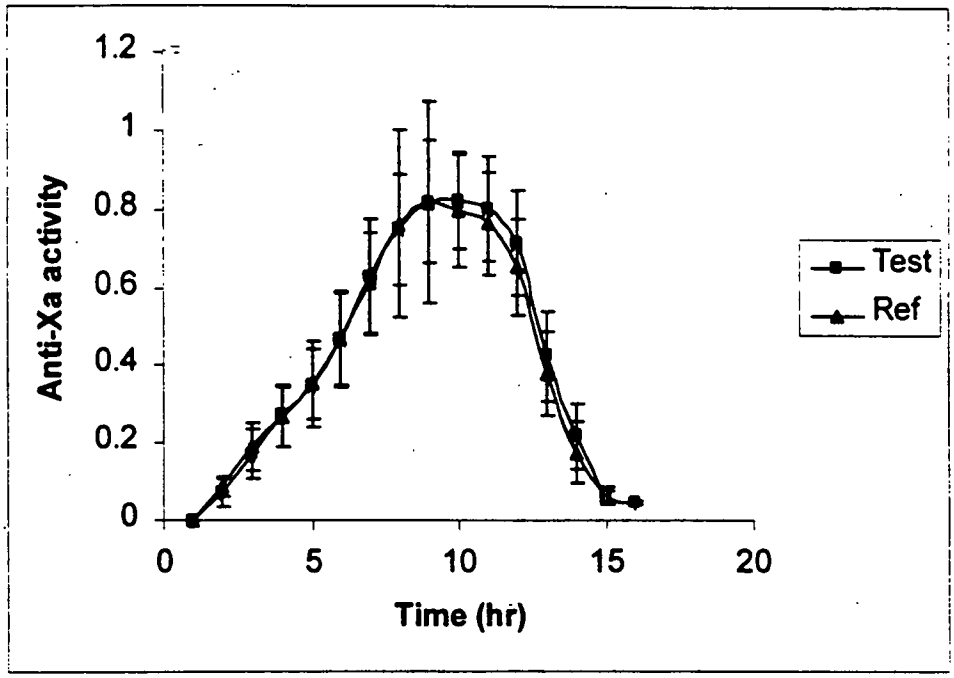


Fig. 1. PK profiles of anti-Xa activity of the tinzaparin reference and test formulations

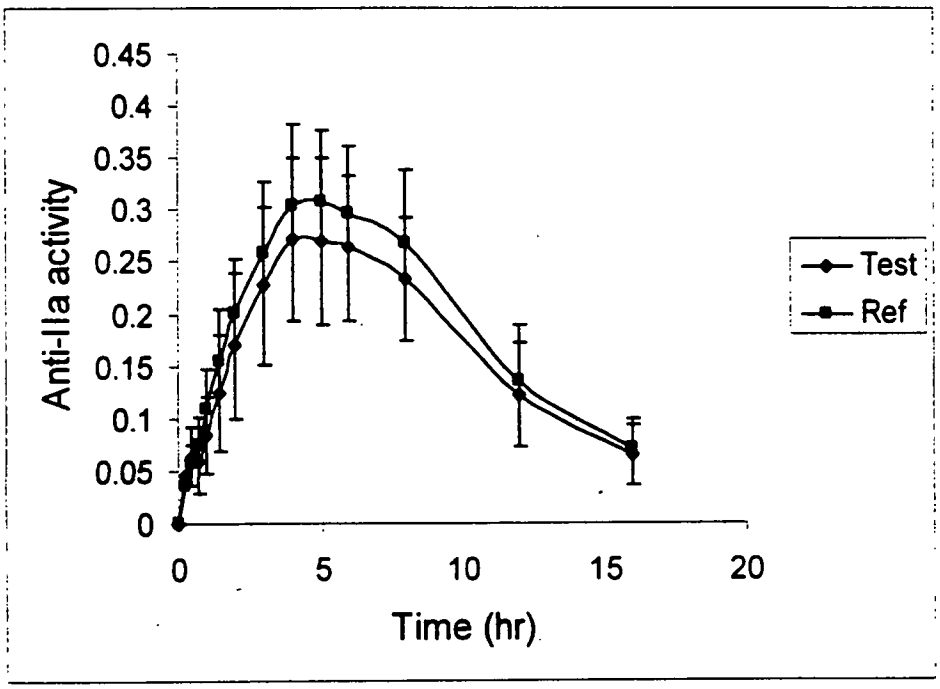


Fig. 2. PK profiles of anti-IIa activity of the tinzaparin reference and test formulations

Reviewer's Comments

- *Anti-Xa/anti-IIa activity ratio is often used as a measure of the ratio of antithrombotic to anticoagulant activities. The anti-Xa/anti-IIa activity ratio based on $AUC_{0-\infty}$ for the two formulations of SC tinzaparin 175 anti-Xa IU/kg ranged between 2.8 and 3.3.*
- *Bioequivalence between the two treatments was tested based on the geometric means of relevant pharmacodynamic parameters. The two formulations pass Schiurmann two one-sided test for bioequivalence on both, anti-IIa and anti-Xa activities. Hence, the two treatments are deemed bioequivalent. In conclusion, tinzaparin formulations composed of as low as 10% and as high as 16% of the < 2000 D fraction would be expected to result in similar anti-Xa and anti-IIa activities.*

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NDA: 20-484/ Study 702-918 **Study Date:** May 1993-Jun 1993

Type of Study: Bioavailability/Bioequivalence/Dose Proportionality

Study DMP 702-918 is entitled,

"A PHASE I PHARMACOKINETIC STUDY OF TINZAPARIN ADMINISTERED INTRAVENOUSLY, AND HEPARIN ADMINISTERED SUBCUTANEOUSLY IN HEALTHY VOLUNTEERS".

Background

During an early stage in the development, several phase I studies were conducted in small numbers of healthy subjects. The pharmacokinetics of tinzaparin was evaluated by pharmacodynamic assessment of anti-Xa and anti-IIa activities. The current study was aimed at providing more accurate biopharmaceutical information on tinzaparin by evaluating pharmacodynamic parameters in a larger number of subjects than previously studied.

Objectives

- Provide accurate estimates of pharmacokinetic parameters of tinzaparin, and to measure activated partial-thromboplastin time (aPTT) after S.C. and I.V. administration.
- Evaluate the absolute bioavailability of S.C. tinzaparin.
- Assess the bioequivalence of tinzaparin with and without preservative (benzyl alcohol) after S.C. administration.
- Evaluate the dose proportionality of tinzaparin over the dose range for prevention of thromboembolism (fixed dose = 4500 anti-Xa IU) and treatment of deep vein thrombosis (fixed dose = 12,250 anti-Xa IU).
- Compare the anticoagulant activity of tinzaparin and heparin administered S.C.

Primary Review Issues:

1. Would the addition of benzyl alcohol , a preservative, to the formulation affect the bioavailabilty of S.C. administered tinzaparin?

2. How large a fraction of tinzaparin is systemically bioavailable after S.C. administration?

3. How does the anticoagulant activity of tinzaparin compare to that of heparin?

4. Is the dose-concentration relationship linear over the proposed therapeutic range?

Study Design

Single-center, open-label, randomized, five-period, five-treatment, crossover study with a Latin-square design

Subjects	30 subjects (23 completed the study)
Duration of Enrollment	5 weeks
Key Inclusion Criteria	Healthy male subjects, 18 to 55 yrs of age Subjects should have a body weight between 65 and 80 kg and within 15% of the ideal weight for height Have no significant diseases or clinically-significant abnormal lab values
Anti-Xa Assay Validation	**Linearity**: The average correlation coefficient of the reference curve (r) was determined as [redacted] **Lower Limit of detection**: [redacted] **Precision**: [redacted] **Accuracy**: For all the samples, the difference between the actual and estimated concentrations was less than 10%
Treatments	Subjects are to receive a single administration of each of the following five treatments: • **Treatment A: Heparin 5000 U, administered S.C.** • **Treatment B: Tinzaparin 4500 anti-Xa IU without preservative, administered S.C.** • **Treatment C: Tinzaparin 4500 anti-Xa IU without preservative, administered I.V.** • **Treatment D: Tinzaparin 12,250 anti-Xa IU with preservative, administered S.C.** • **Treatment E: Tinzaparin 4500 anti-Xa IU with preservative, administered S.C.**
Interperiod Washout	6 days
Sampling Times	Blood samples collected at the following time points:

	IV Administration: **Pre-dose** (at 15 and 0 min prior to drug administration) **Post-dose**: at 5, 10, 15, 30, 45, 60 and 90 min, and at 2, 3, 4, 5, 6, 8, 12, 16 and 24 hrs **S.C. Administration:** **Pre-dose** (at 15 and 0 min prior to drug administration) **Post-dose**: at 15, 30, 45, 60 and 90 min, and at 2, 3, 4, 5, 6, 8, 12, 16, 24 and 32 hrs
Safety	Subjects monitored for adverse events

Analytical assay

Two pharmacodynamic markers were used to determine the activity of ——— in biological samples for the assessment of——— pharmacokinetics: anti-Xa and anti-IIa activities (using amidolytic methods).
The assay was linear in the range ——— for anti-Xa activity and ——— for anti-IIa activity. The lower limit of quantitation was ——— for anti-IIa activity and ——— for anti-Xa activity.

Pharmacodynamic Data

The following pharmacokinetic parameters were determined for both anti-Xa and anti-IIa activities using non-compartmental analysis: C_{max}, t_{max}, $t_{1/2\lambda z}$, Ke, CL, Vd, $AUC_{0\to t}$ and $AUC_{0\to\infty}$ Calculation of the pharmacodynamic parameters was based on baseline-adjusted activity levels.

Statistical Analysis

The mean estimates of the pharmacodynamic parameters and their corresponding standard errors were determined using an ANOVA model adjusted for period, subject and treatment effects. Carryover effects were also tested by adding a first-order carryover variable (subject within sequence) to the ANOVA model. Significance was prospectively defined as $p<0.05$. LSMEANS within SAS was used to determine statistically significant differences between the treatments. Confidence intervals of the ratio of $AUC_{0\to t}$, $AUC_{0\to\infty}$ and C_{max} values were used to test the hypotheses concerning bioavailability, bioequivalence, dose proportionality and comparison to heparin.
A correction factor of 4500/4880 was introduced into the calculations of the pharmacodynamic parameters to correct for the 8.4% overfill in the pre-filled syringes.

Results and Conclusions

Table 5. Summary statistics for pharmacodynamic parameters based on baseline-adjusted **anti-Xa** activity

PD parameter	Summary Statistic	Heparin 5000 IU, S.C. (Trt. A)	Tinzaparin 4500 anti-Xa IU w/o preservative, S.C. (Trt. B)	Tinzaparin 4500 anti-Xa IU w/o preservative, I.V. (Trt. C)	Tinzaparin 12,250 anti-Xa IU w/ preservative, S.C. (Trt. D)	Tinzaparin 4500 anti-Xa IU w/ preservative, S.C. (Trt. E)
C_{max} (IU/ml)	Mean (SD)	0.06 (003)	0.29 (0.06)	1.16 (0.12)	0.85 (0.14)	0.25 (0.05)
	Range				0.62-1.12	
	%CV	51.53	20.51	10.14		20.56
$AUC_{0\rightarrow t}$ (IU·h/ml)	Mean (SD)	0.39 (0.16)	2.24 (0.37)	2.72 (0.40)	9.12 (1.46)	1.88 (0.52)
	Range	0.13-0.83			6.81-12.32	
	%CV		16.57	14.72		27.76
$AUC_{0\rightarrow\infty}$ (IU·h/ml)	Mean (SD)	0.50 (0.19)	2.35 (0.40)	2.75 (0.40)	9.23 (1.47)	1.96 (0.52)
	Range	0.21-0.96			6.91-12.49	
	%CV		17.03	14.45		26.80
t_{max} (h)	Mean (SD)	2.46 (0.93)	3.65 (0.98)	0.15 (0.08)	4.42 (1.27)	3.70 (0.88)
	Range	0.50-4.00			0.75-8.00	
	%CV		26.89	52.00		23.70
CL (L/h)	Mean (SD)	ND	1.97 (0.32)	1.67 (0.22)	1.36 (0.21)	2.42 (0.52)
	Range	ND			0.98-1.77	
	%CV		16.49	13.05		21.39
Vd (L)	Mean (SD)	NA	NA	3.82 (0.41)	NA	NA
	Range	NA	NA		NA	NA
	%CV			10.87		
$t_{1/2}$ (h)	Mean (SD)	ND	4.13 (1.67)	1.60 (0.15)	3.87 (0.68)	3.41 (1.68)
	Range	ND			2.74-5.15	
	%CV		40.55	9.66		49.28

NA=Not Applicable; ND=Not Determined; Trt=Treatment; w/=With; w/o=Without

Table 6. Summary statistics for pharmacodynamic parameters based on baseline-adjusted **anti-IIa** activity

PD parameter	Summary Statistic	Heparin 5000 IU, S.C. (Trt. A)	Tinzaparin 4500 anti-Xa IU w/o preservative, S.C. (Trt. B)	Tinzaparin 4500 anti-Xa IU w/o preservative, I.V. (Trt. C)	Tinzaparin 12,250anti-Xa IU w/ preservative, S.C. (Trt. D)	Tinzaparin 4500 anti-Xa IU w/ preservative, S.C. (Trt. E)
C_{max} (IU/ml)	Mean (SD) Range	0.03 (0.02)	0.09 (0.03)	0.58 (0.07)	0.32 (0.10) 0.16-0.57	0.08 (0.03)
	%CV	64.57	37.41	12.82		31.23
AUC_{0-t} (IU·h/ml)	Mean (SD) Range	0.14 (0.09)	0.62 (0.21)	1.08 (0.19)	2.71 (0.56) 1.72-3.96	0.56 (0.17)
	%CV	62.94	33.56	17.94		31.09
$AUC_{0-\infty}$ (IU·h/ml)	Mean (SD) Range	0.20 (0.18)	0.70 (0.23)	1.10 (0.19)	2.85 (0.66) 1.98-4.88	0.64 (0.19)
	%CV	90.45	32.03	17.65		29.50
t_{max} (h)	Mean (SD) Range	2.70 (1.44)	3.61 (0.89)	0.18 (0.10)	4.47 (1.37) 0.75-8.00	3.67 (1.14)
	%CV	53.24	24.70	57.03		31.15
CL (L/h)	Mean (SD) Range	ND ND	4.49 (2.58)	2.51 (0.39)	2.64 (0.54) 1.48-3.65	4.77 (1.37)
	%CV		57.44	15.67		28.81
Vd (L)	Mean (SD) Range	NA NA	NA NA	4.88 (1.18)	NA NA	NA NA
	%CV			24.26		
$t_{1/2}$ (h)	Mean (SD) Range	ND ND	4.49 (2.50)	1.36 (0.31)	3.35 (1.21) 1.68-6.35	5.05 (3.64)
	%CV		55.64	22.50		72.14

NA=Not Applicable; ND=Not Determined; Trt=Treatment; w/=With; w/o=Without

- Bioavailability of S.C. tinzaparin

The bioavailability of S.C. tinzaparin was assessed by comparing the mean $AUC_{0-\infty}$ estimates of tinzaparin 4500 anti-Xa IU S.C. without preservative (treatment B) to tinzaparin 4500 anti-Xa IU I.V. without preservative (treatment C). The absolute

bioavailability of S.C. tinzaparin was estimated to be 86.7% on anti-Xa activity and 58.7% on anti-IIa activity.-

Table 7a. PD parameters based on **anti-Xa** activity of treatment B vs. treatment C

PD Parameter	Geometric Mean Ratio (%) (Test/reference)	90% Confidence Interval	P-value
C_{max}	24.6	(22.2, 27.2)	0.0001
$AUC_{0\to last}$	83.7	(76.3, 91.9)	0.0021
$AUC_{0\to\infty}$	86.7	(78.7, 95.5)	0.0163

Table 7b. PD parameters based on **anti-IIa** activity of treatment B vs. treatment C

PD Parameter	Geometric Mean Ratio (%) (Test/reference)	90% Confidence Interval	P-value
C_{max}	14.3	(12.5, 16.4)	0.0001
$AUC_{0\to last}$	53.0	(44.8, 62.7)	0.0001
$AUC_{0\to\infty}$	58.6	(48.2, 71.6)	0.0001

- Bioequivalence of S.C. tinzaparin formulations with and without preservative

The bioequivalence of S.C. tinzaparin 4500 anti-Xa IU with preservative (Trt. E) and without preservative (Trt. B) was assessed on anti-Xa and anti-IIa activities.

Table 8a. PD parameters based on **anti-Xa** activity of treatment E vs. treatment B

PD Parameter	Geometric Mean Ratio (%) (Test/reference)	90% Confidence Interval	P-value
C_{max}	91.9	(83.1, 101.6)	0.1663
$AUC_{0\to last}$	88.4	(80.6, 97.0)	0.0292
$AUC_{0\to\infty}$	88.4	(80.3, 97.3)	0.0348

Table 8b. PD parameters based on **anti-IIa** activity of treatment E vs. treatment B

PD Parameter	Geometric Mean Ratio (%) (Test/reference)	90% Confidence Interval	P-value
C_{max}	102.9	(90.1, 117.7)	0.7195
$AUC_{0\to last}$	100.9	(85.4, 119.3)	0.9271
$AUC_{0\to\infty}$	102.3	(84.1, 124.6)	0.8450

Reviewer's Comments: The results show that the 90% CI of the parameter ratios are within the specified limits of 80-125% for bioequivalence. Hence, the presence or

absence of the preservative (benzyl alcohol) does not seem to impact bioavailability of tinzaparin SC formulation.

- Dose proportionality of S.C. tinzaparin formulations

The dose proportionality of S.C. tinzaparin with preservative was assessed by comparing the dose-normalized pharmacodynamic parameters of 12,250 anti-Xa IU formulation (Trt. D) to the parameters for 4500 anti-Xa IU formulation (Trt. E).

Table 9a. PD parameters based on **anti-Xa** activity of treatment D vs. treatment E

PD Parameter	Geometric Mean Ratio (%) (Test/reference)	90% Confidence Interval	P-value
C_{max}	127.1	(114.8, 140.6)	0.0002
$AUC_{0\rightarrow last}$	180.4	(164.4, 197.9)	0.0001
$AUC_{0\rightarrow\infty}$	175.4	(159.3, 193.2)	0.0001

Table 9b. PD parameters based on **anti-IIa** activity of treatment D vs. treatment E

PD Parameter	Geometric Mean Ratio (%) (Test/reference)	90% Confidence Interval	P-value
C_{max}	140.5	(122.9, 160.7)	0.0001
$AUC_{0\rightarrow last}$	182.2	(154.0, 215.5)	0.0001
$AUC_{0\rightarrow\infty}$	165.7	(136.0, 201.9)	0.0001

- Comparison to heparin

Heparin 5000 U, S.C. (Trt. A), was compared to S.C. tinzaparin 4500 anti-Xa IU with preservative (Trt. E). The mean parameter ratios were corrected for the dose ratio (4500/5000).

Table 10a. PD parameters based on **anti-Xa** activity of treatment A vs. treatment E

PD Parameter	Geometric Mean Ratio (%) (Test/reference)	90% Confidence Interval	P-value
C_{max}	18.9	(17.1, 20.9)	0.0001
$AUC_{0\rightarrow last}$	17.6	(16.1, 19.3)	0.0001
$AUC_{0\rightarrow\infty}$	22.1	(20.1, 24.3)	0.0001

Table 10b. PD parameters based on **anti-IIa** activity of treatment A vs. treatment E

PD Parameter	Geometric Mean Ratio (%) (Test/reference)	90% Confidence Interval	P-value
C_{max}	27.3	(23.9, 31.2)	0.0001
$AUC_{0\rightarrow last}$	18.5	(15.7, 21.9)	0.0001
$AUC_{0\rightarrow\infty}$	20.0	(16.4, 24.3)	0.0001

- Anticoagulant activity of treatments

The mean aPTT activity levels were determined over time for each of the five treatments. Following administration of I.V. tinzaparin 4500 anti-Xa IU (Trt. C), aPTT activity reached a maximum of 5.07 U/ml at 5 min. Following S.C. tinzaparin 4500 anti-Xa IU (Trt. B and E), mean aPTT activity-time profiles demonstrated a slow absorption, with maximum activity (3.75 U/ml) occurring by 3 hrs after administration. Administration of S.C. tinzaparin 12,250 anti-Xa IU (Trt. D) resulted in maximum aPTT levels of 4.21 U/ml at 4 hrs post-dose. S.C. heparin 5000 U (Trt. A) demonstrated a slow rise to maximum levels of 3.60 U/ml at 3 hrs after administration.

- Anti-Xa/anti-IIa activity ratios

The mean anti-Xa/anti-IIa activity ratios were assessed at each time point following administration of each of the five treatments.

Table 11. Anti-Xa/anti-IIa activity ratio based on $AUC_{0\rightarrow\infty}$

PD parameter	Summary Statistic	Heparin 5000 IU, S.C. (Trt. A)	Tinzaparin 4500 anti-Xa IU w/o preservative, S.C. (Trt. B)	Tinzaparin 4500 anti-Xa IU w/o preservative, I.V. (Trt. C)	Tinzaparin 12,250 anti-Xa IU w/ preservative, S.C. (Trt. D)	Tinzaparin 4500 anti-Xa IU w/ preservative, S.C. (Trt. E)
$AUC_{0\rightarrow\infty}$	Mean 90% CI	3.41 (2.44-4.76)	3.51 (3.06-4.03)	2.51 (2.42-2.60)	3.28 (3.12-3.44)	3.10 (2.81-3.42)

Reviewer's Comments:

- *The data indicate that there is no dose proportionality between the 4500 anti-Xa IU and 12,250 anti-Xa IU tinzaparin formulations. An increase in the dose of tinzaparin would be expected to result in a greater than proportional increase in anti-Xa and anti-IIa activities.*
- *The anti-Xa/anti-IIa activity ratio in the current study ranged from 2.5 to 3.5. This agrees well with the anti-Xa/anti-IIa activity ratio reported in study 702-001 (2.8-3.3).*

NDA: 20-484/ Study 702-919 **Study Date:** Nov 1984-Feb 1985

Type of Study: PK of Single Dose SC tinzaparin

Introduction

Study DMP 702-919 is entitled,

"INVESTIGATION OF THE PHARMACOKINETICS OF LOW MOLECULAR WEIGHT HEPARIN [redacted]

Background

The current study was conducted early on in the development of tinzaparin by [redacted] back in 1984-1985. It was aimed at investigating the pharmacokinetic and pharmacodynamic characteristics of low molecular weight heparin [redacted] in humans. "Low molecular weight heparin[redacted]" will be referred to as "tinzaparin" throughout the current review.

Upon this reviewer's request for data reanalysis and information relating to analytical methods validation, the Firm submitted a report pursuant to the current study (See DMP 00-010, dated Feb 9, 2000).

Objectives

- Investigate the pharmacokinetics/pharmacodynamics of tinzaparin after a single S.C. injection.
- Compare the pharmacodynamic activity of tinzaparin to that of conventional heparin.
- Compare the bioavailability of two different lots of conventional heparin to determine if any significant lot to lot variation exists (Lot 5864 & lot OR314).

Primary Review Issues:

1. **How does the anticoagulant activity of tinzaparin compare to that of heparin?**
2. **What are the primary pharmacokinetic and pharmacodynamic characteristics of tinzaparin?**
3. **Do different heparin lots have different systemic bioavailabilities?**

Study Design

Single dose, randomized, crossover study in healthy subjects. The study was double-blinded for S.C. injections, but open-label for I.V. injections.

Subjects 6 subjects (4 men and 2 women); age 27-39 yrs

Key Inclusion Criteria	Healthy male and female subjects, over 18 yrs of age Subjects should have a body weight between 65 and 80 kg and within 15% of the ideal weight for height
Key Exclusion Criteria	History of allergy or hypersensitivity to heparin or any of its components Evidence of history of diseases associated with bleeding risk or bleeding disorder Any disease or condition that might compromise the cardiovascular, or renal, systems
Treatments	Subjects are randomized to receive a single administration of each of the following six treatments: • **Treatment A: Tinzaparin 2,500 anti-Xa IU, administered S.C.** • **Treatment B: Tinzaparin 5,000 anti-Xa IU, administered S.C.** • **Treatment C: Heparin 5000 anti-Xa IU, administered S.C., lot 5864** • **Treatment D: Tinzaparin 10,000 anti-Xa IU, administered S.C.** • **Treatment E: Heparin 5000 anti-Xa IU, administered S.C., lot OR314** • **Treatment F: Tinzaparin 5,000 anti-Xa IU, administered I.V.**
Washout	7 days
Sampling Times	Serum samples collected at the following time points: **IV administration:** **Pre-dose** (at 0 min prior to drug administration) **Post-dose**: at 2, 5, 10, 15, 30 min, and at 1, 2, 4, 6, 8 and 24 hrs **S.C. Administration:** **Pre-dose** (at 0 min prior to drug administration) **Post-dose**: at 15 and 30 min, and at 1, 2, 4, 6, 8 and 24 hrs
Safety	Subjects monitored for adverse events.

Pharmacodynamic Data

Three primary pharmacokinetic / pharmacodynamic parameters were used to determine the activity of tinzaparin in biological samples for the assessment of tinzaparin pharmacodynamics: anti-Xa and anti-IIa activities (using amidolytic methods) and aPTT. In addition, several secondary pharmacodynamic parameters were determined including, antithrombin III, platelet count, plasminogen, tPA, tPA inhibitor, α_2-antiplasmin and ECLT (Euglobulin clot lysis time).

Statistical Analysis

A formal statistical analysis of the data to compare various treatments was not conducted due to the limited sample size and marked variability in the study data.

Results

No significant changes were observed for any of the secondary pharmacodynamic parameters with tinzaparin or heparin treatment.

The results of data reanalysis are shown below in tables 12 and 13.

1. Anti-Xa Activity

Table 12. Mean (± S.D.) pharmacodynamic parameters of the anti-Xa activity (n = 36)

Treatment	Dose/Route IU/ IV or SC	C_{max} (IU/ml)	T_{max} (hr)	AUC_{0-t} (IU·hr/ ml)	$T_{1/2}$ (h)	Vd (L)	CL (L/hr)
A (Tinzaparin)	2500/SC	0.13 (0.04)	0.38 (0.14)	0.27 (0.10)	2.07 (0.78)	---	---
B (Tinzaparin)	5000/SC	0.28 (0.16)	0.58 (0.2)	0.47 (0.21)	2.29 (1.09)	---	---
C (Heparin, Lot #5864)	5000/SC	0.05 (0.01)	0.13 (0.09)	0.10 (0.06)	4.26 (1.11)	---	---
D (Tinzaparin)	10000/SC	0.55 (0.19)	0.58 (0.20)	1.33 (0.49)	2.42 (1.87)	---	---
E (Heparin, Lot #OR 314)	5000/SC	0.04 (0.02)	0.4 (0.31)	0.04 (0.03)	1.15 (0.59)	---	---
F (Tinzaparin)	5000/IV	1.3 (0.27)	0.06 (0.05)	3.03 (1.44)	3.24 (2.45)	6.74 (2.16)	1.92 (1.02)

Anti-IIa Activity

Table 13. Mean (± S.D.) pharmacodynamic parameters of the anti-IIa activity (n = 36)

Treatment	Dose/Route IU/ IV or SC	C_{max} (IU/ml)	T_{max} (hr)	AUC_{0-t} (IU·hr/ml)	$T_{1/2}$ (h)	Vd (L)	CL (L/hr)
A (Tinzaparin)	2500/SC	0.02 (0.01)	0.24 (0.14)	0.02 (0.01)	2.53 (1.57)	---	---
B (Tinzaparin)	5000/SC	0.04 (0.02)	0.75 (0.67)	0.07 (0.04)	3.63 (4.62)	---	---
C (Heparin, Lot #5864)	5000/SC	0.02 (0.02)	0.27 (0.20)	0.04 (0.03)	5.95 (6.67)	---	---
D (Tinzaparin)	10000/SC	0.08 (0.03)	0.67 (0.26)	0.16 (0.06)	3.4 (2.71)	---	---
E (Heparin, Lot #OR 314)	5000/SC	0.04 (0.02)	0.24 (0.17)	0.08 (0.08)	5.2 (5.13)	---	---
F (Tinzaparin)	5000/IV	0.28 (0.06)	0.03	0.50 (0.19)	1.39 (0.71)	20.39 (10.39)	11.05 (5.54)

Reviewer's Comments

The compartmental pharmacokinetic approach utilized for calculation of the basic pharmacokinetic parameters in the original study report was deemed to be of little value compared to the non-compatmental approach. This is particularly an important point since the study data was extracted from a small number of subjects and thus expectedly, was associated with high variability. Hence, this reviewer requested recalculation of the relevant pharmacokinetic parameters using a non-compartmental approach.

Overall, the two heparin formulations from different lots showed similar anti-IIa and anti-Xa plasma profiles despite significant differences in the profiles of terminal phase, which might be due to the inherent variability in heparin composition and the analytical assay. Bioavailability calculations were not performed due to large variability in the data that lead to poor characterization of the terminal elimination phase.

Administration of varying doses of tinzaparin resulted in dose-proportional increases in anti-Xa and anti-IIa activity levels. However, a small deviation towards a greater than proportional increase in AUC with increasing tinzaparin doses was noted for both anti-Xa and anti-IIa activity levels. This was particularly true for tinzaparin 5,000 anti-Xa IU and 10,000 anti-Xa IU formulations.

A comparison between the tinzaparin 5000 IU, SC treatment and corresponding heparin treatments with respect to their anti-Xa and anti-IIa levels showed that tinzaparin generally results in a higher anti-Xa activity, but a lower anti-IIa activity.

The study results point to a trend for a linear correlation between body weight and AUC of anti-Xa and anti-IIa. However, due to the limited sample size in this study, this has yet to be confirmed.

NDA: 20-484/ Study 702-920 Study Date: Feb 1985-May 1985

Type of Study: Safety and PK of IV Tinzaparin

Study DMP 702-920 is entitled,

"SAFETY EVALUATION AND PHARMACOKINETICS AFTER INTRAVENOUS ADMINISTRATION OF LOW MOLECULAR WEIGHT HEPARIN [redacted]

Objectives

- Compare the safety and the pharmacokinetics of tinzaparin and heparin after I.V. injection in healthy subjects.

Primary Review Issues

- **How does tinzaparin compare to heparin in terms of safety and pharmacodynamic activity?**

Study Design

Single dose, single-blind, non-randomized, crossover comparative study

Subjects	6 healthy male subjects; age 21-38 yrs
Key Exclusion Criteria	History of allergy or hypersensitivity to heparin or any of its components Evidence of history of diseases associated with bleeding risk or bleeding disorder Any disease or condition that might compromise the cardiovascular, or renal, systems
Treatments	Subjects are to receive a single administration of each of the following four treatments in the following order: • **Treatment A: Heparin 5,000 IU, administered I.V.** • **Treatment B: Tinzaparin 2,500 anti-Xa IU, administered I.V.** • **Treatment C: Tinzaparin 5,000 anti-Xa IU, administered I.V.** • **Treatment D: Tinzaparin 4,000 anti-Xa IU, administered I.V.**

Assay Validation	Precision (anti-Xa activity): —— Precision (anti-Xa activity): —— LOD and LOQ were not provided
Washout	(7-14) days
Sampling Times	Serum samples collected at the following time points: **Pre-dose** (at 0 min prior to drug administration) **Post-dose**: at 2, 5, 10, 15, and 30 min, and at 1, 2, 4, 6, 8, 11.5 and 24 hrs

Pharmacodynamic Data

Three pharmacokinetic / pharmacodynamic parameters were used to determine the activity of tinzaparin in biological samples for the assessment of tinzaparin pharmacodynamics: anti-Xa and anti-IIa activities (using amidolytic methods) and aPTT. A first-order elimination model was used to estimate half-life and volume of distribution for each treatment.

Safety Analysis

Safety was evaluated by analysis of haematological, biochemical and urinalysis parameters before injection and at 2 and 24 hr after injection of each treatment. Safety was also assessed by incidence of adverse events.

Statistical Analysis

The pharmacokinetic parameters were calculated from each subject individually, then mean values and 95% confidence intervals were calculated from the individual parameters.
Analysis of variance and covariance was utilized to evaluate the effects of treatments and sampling times on the safety parameters.

Results and Conclusions

Table 14. Pharmacodynamic parameters based on baseline-adjusted **anti-Xa** activity

PD parameter	Treatment A	Treatment B	Treatment C	Treatment D
$t_{1/2}$ (min) Mean 90% CI	 60 (48-80)	 59 (46-81)	 116 (78-122)	 78 (59-115)
Vd (L) Mean 90% CI	 4.6 (3.7-6.0)	 3.9 (2.7-5.0)	 3.6 (3.0-4.4)	 2.4 (2.1-2.9)

Table 15. Pharmacodynamic parameters based on baseline-adjusted **anti-IIa** activity

PD parameter	Treatment A	Treatment B	Treatment C	Treatment D
$t_{1/2}$ (min) Mean 90% CI	49 (41-60)	68 (52.97)	80 (61-118)	62 (50-82)
Vd (L) Mean 90% CI	5.1 (4.5-5.9)	7.2 (4.4-21)	6.7 (5.3-9.2)	5.5 (4.3-7.6)

Table 16. Pharmacodynamic parameters based on baseline-adjusted **aPTT** activity

PD parameter	Treatment A	Treatment B	Treatment C	Treatment D
$t_{1/2}$ (min) Mean 90% CI	48 (34-80)	35 (26-52)	57 (59-109)	32 (26-44)
Vd (L) Mean 90% CI	3.9 (3.4-4.7)	5.9 (3.1-5.9)	4.5 (3.4-6.4)	2.8 (2.3-3.7)

Reviewer's Comments:

The Firm only determined half-life and volume of distribution, while other relevant pharmacokinetic parameters as CL and AUC were not determined. In addition, due to the limited sample size employed in the study and the large variability in the data, a meaningful comparison between the four treatments in the study is not feasible.

NDA: 20-484/ Study 702-921 **Study Date:** Apr 1986-Sep 1986

Type of Study: Safety of Single Dose SC Tinzaparin

Introduction

Study DMP 702-921 is entitled,

"PHASE I PHARMACODYNAMICS AND EFFECT OF LHN-1 ON COAGULATION PARAMETERS IN NORMAL, HEALTHY VOLUNTEERS IN A 5 DAY DOSE REGIMEN "

Objectives

- Compare the effects of 5 SC tinzaparin dosing regimens on the coagulation mechanisms in healthy male subjects.
- Determine the clinical and haematological effects of 5 SC tinzaparin dosing regimens.

Primary Review Issues:

- **How do once and twice daily multiple dosing regimens of tinzaparin compare with respect to their hematological effects?**

Study Design

Open-label, non-randomized, five-period, parallel group study

Subjects	40 subjects
Duration of Study	5 days
Key Inclusion Criteria	Healthy male subjects, 18 to 40 yrs of age Subjects should have a body weight between 65 and 80 kg and within 20% of the ideal weight for height Have no significant diseases or clinically-significant abnormal lab values
Treatments	Subjects were divided into 5 groups and each group was to receive one of the following five SC treatments for 5 consecutive days: • **Treatment A: Tinzaparin 2,500 anti-Xa IU QD** • **Treatment B: Tinzaparin 5,000 anti-Xa IU QD**

- **Treatment C: Tinzaparin 7,500 anti-Xa IU QD**
- **Treatment D: Tinzaparin 2,500 anti-Xa IU BID**
- **Treatment E: Tinzaparin 5,000 anti-Xa IU BID**

Assay Validation	Precision (anti-Xa activity): ~~~ Precision (anti-Xa activity): Not provided LOD and LOQ were not provided
Sampling Times	Plasma samples were collected at the following time points: **Pre-dose** (at 0 min prior to drug administration on days 1-5) **Post-dose**: **Day 1**: at 1, 2, 3 and 4 hrs **Day 2-4:** at 3 hrs **Day 5:** at 1, 2, 3, 8 and 24 hrs
Safety	Subjects monitored for adverse events

Pharmacodynamic Data

The only relevant pharmacodynamic parameters to be determined during the study were anti-Xa activity and aPTT.

Results and Conclusions

No pharmacokinetic parameters were determined.
Significant increases in aPTT and prolongation of thrombin time was observed in all treatment groups. APTT levels returned to normal within 24 hrs after the last dose. As for anti-Xa activity, a significant increase was observed 3 hrs after injection after both once and twice daily dosing. However, only twice daily dosing resulted in a measurable increase in anti-Xa activity on the morning just prior to the next daily dose.

Reviewer's Comments:

Very little, if any, conclusions can be made based on the study data. This is primarily due to the limiting deficiencies in the study design, including infrequent and small number of sampling time points, resulting in incomplete characterization of the pharmacokinetic profiles of the treatments. In addition, anti-IIa, an important primary pharmacodynamic parameter, was not determined in the course of the study.

NDA: 20-484/ Study 702-922 Study Date: Oct 1987-Apr 1988

Type of Study: Safety of Multiple Dose SC Tinzaparin

Introduction

Study DMP 702-922 is entitled,

"TOLERANCE AND PHARMACODYNAMIC STUDY OF LOGIPARIN, A LOW MOLECULAR WEIGHT HEPARIN COMPOUND, IN HEALTHY MALE VOLUNTEERS"

Objectives

- Investigate the tolerance and pharmacodynamic effects of three different doses of tinzaparin in healthy male subjects

Primary Review Issues:

1. **How comparable are the tolerance and pharmacodynamic profiles of three different multiple dosing regimens of tinzaparin?**
2. **How effective is protamine sulfate in neutralizing the effects of tinzaparin?**

Study Design

Open-label, non-randomized, parallel group study

Subjects	18 subjects
Duration of Study	5 days
Key Inclusion Criteria	Healthy male subjects, 18 to 45 yrs of age Subjects should have a body weight within 10% of their ideal weight Have no significant diseases or clinically-significant abnormal lab values
Treatments	Subjects were divided into 3 groups and each group was to receive one of the following three SC treatments for 5 consecutive days: • **Group 1: Tinzaparin 150 anti-Xa IU/kg BID** • **Group 2: Tinzaparin 250 anti-Xa IU/kg QD (Discontinued after the first dose due to large changes in coagulation parameters on day 1 following dosing in 4 patients)**

- **Group 3: Tinzaparin 200 anti-Xa IU/kg QD**

Protamine sulfate, IV to be administered after the last dose of tinzaparin in each subject

Sampling Times — Blood samples collected at the following time points:
Pre-dose (at day -1)
Post-morning dose on days 1-5: at 1, 4, 12, 16, 20 and 24 hrs
Additional samples collected at 4.25 and 6 hrs after the last dose in group 1, and at 4.25, 6 and 8 hrs after the last dose in group 3

Safety — Subjects monitored for adverse events

Pharmacodynamic Markers

The relevant pharmacodynamic parameters to be determined during the study were anti-Xa activity, anti-IIa activity, Thrombin time and aPTT.

Pharmacokinetic Analysis

No pharmacokinetic analysis of the data was performed by the Firm.

Statistical Analysis

Groups 1 and 3 were analyzed separately. A two factor (subject and time) analysis of variance was performed on each parameter.
To determine changes over the five treatment days, a series of Dunnet's t-tests were conducted comparing day –1 mean values with all subsequent mean values for the parameter.
The neutralizing effects of protamine sulfate on day 5 was assessed by comparing the mean value obtained immediately before administration of protamine sulfate and that 15 min after administration.

Results and Conclusions

Pharmacodynamic parameters

aPTT

Significant increases in aPTT were observed 4 hrs after both doses in group1 and 1 hr after the morning dose on each study day. As for group 3, significant increases in aPTT were observed 1 and 4 hrs after the dose on each study day and at 6 and 8 hrs post dose on day 5.
The maximum effect was noted 4 hrs post dose with aPTT values returning to normal at 12 hrs post dose.
After protamine sulfate administration to both groups, aPTT levels were significantly reduced in group 3, but not in group 1. However, at 6 hrs post dose on day 5 in group 3, no reduction in aPTT level was visible.

Clinical Pharmacology and Biopharmaceutics Review

NDA: 20-484 **Submission Date**: 7/1/1999, 2/9/2000, 2/22/2000

Trade Name: innohep®

Active Ingredient: Tinzaparin Sodium

Sponsor: Dupont Pharmaceuticals Company

Reviewer: Suliman I. Al-Fayoumi, Ph.D.

Type of Submission: Original NDA-NME (1S)

Synopsis

The pharmacokinetics (PK) of IV and SC tinzaparin, measured as anticoagulant activity (anti-Xa and anti-IIa activities), have been characterized in healthy subjects as well as in the relevant patient population. The sponsor demonstrated bioequivalence between the To-Be-Marketed and Clinical Trial formulations. The effects of various covariates such as age, weight and renal function, have been examined on tinzaparin PK in the relevant patient population using population PK analysis.

Recommendation

NDA 20-484 for tinzaparin sodium (innohep®) was submitted on 7/1/1999. The Human Pharmacokinetics and Bioavailability section of the application has been reviewed by the Office of Clinical Pharmacology and Biopharmaceutics (OCPB/Division of Pharmaceutical Evaluation II). From the view point of OCPB, the submission is acceptable.

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Suresh Doddapaneni, Ph.D., Team leader _____

cc: HFD-180: NDA 20-484 (1x); DIV FILE (1x); KOLIVER (1x); SDODDAPANENI (1x); SALFAYOUMI (1x); HFD-870 SHUANG (1x); CDR: ATTN Zom Zadeng

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I. Introduction

Tinzaparin is a low molecular weight heparin (LMH) that is produced by controlled enzymatic depolymerization of unfractionated porcine heparin using heparinase. Tinzaparin inhibits reactions that lead to the clotting of blood and formation of fibrin clots both *in vitro* and *in vivo*. It acts as potent co-inhibitor of several activated coagulation factors, in particular factors Xa and IIa (thrombin).

Tinzaparin sodium is currently approved in over 20 countries for treatment of deep vein thrombosis (DVT), and perioperative thromboembolism prophylaxis in orthopedic (hip/knee replacement) or general surgery settings. Three other LMHs, enoxaparin sodium (NDA 20-164), dalteparin sodium (NDA 20-287) and ardeparin sodium (NDA 20-227), are currently marketed in the US.

NDA 20-484 for tinzaparin sodium (innohep®) was submitted to the Agency on 7/1/1999. The sponsor's proposed indications include the treatment of acute symptomatic DVT with and without pulmonary embolism when administered in conjunction with warfarin sodium.

For the treatment of DVT, a tinzaparin dose of 175 anti-Xa IU/kg of body weight is proposed, given SC once daily for at least 6 days and until the patient is adequately anticoagulated with warfarin.

Due to difficulty in measuring the chemical entity in blood, kinetics of LMHs are characterized by measurement of two commonly used biomarkers, anti-factor Xa (anti-Xa) and anti-thrombin (anti-IIa) activities.

The sponsor has submitted 11 pharmacokinetic (PK)/pharmacodynamic (PD) studies in support of this submission, including two additional population PK analyses, which were aimed at assessing the PK of anti-Xa activity of tinzaparin in the targeted patient population as well as, evaluating the influence of several covariates such as body weight , age and renal function on tinzaparin clearance. A Question-Based Review of the NDA yielded the following information:

II. Analytical Assay

Is the analytical assay methodology adequately validated?

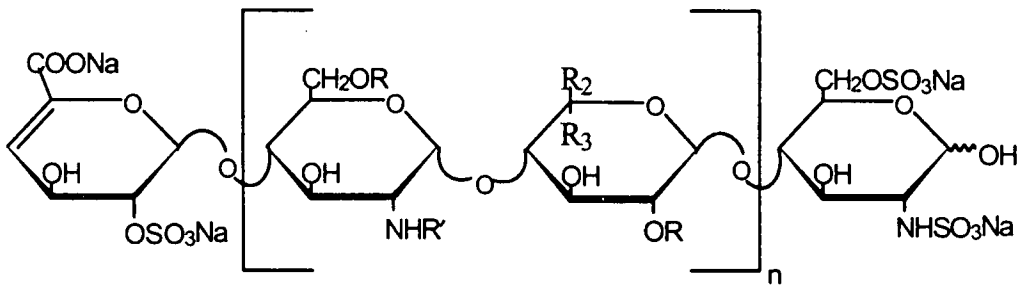
Since tinzaparin plasma concentrations can't be directly measured, several pharmacodynamic biomarkers are used to determine the activity of tinzaparin in blood for the assessment of tinzaparin pharmacokinetics; the most relevant of which are anti-Xa and anti-IIa activities (determined using amidolytic (chromogenic) methods).

The amidolytic methods are based on an enzymatic reaction in which the target enzyme interacts with a specific substrate. This substrate is an oligopeptide, which contains a specific site for binding to a given enzyme, with an attached chromophore end group: paranitroaniline. The binding of the substrate with the target enzyme (factor Xa or factor IIa) releases the chromophore group, which can be quantified spectrophotometrically at 405 nm.

The development of tinzaparin has spanned the evolution of chromogenic assay methodologies for LMH. Hence, out of all the PK/PD studies submitted in the NDA application, only two studies included full analytical methods validation reports (studies DMP 702-001 & DMP 702-918). PK studies with tinzaparin have come from 7 different laboratories using 8 methods for anti-Xa activity and 4 methods for anti-IIa activity. Some of the limitations posed by the use of invalidated chromogenic assays include: 1) unspecified limits of quantitation, 2) activity greater than the upper limit of quantitation were extrapolated from the standard curve, 3) large baseline variability. Appendix A contains sponsor's clarification regarding this reviewer's query on the aforementioned aspects. In addition, table 1.4 in Appendix B contains a summary of the available analytical methods validation data for all the submitted PK studies.

III. Formulation

What is the composition of the SC tinzaparin formulation?



Tinzaparin sodium

n= 1 to 25, R = H or SO_3Na, R' = H or SO_3Na or $COCH_3$

R_2 = H and R_3 = COONa or R_2 = COONa and R_3= H

The average molecular weight for tinzaparin ranges between 5,500 and 7,500 daltons.
The molecular weight distribution is:
<2,000 daltons < 10%
2,000 to 8,000 daltons 60% to 72%
8,000 daltons 22% to 36%

In early supportive PK studies, innohep® SC injections consisted of aqueous tinzaparin solutions preserved with benzyl alcohol (10 mg/ml) and were produced [redacted] In more recent PK studies, several changes were made to the formulation including; variation in benzyl alcohol content from 0 to 13.5 mg/ml (see study

DMP 702-918), the addition of sodium metabisulfite and the use of [redacted] in the formulation process. However, none of these variations has been shown to alter bioavailability of the formulation.
A list of the tinzaparin batches used in the PK studies is included in table 1.3 in Appendix B.

IV. Bioavailbility

How large a fraction of tinzaparin is systemically available after SC administration?

The absolute bioavailability (following administration of 4,500 anti-Xa IU SC and IV formulations) is 86.7% based on anti-Xa activity (see study DMP 702-918).

V. Bioequivalence

Is the To-Be-Marketed formulation equivalent to the Clinical Trial formulation?

The to-be-marketed tinzaparin containing < 10% of the molecules in the sub-2k D[1] fraction appeared to be bioequivalent to a specially-produced tinzaparin-like LMH with a fraction of sub-2k D molecules of approximately 16%, which corresponds to the highest fraction used in clinical trials (15.2%) (see study DMP 702-001).

VI. Dose Proportionality

Is there dose proportionality for the anti-Xa activity?

The administration of 4,500 anti-Xa IU and 12,250 anti-Xa IU doses of tinzaparin resulted in increases in anti-Xa activity that were greater than dose proportional (based on $AUC_{0-\infty}$) (see study DMP 702-918).

VII. Pharmacokinetics

The sponsor has characterized the single dose PK of tinzaparin (see study DMP 702-918). In addition, a study is currently ongoing to characterize the multiple dose PK of tinzaparin 175 anti-Xa IU/kg, SC in healthy subjects (Study DMP 702-945).

a) Absorption

Plasma levels of anti-Xa activity increase in the first 2 to 3 hours following SC injection of tinzaparin and reach a maximum within 4 to 5 hours. Maximum concentrations (C_{max}) of 0.25 and 0.87 IU/mL are achieved following a single SC fixed dose of 4,500 IU (approximately 64.3 IU/kg) and weight-adjusted dose of 175 IU/kg of tinzaparin respectively.

b) Distribution

Tinzaparin has a volume of distribution of 3.1 to 5.0 L.

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[1] **The sub-2k D molecules is considered to be the primary pharmacologically-inactive fraction of LMH.**

c) Elimination

The elimination half-life following SC administration of 4,500 IU tinzaparin is approximately 3.4 hours based on anti-Xa activity. Following a single SC administration of 175 IU/kg of tinzaparin, the elimination half-life based on anti-Xa activity is approximately 3.9 hours. Clearance following IV administration of 4,500 IU tinzaparin is approximately 1.7 L/hr. The primary route of elimination is renal.

VIII. Overdose

In case of overdosage, can protamine sulfate be used to effectively neutralize tinzaparin?

Overdosage of tinzaparin may lead to bleeding complications. Protamine sulfate can be administered to neutralize excess tinzaparin. Two studies were conducted to examine the neutralizing effect of protamine sulfate (IV infusion of 1 mg/100 anti-Xa IU) on tinzaparin PK. As with other LMHs, protamine did almost completely neutralize anti-IIa activity, but not anti-Xa activity (maximum about 60%) (see studies DMP 702-922 & 702-924).

IX. Interaction with Aspirin

A single drug-drug interaction study was conducted to examine the effect of concomitant administration of aspirin on tinzaparin PK. However, the results were inconclusive due to inadequate study design (see study DMP 702-923). Upon discussing the issue with Dr. Lilia Talarico, the GI & Coagulation division director, it was decided that, based on current experience with other LMHs, a repeat of the study was not necessary.

X. Special Populations

a) Elderly population

Is there a need for dose adjustment in geriatric patients?

Population PK analysis was utilized to determine the effect of age on tinzaparin PK (see Pop PK Analysis report). Tinzaparin clearance does not seem to depend on age. However, tinzaparin should be used with care in these patients, since renal function generally declines with age.

b) Pediatric population

Is there a need for dose adjustment in pediatric patients?

No adequate and well-controlled clinical or PK studies have been conducted in the pediatric population. The sponsor has included the following statement in the package insert under PRECAUTIONS, "Safety and effectiveness of INNOHEP in patients below the age of 18 years have not been established".

c) Renal Impairment patients

Is there a need for dose adjustment in renal impairment patients?

A study was conducted to determine tinzaparin PK in chronic renal failure (RF) patients on haemodialysis. Compared to healthy subjects, the major PK parameters (CL, V_d and AUC) were similar, except for half-life (5.2 hrs in RF patients compared to 1.6 hrs in healthy subjects). In addition, the population PK analysis in the target patient population investigated the effect of renal function on tinzaparin PK (see study DMP 702-925 & Pop PK Analysis report). Evaluation of individual clearance estimates showed that in severe renal failure (CLcr < 30 ml/min), clearance of tinzaparin was reduced by 24% compared to normal. The sponsor indicated in the proposed package insert under the SPECIAL POPULATIONS section as follows; "Patients with severe renal impairment should be dosed with caution".

d) Hepatic Impairment Patients

Is there a need for dose adjustment in hepatic impairment patients?

No studies have been conducted to assess the effect of hepatic impairment on tinzaparin PK, as the hepatic route is not a major route of elimination of LMHs.

e) Pregnancy

Does tinzaparin cross the placenta into the fetus during pregnancy

Two studies were conducted to determine whether tinzaparin crosses the placenta into the fetus during pregnancy. A clear discrepancy was observed between the results of the two studies, which might be related to the different routes of administration employed in those studies (see studies DMP 702-926 & 702-927). Overall, results of the two studies were inconclusive.

f) Obesity

Is there a need for dose adjustment in obese patients?

Population PK analysis was utilized to determine the effect of body weight on tinzaparin PK (see Pop PK Analysis report). Obese patients (BMI > 30 kg/m^2) had tinzaparin clearance that was 22% lower than normal. Dosing based on body weight is generally sufficient to normalize the differences in tinzaparin PK among patients of varying weights. However, the sponsor indicates in the package insert under SPECIAL POPULATIONS section that "Clinical trial experience is limited in patients with a BMI >40 kg/m^2".

g) Nursing Women

Is there a need for dose adjustment in nursing women?

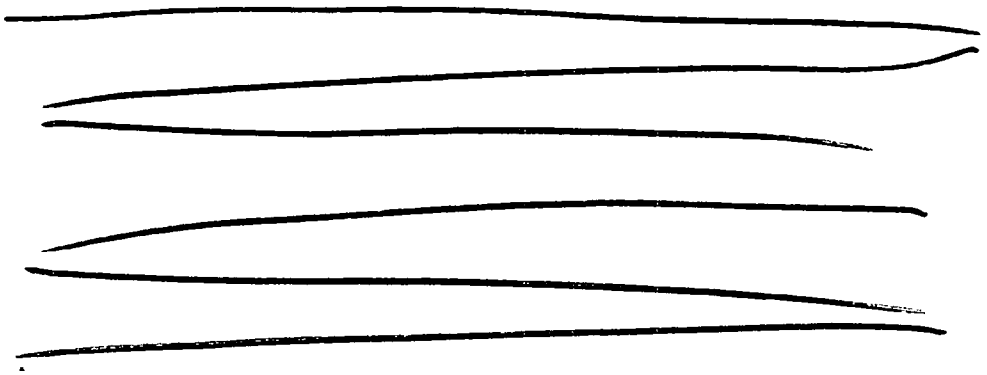
No studies have examined excretion of tinzaparin in human milk. Hence, caution should be exercised when tinzaparin is administered to nursing women.

h) Pharmacokinetics in Patients

Is tinzaparin PK different in the target patient population from that of healthy subjects?

Population PK analysis was utilized to determine tinzaparin PK in the target patient population (see Pop PK Analysis report). Estimates of the average PK parameters for tinzaparin were similar to those obtained previously in Phase I studies: CL = 0.0193 L/h/kg (CI = 0.013-0.026), V_c = 0.143 L/kg (CI = 0.105-0.181) and $t_{1/2}$ = 5.1 hrs (CI = 3.5-6.7).

IX. Proposed Package Insert (Pages 10-13, CPB-related only)



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4 page(s) of revised draft labeling has been redacted from this portion of the review.

NDA: 20-484/ Study 702-001 **Study Date:** Dec 1998-Jan 1999

Type of Study: Bioequivalence

Study DMP 702-001 is entitled,

"A PHASE I, SINGLE DOSE, CROSSOVER, BIOEQUIVALENCE STUDY COMPARING TO BE MARKETED TINZAPARIN AND TINZAPARIN-LIKE LOW MOLECULAR WEIGHT HEPARIN IN HEALTHY VOLUNTEERS".

Background

Tinzaparin was developed jointly by two pharmaceutical companies, Leo Pharmaceutical [redacted] Many early clinical trials were conducted by [redacted] However, the company discontinued its anticoagulation business in 1995; thereafter, Leo became the sole tinzaparin supplier.

Low molecular weight heparins such as tinzaparin, are polydisperse polysaccharides which include biologically inactive species. The fraction of heparin sub-2k D molecules is generally regarded to be without physiological activity. Due to different volumes of ethanol during the fractionating stage of the manufacturing process of tinzaparin, the fraction of molecules below 2000 D varied between 2.5% and 15.2% in the preparations used in the clinical development of the drug. The to-be-marketed tinzaparin will have less than 10% of the sub-2k D fraction. The remaining molecular distribution (2000-8000 D and > 8000 D) is unchanged.

Objectives

- To demonstrate bioequivalence between tinzaparin formulations containing varying fractions (<10%-16%) of sub-2k D molecules, thought to be the primary pharmacologically-inactive fraction of LMH.

The present study will compare the bioequivalence of the to-be-marketed tinzaparin containing < 10% of the molecules in the sub-2k D fraction to a specially-produced tinzaparin-like low molecular weight heparin with a fraction of sub-2k D molecules of approximately 16%, which corresponds to the highest fraction used in clinical trials (15.2%). Bioequivalence will be assessed based on anti-Xa activity, which is accepted as a pharmacodynamic measure to characterize low molecular weight heparins.

Primary Review Issue

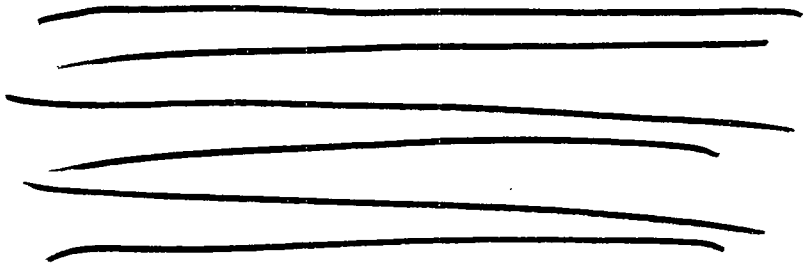
- **Is the sub-2k D fraction in tinzaparin (10-16 %) critical for anti-Xa activity of tinzaparin?**

Study Design

Single dose, open-label, randomized, two-period, crossover study in healthy subjects

Subjects	30 subjects: 22 men and 8 women, (age 41 ± 13 yrs)
Duration of enrollment	2 months
Key Inclusion Criteria	Healthy male and female subjects, over 18 yrs of age Subjects should have a body weight within 15% of the appropriate weight range Female subjects should be non-lactating and of non-childbearing potential Have no significant diseases or clinically significant abnormal lab values
Key Exclusion Criteria	History of allergy or hypersensitivity to heparin or any of its components Evidence of history of diseases associated with bleeding risk or bleeding disorder Any disease or condition that might compromise the cardiovascular, hematological, renal, hepatic, pulmonary, endocrine, central nervous or gastrointestinal systems Any medication that could induce or inhibit hepatic microsomal enzymes within one month of the start of the study Consumption of any medication (including OTC drugs) within 2 weeks of the study start
Study Procedure	Subjects are randomly allocated into one of the two treatment sequences: • **Reference Product**: The to-be marketed tinzaparin formulation (20,000 IU anti-Xa/ml) • **Test Product**: Clinical trial tinzaparin formulation (20,000 IU anti-Xa/ml)
Interperiod Washout	(7-14) days
Sampling	Serum samples collected at the following time points (for both treatments): **Pre-dose** (at 15 min prior to drug administration) **Post-dose**: at 0 min, 15, 30, 45, 60, and 90 min and at 2, 3, 4, 6, 8, 12, 16, 24 and 30 hrs

Anti-Xa
Assay Validation



Safety — Subjects monitored for adverse events. No subject discontinuations were reported in this study.

Pharmacodynamic Data

Two pharmacokinetic / pharmacodynamic parameters were used to determine the activity of tinzaparin in biological samples for the assessment of tinzaparin pharmacodynamics: anti-Xa and anti-IIa activities (using amidolytic methods).
The following pharmacokinetic parameters were determined using non-compartmental analysis: C_{max}, t_{max}, $t_{1/2\lambda z}$, $AUC_{0\rightarrow t}$ and $AUC_{0\rightarrow\infty}$.

Statistical Analysis

Assuming a coefficient of variation of 17% and a difference from the reference of no more than 5%, a sample size of at least 20 subjects was determined to provide at least 90% power to establish bioequivalence between the reference and test formulations at a significance level of
0.05.

Prior to testing for bioequivalence, the presence of carry-over effect was assessed using an analysis of variance (ANOVA) model for a two-period crossover. Effects for treatment, sequence, period and subject nested within sequence were included in the model.
Summary statistics (n, mean, SD, median, minimum and maximum) were provided for each pharmacokinetic parameter except for t_{max}, for which median, minimum and maximum values were determined.
Bioequivalence of the test and reference formulations was established if the 90% confidence intervals on the difference in the mean log-transformed C_{max}, $AUC_{0\rightarrow t}$ and $AUC_{0\rightarrow\infty}$ fell between 80% and 125%.

Results

1. Anti-Xa Activity

Table 1. Mean (± S.D.) pharmacodynamic parameters of the anti-Xa activity (n = 30)

Treatment	C_{max}	$AUC_{0\rightarrow last}$	$AUC_{0\rightarrow\infty}$
Test	0.887	9.769	10.322

	(0.141)	(1.628)	(1.558)
Reference	0.869 (0.236)	8.643 (1.537)	9.552 (1.587)

Table 2. Schuirmann confidence intervals for anti-Xa activity PD parameters

Parameter	Treatment Comparison	Estimate %	Confidence Interval
Ln A_{max}	Test vs. Reference	103.5	(97.4-110.0)
Ln $AUC_{0\rightarrow last}$	Test vs. Reference	113.2	(108.5-118.1)
Ln $AUC_{0\rightarrow\infty}$	Test vs. Reference	108.6	(104.8-112.5)

1. Anti-IIa Activity

Table 3. Mean (± S.D.) pharmacodynamic parameters of the anti-IIa activity (n = 30)

Treatment	C_{max}	$AUC_{0\rightarrow last}$	$AUC_{0\rightarrow\infty}$
Test	0.330 (0.073)	2.901 (0.582)	3.531 (0.669)
Reference	0.301 (0.074)	2.507 (0.538)	3.114 (0.652)

Table 4. Schuirmann confidence intervals for anti-IIa activity PD parameters

Parameter	Treatment Comparison	Estimate %	Confidence Interval
Ln C_{max}	Test vs. Reference	90.8	(84.1-98.1)
Ln $AUC_{0\rightarrow last}$	Test vs. Reference	86.2	(81.3-91.3)
Ln $AUC_{0\rightarrow\infty}$	Test vs. Reference	87.3	(82.2-92.7)

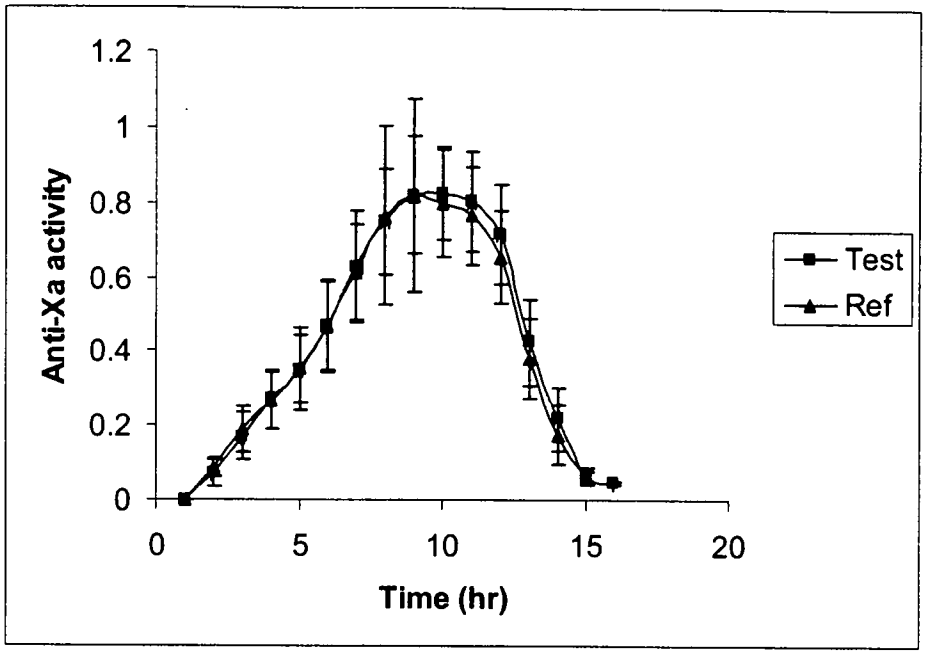


Fig. 1. PK profiles of anti-Xa activity of the tinzaparin reference and test formulations

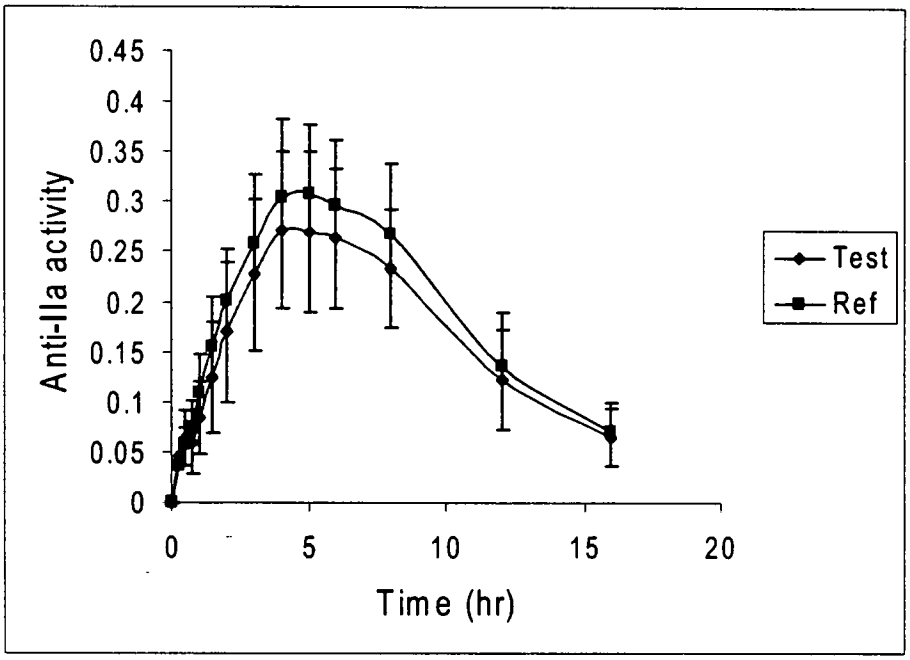


Fig. 2. PK profiles of anti-IIa activity of the tinzaparin reference and test formulations

Reviewer's Comments

- *Anti-Xa/anti-IIa activity ratio is often used as a measure of the ratio of antithrombotic to anticoagulant activities. The anti-Xa/anti-IIa activity ratio based on $AUC_{0-\infty}$ for the two formulations of SC tinzaparin 175 anti-Xa IU/kg ranged between 2.8 and 3.3.*
- *Bioequivalence between the two treatments was tested based on the geometric means of relevant pharmacodynamic parameters. The two formulations pass Schiurmann two one-sided test for bioequivalence on both, anti-IIa and anti-Xa activities. Hence, the two treatments are deemed bioequivalent. In conclusion, tinzaparin formulations composed of as low as 10% and as high as 16% of the < 2000 D fraction would be expected to result in similar anti-Xa and anti-IIa activities.*

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ON ORIGINAL

NDA: 20-484/ Study 702-918 **Study Date:** May 1993-Jun 1993

Type of Study: Bioavailability/Bioequivalence/Dose Proportionality

Study DMP 702-918 is entitled,

"A PHASE I PHARMACOKINETIC STUDY OF TINZAPARIN ADMINISTERED INTRAVENOUSLY, AND HEPARIN ADMINISTERED SUBCUTANEOUSLY IN HEALTHY VOLUNTEERS".

Background

During an early stage in the development, several phase I studies were conducted in small numbers of healthy subjects. The pharmacokinetics of tinzaparin was evaluated by pharmacodynamic assessment of anti-Xa and anti-IIa activities. The current study was aimed at providing more accurate biopharmaceutical information on tinzaparin by evaluating pharmacodynamic parameters in a larger number of subjects than previously studied.

Objectives

- Provide accurate estimates of pharmacokinetic parameters of tinzaparin, and to measure activated partial-thromboplastin time (aPTT) after S.C. and I.V. administration.
- Evaluate the absolute bioavailability of S.C. tinzaparin.
- Assess the bioequivalence of tinzaparin with and without preservative (benzyl alcohol) after S.C. administration.
- Evaluate the dose proportionality of tinzaparin over the dose range for prevention of thromboembolism (fixed dose = 4500 anti-Xa IU) and treatment of deep vein thrombosis (fixed dose = 12,250 anti-Xa IU).
- Compare the anticoagulant activity of tinzaparin and heparin administered S.C.

Primary Review Issues:

1. Would the addition of benzyl alcohol , a preservative, to the formulation affect the bioavailabilty of S.C. administered tinzaparin?

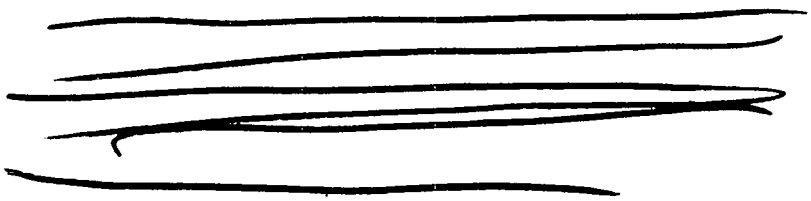
2. How large a fraction of tinzaparin is systemically bioavailable after S.C. administration?

3. How does the anticoagulant activity of tinzaparin compare to that of heparin?

4. Is the dose-concentration relationship linear over the proposed therapeutic range?

Study Design

Single-center, open-label, randomized, five-period, five-treatment, crossover study with a Latin-square design

Subjects	30 subjects (23 completed the study)
Duration of Enrollment	5 weeks
Key Inclusion Criteria	Healthy male subjects, 18 to 55 yrs of age Subjects should have a body weight between 65 and 80 kg and within 15% of the ideal weight for height Have no significant diseases or clinically-significant abnormal lab values
Anti-Xa Assay Validation	
Treatments	Subjects are to receive a single administration of each of the following five treatments: • **Treatment A: Heparin 5000 U, administered S.C.** • **Treatment B: Tinzaparin 4500 anti-Xa IU without preservative, administered S.C.** • **Treatment C: Tinzaparin 4500 anti-Xa IU without preservative, administered I.V.** • **Treatment D: Tinzaparin 12,250 anti-Xa IU with preservative, administered S.C.** • **Treatment E: Tinzaparin 4500 anti-Xa IU with preservative, administered S.C.**
Interperiod Washout	6 days
Sampling Times	Blood samples collected at the following time points: **IV Administration:**

Pre-dose (at 15 and 0 min prior to drug administration)
Post-dose: at 5, 10, 15, 30, 45, 60 and 90 min, and at 2, 3, 4, 5, 6, 8, 12, 16 and
24 hrs

S.C. Administration:
Pre-dose (at 15 and 0 min prior to drug administration)
Post-dose: at 15, 30, 45, 60 and 90 min, and at 2, 3, 4, 5, 6, 8, 12, 16, 24 and
32 hrs

Safety — Subjects monitored for adverse events

Analytical assay

Two pharmacodynamic markers were used to determine the activity of [redacted] biological samples for the assessment of [redacted] pharmacokinetics: anti-Xa and anti-IIa activities (using amidolytic methods).
The assay was linear in the range of [redacted] for anti-Xa activity and [redacted] for anti-IIa activity. The lower limit of quantitation was 0.051 IU/ml for anti-IIa activity and [redacted] for anti-Xa activity.

Pharmacodynamic Data

The following pharmacokinetic parameters were determined for both anti-Xa and anti-IIa activities using non-compartmental analysis: C_{max}, t_{max}, $t_{1/2\lambda z}$, Ke, CL, Vd, $AUC_{0\rightarrow t}$ and $AUC_{0\rightarrow\infty}$ Calculation of the pharmacodynamic parameters was based on baseline-adjusted activity levels.

Statistical Analysis

The mean estimates of the pharmacodynamic parameters and their corresponding standard errors were determined using an ANOVA model adjusted for period, subject and treatment effects. Carryover effects were also tested by adding a first-order carryover variable (subject within sequence) to the ANOVA model. Significance was prospectively defined as $p<0.05$. LSMEANS within SAS was used to determine statistically significant differences between the treatments. Confidence intervals of the ratio of $AUC_{0\rightarrow t}$, $AUC_{0\rightarrow\infty}$ and C_{max} values were used to test the hypotheses concerning bioavailability, bioequivalence, dose proportionality and comparison to heparin.
A correction factor of 4500/4880 was introduced into the calculations of the pharmacodynamic parameters to correct for the [redacted] overfill in the pre-filled syringes.

Results and Conclusions

Table 5. Summary statistics for pharmacodynamic parameters based on baseline-adjusted **anti-Xa** activity

PD parameter	Summary Statistic	Heparin 5000 IU, S.C. (Trt. A)	Tinzaparin 4500 anti-Xa IU w/o preservative, S.C. (Trt. B)	Tinzaparin 4500 anti-Xa IU w/o preservative, I.V. (Trt. C)	Tinzaparin 12,250 anti-Xa IU w/ preservative, S.C. (Trt. D)	Tinzaparin 4500 anti-Xa IU w/ preservative, S.C. (Trt. E)
C_{max} (IU/ml)	Mean (SD) Range	0.06 (003)	0.29 (0.06)	1.16 (0.12)	0.85 (0.14) 0.62-1.12	0.25 (0.05)
	%CV	51.53	20.51	10.14		20.56
AUC_{0-t} (IU·h/ml)	Mean (SD) Range	0.39 (0.16) 0.13-0.83	2.24 (0.37)	2.72 (0.40)	9.12 (1.46) 6.81-12.32	1.88 (0.52)
	%CV		16.57	14.72		27.76
$AUC_{0-\infty}$ (IU·h/ml)	Mean (SD) Range	0.50 (0.19) 0.21-0.96	2.35 (0.40)	2.75 (0.40)	9.23 (1.47) 6.91-12.49	1.96 (0.52)
	%CV		17.03	14.45		26.80
t_{max} (h)	Mean (SD) Range	2.46 (0.93) 0.50-4.00	3.65 (0.98)	0.15 (0.08)	4.42 (1.27) 0.75-8.00	3.70 (0.88)
	%CV		26.89	52.00		23.70
CL (L/h)	Mean (SD) Range	ND ND	1.97 (0.32)	1.67 (0.22)	1.36 (0.21) 0.98-1.77	2.42 (0.52)
	%CV		16.49	13.05		21.39
Vd (L)	Mean (SD) Range	NA NA	NA NA	3.82 (0.41)	NA NA	NA NA
	%CV			10.87		
$t_{1/2}$ (h)	Mean (SD) Range	ND ND	4.13 (1.67)	1.60 (0.15)	3.87 (0.68) 2.74-5.15	3.41 (1.68)
	%CV		40.55	9.66		49.28

NA=Not Applicable; ND=Not Determined; Trt=Treatment; w/=With; w/o=Without

Table 6. Summary statistics for pharmacodynamic parameters based on baseline-adjusted **anti-IIa** activity

PD parameter	Summary Statistic	Heparin 5000 IU, S.C. (Trt. A)	Tinzaparin 4500 anti-Xa IU w/o preservative, S.C. (Trt. B)	Tinzaparin 4500 anti-Xa IU w/o preservative, I.V. (Trt. C)	Tinzaparin 12,250anti-Xa IU w/ preservative, S.C. (Trt. D)	Tinzaparin 4500 anti-Xa IU w/ preservative, S.C. (Trt. E)
C_{max} (IU/ml)	Mean	0.03	0.09	0.58	0.32 (0.10)	0.08
	(SD)	(0.02)	(0.03)	(0.07)	0.16-0.57	(0.03)
	Range					
	%CV	64.57	37.41	12.82		31.23
$AUC_{0\rightarrow t}$ (IU·h/ml)	Mean	0.14	0.62	1.08	2.71 (0.56)	0.56
	(SD)	(0.09)	(0.21)	(0.19)	1.72-3.96	(0.17)
	Range					
	%CV	62.94	33.56	17.94		31.09
$AUC_{0\rightarrow\infty}$ (IU·h/ml)	Mean	0.20	0.70	1.10	2.85 (0.66)	0.64
	(SD)	(0.18)	(0.23)	(0.19)	1.98-4.88	(0.19)
	Range					
	%CV	90.45	32.03	17.65		29.50
t_{max} (h)	Mean	2.70	3.61	0.18	4.47 (1.37)	3.67
	(SD)	(1.44)	(0.89)	(0.10)	0.75-8.00	(1.14)
	Range					
	%CV	53.24	24.70	57.03		31.15
CL (L/h)	Mean	ND	4.49	2.51	2.64 (0.54)	4.77
	(SD)	ND	(2.58)	(0.39)	1.48-3.65	(1.37)
	Range					
	%CV		57.44	15.67		28.81
Vd (L)	Mean	NA	NA	4.88	NA	NA
	(SD)	NA	NA	(1.18)	NA	NA
	Range					
	%CV			24.26		
$t_{1/2}$ (h)	Mean	ND	4.49	1.36	3.35 (1.21)	5.05
	(SD)	ND	(2.50)	(0.31)	1.68-6.35	(3.64)
	Range					
	%CV		55.64	22.50		72.14

NA=Not Applicable; ND=Not Determined; Trt=Treatment; w/=With; w/o=Without

- Bioavailability of S.C. tinzaparin

The bioavailability of S.C. tinzaparin was assessed by comparing the mean $AUC_{0\rightarrow\infty}$ estimates of tinzaparin 4500 anti-Xa IU S.C. without preservative (treatment B) to tinzaparin 4500 anti-Xa IU I.V. without preservative (treatment C). The absolute bioavailability of S.C. tinzaparin was estimated to be [redacted] on anti-Xa activity and [redacted] on anti-IIa activity.-

Table 7a. PD parameters based on **anti-Xa** activity of treatment B vs. treatment C

PD Parameter	Geometric Mean Ratio (%) (Test/reference)	90% Confidence Interval	P-value
C_{max}	24.6	(22.2, 27.2)	0.0001
$AUC_{0\rightarrow last}$	83.7	(76.3, 91.9)	0.0021
$AUC_{0\rightarrow\infty}$	86.7	(78.7, 95.5)	0.0163

Table 7b. PD parameters based on **anti-IIa** activity of treatment B vs. treatment C

PD Parameter	Geometric Mean Ratio (%) (Test/reference)	90% Confidence Interval	P-value
C_{max}	14.3	(12.5, 16.4)	0.0001
$AUC_{0\rightarrow last}$	53.0	(44.8, 62.7)	0.0001
$AUC_{0\rightarrow\infty}$	58.6	(48.2, 71.6)	0.0001

- Bioequivalence of S.C. tinzaparin formulations with and without preservative

The bioequivalence of S.C. tinzaparin 4500 anti-Xa IU with preservative (Trt. E) and without preservative (Trt. B) was assessed on anti-Xa and anti-IIa activities.

Table 8a. PD parameters based on **anti-Xa** activity of treatment E vs. treatment B

PD Parameter	Geometric Mean Ratio (%) (Test/reference)	90% Confidence Interval	P-value
C_{max}	91.9	(83.1, 101.6)	0.1663
$AUC_{0\rightarrow last}$	88.4	(80.6, 97.0)	0.0292
$AUC_{0\rightarrow\infty}$	88.4	(80.3, 97.3)	0.0348

Table 8b. PD parameters based on **anti-IIa** activity of treatment E vs. treatment B

PD Parameter	Geometric Mean Ratio (%) (Test/reference)	90% Confidence Interval	P-value
C_{max}	102.9	(90.1, 117.7)	0.7195
$AUC_{0\rightarrow last}$	100.9	(85.4, 119.3)	0.9271
$AUC_{0\rightarrow\infty}$	102.3	(84.1, 124.6)	0.8450

Reviewer's Comments: The results show that the 90% CI of the parameter ratios are within the specified limits of 80-125% for bioequivalence. Hence, the presence or absence of the preservative (benzyl alcohol) does not seem to impact bioavailability of tinzaparin SC formulation.

- Dose proportionality of S.C. tinzaparin formulations

The dose proportionality of S.C. tinzaparin with preservative was assessed by comparing the dose-normalized pharmacodynamic parameters of 12,250 anti-Xa IU formulation (Trt. D) to the parameters for 4500 anti-Xa IU formulation (Trt. E).

Table 9a. PD parameters based on **anti-Xa** activity of treatment D vs. treatment E

PD Parameter	Geometric Mean Ratio (%) (Test/reference)	90% Confidence Interval	P-value
C_{max}	127.1	(114.8, 140.6)	0.0002
$AUC_{0\rightarrow last}$	180.4	(164.4, 197.9)	0.0001
$AUC_{0\rightarrow\infty}$	175.4	(159.3, 193.2)	0.0001

Table 9b. PD parameters based on **anti-IIa** activity of treatment D vs. treatment E

PD Parameter	Geometric Mean Ratio (%) (Test/reference)	90% Confidence Interval	P-value
C_{max}	140.5	(122.9, 160.7)	0.0001
$AUC_{0\rightarrow last}$	182.2	(154.0, 215.5)	0.0001
$AUC_{0\rightarrow\infty}$	165.7	(136.0, 201.9)	0.0001

- Comparison to heparin

Heparin 5000 U, S.C. (Trt. A), was compared to S.C. tinzaparin 4500 anti-Xa IU with preservative (Trt. E). The mean parameter ratios were corrected for the dose ratio (4500/5000).

Table 10a. PD parameters based on **anti-Xa** activity of treatment A vs. treatment E

PD Parameter	Geometric Mean Ratio (%) (Test/reference)	90% Confidence Interval	P-value
C_{max}	18.9	(17.1, 20.9)	0.0001
$AUC_{0\rightarrow last}$	17.6	(16.1, 19.3)	0.0001
$AUC_{0\rightarrow\infty}$	22.1	(20.1, 24.3)	0.0001

Table 10b. PD parameters based on **anti-IIa** activity of treatment A vs. treatment E

PD Parameter	Geometric Mean Ratio (%) (Test/reference)	90% Confidence Interval	P-value
C_{max}	27.3	(23.9, 31.2)	0.0001
$AUC_{0\rightarrow last}$	18.5	(15.7, 21.9)	0.0001
$AUC_{0\rightarrow\infty}$	20.0	(16.4, 24.3)	0.0001

- Anticoagulant activity of treatments

The mean aPTT activity levels were determined over time for each of the five treatments. Following administration of I.V. tinzaparin 4500 anti-Xa IU (Trt. C), aPTT activity reached a maximum of 5.07 U/ml at 5 min. Following S.C. tinzaparin 4500 anti-Xa IU (Trt. B and E), mean aPTT activity-time profiles demonstrated a slow absorption, with maximum activity (3.75 U/ml) occurring by 3 hrs after administration. Administration of S.C. tinzaparin 12,250 anti-Xa IU (Trt. D) resulted in maximum aPTT levels of 4.21 U/ml at 4 hrs post-dose. S.C. heparin 5000 U (Trt. A) demonstrated a slow rise to maximum levels of 3.60 U/ml at 3 hrs after administration.

- Anti-Xa/anti-IIa activity ratios

The mean anti-Xa/anti-IIa activity ratios were assessed at each time point following administration of each of the five treatments.

Table 11. Anti-Xa/anti-IIa activity ratio based on $AUC_{0\rightarrow\infty}$

PD parameter	Summary Statistic	Heparin 5000 IU, S.C. (Trt. A)	Tinzaparin 4500 anti-Xa IU w/o preservative, S.C. (Trt. B)	Tinzaparin 4500 anti-Xa IU w/o preservative, I.V. (Trt. C)	Tinzaparin 12,250 anti-Xa IU w/ preservative, S.C. (Trt. D)	Tinzaparin 4500 anti-Xa IU w/ preservative, S.C. (Trt. E)
$AUC_{0\rightarrow\infty}$	Mean 90% CI	3.41 (2.44-4.76)	3.51 (3.06-4.03)	2.51 (2.42-2.60)	3.28 (3.12-3.44)	3.10 (2.81-3.42)

Reviewer's Comments:

- *The data indicate that there is no dose proportionality between the 4500 anti-Xa IU and 12,250 anti-Xa IU tinzaparin formulations. An increase in the dose of tinzaparin would be expected to result in a greater than proportional increase in anti-Xa and anti-IIa activities.*
- *The anti-Xa/anti-IIa activity ratio in the current study ranged from 2.5 to 3.5. This agrees well with the anti-Xa/anti-IIa activity ratio reported in study 702-001 (2.8-3.3).*

NDA: 20-484/ Study 702-919 **Study Date:** Nov 1984-Feb 1985

Type of Study: PK of Single Dose SC tinzaparin

Introduction

Study DMP 702-919 is entitled,

"INVESTIGATION OF THE PHARMACOKINETICS OF LOW MOLECULAR WEIGHT HEPARIN [redacted]

Background

The current study was conducted early on in the development of tinzaparin by [redacted] back in 1984-1985. It was aimed at investigating the pharmacokinetic and pharmacodynamic characteristics of low molecular weight heparin [redacted] in humans. "Low molecular weight heparin [redacted]" will be referred to as "tinzaparin" throughout the current review.

Upon this reviewer's request for data reanalysis and information relating to analytical methods validation, the Firm submitted a report pursuant to the current study (See DMP 00-010, dated Feb 9, 2000).

Objectives

- Investigate the pharmacokinetics/pharmacodynamics of tinzaparin after a single S.C. injection.
- Compare the pharmacodynamic activity of tinzaparin to that of conventional heparin.
- Compare the bioavailability of two different lots of conventional heparin to determine if any significant lot to lot variation exists (Lot 5864 & lot OR314).

Primary Review Issues:

1. **How does the anticoagulant activity of tinzaparin compare to that of heparin?**
2. **What are the primary pharmacokinetic and pharmacodynamic characteristics of tinzaparin?**
3. **Do different heparin lots have different systemic bioavailabilities?**

Study Design

Single dose, randomized, crossover study in healthy subjects. The study was double-blinded for S.C. injections, but open-label for I.V. injections.

Subjects 6 subjects (4 men and 2 women); age 27-39 yrs

Key Inclusion Criteria	Healthy male and female subjects, over 18 yrs of age Subjects should have a body weight between 65 and 80 kg and within 15% of the ideal weight for height
Key Exclusion Criteria	History of allergy or hypersensitivity to heparin or any of its components Evidence of history of diseases associated with bleeding risk or bleeding disorder Any disease or condition that might compromise the cardiovascular, or renal, systems
Treatments	Subjects are randomized to receive a single administration of each of the following six treatments: • **Treatment A: Tinzaparin 2,500 anti-Xa IU, administered S.C.** • **Treatment B: Tinzaparin 5,000 anti-Xa IU, administered S.C.** • **Treatment C: Heparin 5000 anti-Xa IU, administered S.C., lot 5864** • **Treatment D: Tinzaparin 10,000 anti-Xa IU, administered S.C.** • **Treatment E: Heparin 5000 anti-Xa IU, administered S.C., lot OR314** • **Treatment F: Tinzaparin 5,000 anti-Xa IU, administered I.V.**
Washout	7 days
Sampling Times	Serum samples collected at the following time points: **IV administration:** **Pre-dose** (at 0 min prior to drug administration) **Post-dose**: at 2, 5, 10, 15, 30 min, and at 1, 2, 4, 6, 8 and 24 hrs **S.C. Administration:** **Pre-dose** (at 0 min prior to drug administration) **Post-dose**: at 15 and 30 min, and at 1, 2, 4, 6, 8 and 24 hrs
Safety	Subjects monitored for adverse events.

Pharmacodynamic Data

Three primary pharmacokinetic / pharmacodynamic parameters were used to determine the activity of tinzaparin in biological samples for the assessment of tinzaparin pharmacodynamics: anti-Xa and anti-IIa activities (using amidolytic methods) and aPTT. In addition, several secondary pharmacodynamic parameters were determined including, antithrombin III, platelet count, plasminogen, tPA, tPA inhibitor, α_2-antiplasmin and ECLT (Euglobulin clot lysis time).

Statistical Analysis

A formal statistical analysis of the data to compare various treatments was not conducted due to the limited sample size and marked variability in the study data.

Results

No significant changes were observed for any of the secondary pharmacodynamic parameters with tinzaparin or heparin treatment.

The results of data reanalysis are shown below in tables 12 and 13.

1. Anti-Xa Activity

Table 12. Mean (± S.D.) pharmacodynamic parameters of the anti-Xa activity (n = 36)

Treatment	Dose/Route IU/ IV or SC	C_{max} (IU/ml)	T_{max} (hr)	$AUC_{0\rightarrow t}$ (IU·hr/ml)	$T_{1/2}$ (h)	Vd (L)	CL (L/hr)
A (Tinzaparin)	2500/SC	0.13 (0.04)	0.38 (0.14)	0.27 (0.10)	2.07 (0.78)	---	---
B (Tinzaparin)	5000/SC	0.28 (0.16)	0.58 (0.2)	0.47 (0.21)	2.29 (1.09)	---	---
C (Heparin, Lot #5864)	5000/SC	0.05 (0.01)	0.13 (0.09)	0.10 (0.06)	4.26 (1.11)	---	---
D (Tinzaparin)	10000/SC	0.55 (0.19)	0.58 (0.20)	1.33 (0.49)	2.42 (1.87)	---	---
E (Heparin, Lot #OR 314)	5000/SC	0.04 (0.02)	0.4 (0.31)	0.04 (0.03)	1.15 (0.59)	---	---
F (Tinzaparin)	5000/IV	1.3 (0.27)	0.06 (0.05)	3.03 (1.44)	3.24 (2.45)	6.74 (2.16)	1.92 (1.02)

Anti-IIa Activity

Table 13. Mean (± S.D.) pharmacodynamic parameters of the anti-IIa activity (n = 36)

Treatment	Dose/Route IU/ IV or SC	C_{max} (IU/ml)	T_{max} (hr)	AUC_{0-t} (IU·hr/ml)	$T_{1/2}$ (h)	Vd (L)	CL (L/hr)
A (Tinzaparin)	2500/SC	0.02 (0.01)	0.24 (0.14)	0.02 (0.01)	2.53 (1.57)	---	---
B (Tinzaparin)	5000/SC	0.04 (0.02)	0.75 (0.67)	0.07 (0.04)	3.63 (4.62)	---	---
C (Heparin, Lot #5864)	5000/SC	0.02 (0.02)	0.27 (0.20)	0.04 (0.03)	5.95 (6.67)	---	---
D (Tinzaparin)	10000/SC	0.08 (0.03)	0.67 (0.26)	0.16 (0.06)	3.4 (2.71)	---	---
E (Heparin, Lot #OR 314)	5000/SC	0.04 (0.02)	0.24 (0.17)	0.08 (0.08)	5.2 (5.13)	---	---
F (Tinzaparin)	5000/IV	0.28 (0.06)	0.03	0.50 (0.19)	1.39 (0.71)	20.39 (10.39)	11.05 (5.54)

Reviewer's Comments

The compartmental pharmacokinetic approach utilized for calculation of the basic pharmacokinetic parameters in the original study report was deemed to be of little value compared to the non-compatmental approach. This is particularly an important point since the study data was extracted from a small number of subjects and thus expectedly, was associated with high variability. Hence, this reviewer requested recalculation of the relevant pharmacokinetic parameters using a non-compartmental approach.

Overall, the two heparin formulations from different lots showed similar anti-IIa and anti-Xa plasma profiles despite significant differences in the profiles of terminal phase, which might be due to the inherent variability in heparin composition and the analytical assay. Bioavailability calculations were not performed due to large variability in the data that lead to poor characterization of the terminal elimination phase.

Administration of varying doses of tinzaparin resulted in dose-proportional increases in anti-Xa and anti-IIa activity levels. However, a small deviation towards a greater than proportional increase in AUC with increasing tinzaparin doses was noted for both anti-Xa and anti-IIa activity levels. This was particularly true for tinzaparin 5,000 anti-Xa IU and 10,000 anti-Xa IU formulations.

A comparison between the tinzaparin 5000 IU, SC treatment and corresponding heparin treatments with respect to their anti-Xa and anti-IIa levels showed that tinzaparin generally results in a higher anti-Xa activity, but a lower anti-IIa activity.

The study results point to a trend for a linear correlation between body weight and AUC of anti-Xa and anti-IIa. However, due to the limited sample size in this study, this has yet to be confirmed.

NDA: 20-484/ Study 702-920 Study Date: Feb 1985-May 1985

Type of Study: Safety and PK of IV Tinzaparin

Study DMP 702-920 is entitled,

"SAFETY EVALUATION AND PHARMACOKINETICS AFTER INTRAVENOUS ADMINISTRATION OF LOW MOLECULAR WEIGHT HEPARIN [redacted]

Objectives

- Compare the safety and the pharmacokinetics of tinzaparin and heparin after I.V. injection in healthy subjects.

Primary Review Issues

- **How does tinzaparin compare to heparin in terms of safety and pharmacodynamic activity?**

Study Design

Single dose, single-blind, non-randomized, crossover comparative study

Subjects	6 healthy male subjects; age 21-38 yrs
Key Exclusion Criteria	History of allergy or hypersensitivity to heparin or any of its components Evidence of history of diseases associated with bleeding risk or bleeding disorder Any disease or condition that might compromise the cardiovascular, or renal, systems
Treatments	Subjects are to receive a single administration of each of the following four treatments in the following order: • **Treatment A: Heparin 5,000 IU, administered I.V.** • **Treatment B: Tinzaparin 2,500 anti-Xa IU, administered I.V.** • **Treatment C: Tinzaparin 5,000 anti-Xa IU, administered I.V.** • **Treatment D: Tinzaparin 4,000 anti-Xa IU, administered I.V.**
Assay Validation	Precision (anti-Xa activity): [redacted]

Precision (anti-Xa activity): [redacted]
LOD and LOQ were not provided

Washout: (7-14) days

Sampling Times: Serum samples collected at the following time points:

Pre-dose (at 0 min prior to drug administration)
Post-dose: at 2, 5, 10, 15, and 30 min, and at 1, 2, 4, 6, 8, 11.5 and 24 hrs

Pharmacodynamic Data

Three pharmacokinetic / pharmacodynamic parameters were used to determine the activity of tinzaparin in biological samples for the assessment of tinzaparin pharmacodynamics: anti-Xa and anti-IIa activities (using amidolytic methods) and aPTT. A first-order elimination model was used to estimate half-life and volume of distribution for each treatment.

Safety Analysis

Safety was evaluated by analysis of haematological, biochemical and urinalysis parameters before injection and at 2 and 24 hr after injection of each treatment. Safety was also assessed by incidence of adverse events.

Statistical Analysis

The pharmacokinetic parameters were calculated from each subject individually, then mean values and 95% confidence intervals were calculated from the individual parameters. Analysis of variance and covariance was utilized to evaluate the effects of treatments and sampling times on the safety parameters.

Results and Conclusions

Table 14. Pharmacodynamic parameters based on baseline-adjusted **anti-Xa** activity

PD parameter	Treatment A	Treatment B	Treatment C	Treatment D
$t_{1/2}$ (min)				
Mean	60	59	116	78
90% CI	(48-80)	(46-81)	(78-122)	(59-115)
Vd (L)				
Mean	4.6	3.9	3.6	2.4
90% CI	(3.7-6.0)	(2.7-5.0)	(3.0-4.4)	(2.1-2.9)

Table 15. Pharmacodynamic parameters based on baseline-adjusted **anti-IIa** activity

PD parameter	Treatment A	Treatment B	Treatment C	Treatment D
$t_{1/2}$ (min) Mean 90% CI	49 (41-60)	68 (52.97)	80 (61-118)	62 (50-82)
Vd (L) Mean 90% CI	5.1 (4.5-5.9)	7.2 (4.4-21)	6.7 (5.3-9.2)	5.5 (4.3-7.6)

Table 16. Pharmacodynamic parameters based on baseline-adjusted **aPTT** activity

PD parameter	Treatment A	Treatment B	Treatment C	Treatment D
$t_{1/2}$ (min) Mean 90% CI	48 (34-80)	35 (26-52)	57 (59-109)	32 (26-44)
Vd (L) Mean 90% CI	3.9 (3.4-4.7)	5.9 (3.1-5.9)	4.5 (3.4-6.4)	2.8 (2.3-3.7)

Reviewer's Comments:

The Firm only determined half-life and volume of distribution, while other relevant pharmacokinetic parameters as CL and AUC were not determined. In addition, due to the limited sample size employed in the study and the large variability in the data, a meaningful comparison between the four treatments in the study is not feasible.

NDA: 20-484/ Study 702-921 Study Date: Apr 1986-Sep 1986

Type of Study: Safety of Single Dose SC Tinzaparin

Introduction

Study DMP 702-921 is entitled,

"PHASE I PHARMACODYNAMICS AND EFFECT OF LHN-1 ON COAGULATION PARAMETERS IN NORMAL, HEALTHY VOLUNTEERS IN A 5 DAY DOSE REGIMEN "

Objectives

- Compare the effects of 5 SC tinzaparin dosing regimens on the coagulation mechanisms in healthy male subjects.
- Determine the clinical and haematological effects of 5 SC tinzaparin dosing regimens.

Primary Review Issues:

- **How do once and twice daily multiple dosing regimens of tinzaparin compare with respect to their hematological effects?**

Study Design

Open-label, non-randomized, five-period, parallel group study

Subjects	40 subjects
Duration of Study	5 days
Key Inclusion Criteria	Healthy male subjects, 18 to 40 yrs of age Subjects should have a body weight between 65 and 80 kg and within 20% of the ideal weight for height Have no significant diseases or clinically-significant abnormal lab values
Treatments	Subjects were divided into 5 groups and each group was to receive one of the following five SC treatments for 5 consecutive days: • **Treatment A: Tinzaparin 2,500 anti-Xa IU QD** • **Treatment B: Tinzaparin 5,000 anti-Xa IU QD**

- **Treatment C: Tinzaparin 7,500 anti-Xa IU QD**
- **Treatment D: Tinzaparin 2,500 anti-Xa IU BID**
- **Treatment E: Tinzaparin 5,000 anti-Xa IU BID**

Assay Validation	Precision (anti-Xa activity): [redacted] Precision (anti-Xa activity): Not provided LOD and LOQ were not provided
Sampling Times	Plasma samples were collected at the following time points: **Pre-dose** (at 0 min prior to drug administration on days 1-5) **Post-dose**: **Day 1**: at 1, 2, 3 and 4 hrs **Day 2-4:** at 3 hrs **Day 5:** at 1, 2, 3, 8 and 24 hrs
Safety	Subjects monitored for adverse events

Pharmacodynamic Data

The only relevant pharmacodynamic parameters to be determined during the study were anti-Xa activity and aPTT.

Results and Conclusions

No pharmacokinetic parameters were determined.
Significant increases in aPTT and prolongation of thrombin time was observed in all treatment groups. APTT levels returned to normal within 24 hrs after the last dose. As for anti-Xa activity, a significant increase was observed 3 hrs after injection after both once and twice daily dosing. However, only twice daily dosing resulted in a measurable increase in anti-Xa activity on the morning just prior to the next daily dose.

Reviewer's Comments:

Very little, if any, conclusions can be made based on the study data. This is primarily due to the limiting deficiencies in the study design, including infrequent and small number of sampling time points, resulting in incomplete characterization of the pharmacokinetic profiles of the treatments. In addition, anti-IIa, an important primary pharmacodynamic parameter, was not determined in the course of the study.

NDA: **20-484/ Study 702-922** **Study Date:** Oct 1987-Apr 1988

Type of Study: Safety of Multiple Dose SC Tinzaparin

Introduction

Study DMP 702-922 is entitled,

"TOLERANCE AND PHARMACODYNAMIC STUDY OF LOGIPARIN, A LOW MOLECULAR WEIGHT HEPARIN COMPOUND, IN HEALTHY MALE VOLUNTEERS"

Objectives

- Investigate the tolerance and pharmacodynamic effects of three different doses of tinzaparin in healthy male subjects

Primary Review Issues:

1. **How comparable are the tolerance and pharmacodynamic profiles of three different multiple dosing regimens of tinzaparin?**
2. **How effective is protamine sulfate in neutralizing the effects of tinzaparin?**

Study Design

Open-label, non-randomized, parallel group study

Subjects	18 subjects
Duration of Study	5 days
Key Inclusion Criteria	Healthy male subjects, 18 to 45 yrs of age Subjects should have a body weight within 10% of their ideal weight Have no significant diseases or clinically-significant abnormal lab values
Treatments	Subjects were divided into 3 groups and each group was to receive one of the following three SC treatments for 5 consecutive days: • **Group 1: Tinzaparin 150 anti-Xa IU/kg BID** • **Group 2: Tinzaparin 250 anti-Xa IU/kg QD (Discontinued after the first dose due to large changes in coagulation parameters on day 1 following dosing in 4 patients)**

- **Group 3: Tinzaparin 200 anti-Xa IU/kg QD**

  Protamine sulfate, IV to be administered after the last dose of tinzaparin in each subject

Sampling Times	Blood samples collected at the following time points: **Pre-dose** (at day -1) **Post-morning dose on days 1-5**: at 1, 4, 12, 16, 20 and 24 hrs **Additional samples collected** at 4.25 and 6 hrs after the last dose in group 1, and at 4.25, 6 and 8 hrs after the last dose in group 3
Safety	Subjects monitored for adverse events

Pharmacodynamic Markers

The relevant pharmacodynamic parameters to be determined during the study were anti-Xa activity, anti-IIa activity, Thrombin time and aPTT.

Pharmacokinetic Analysis

No pharmacokinetic analysis of the data was performed by the Firm.

Statistical Analysis

Groups 1 and 3 were analyzed separately. A two factor (subject and time) analysis of variance was performed on each parameter.
To determine changes over the five treatment days, a series of Dunnet's t-tests were conducted comparing day –1 mean values with all subsequent mean values for the parameter.
The neutralizing effects of protamine sulfate on day 5 was assessed by comparing the mean value obtained immediately before administration of protamine sulfate and that 15 min after administration.

Results and Conclusions

Pharmacodynamic parameters

aPTT

Significant increases in aPTT were observed 4 hrs after both doses in group1 and 1 hr after the morning dose on each study day. As for group 3, significant increases in aPTT were observed 1 and 4 hrs after the dose on each study day and at 6 and 8 hrs post dose on day 5.
The maximum effect was noted 4 hrs post dose with aPTT values returning to normal at 12 hrs post dose.
After protamine sulfate administration to both groups, aPTT levels were significantly reduced in group 3, but not in group 1. However, at 6 hrs post dose on day 5 in group 3, no reduction in aPTT level was visible.

Anti-Xa Activity

In group 1, significant increases were observed in anti-Xa activity 4 hrs after the morning dose on each study day and 4 hrs after the evening dose on days 1-4. They were also observed 1 hr after the morning dose on days 2-5 and at 8 hrs after the evening dose on days 3 and 4.
Significant increases were observed in anti-Xa activity in group 3, 4 hrs after the dose on each study day, 1 hr after the dose on days 1, 3, 4 and 5, 12 hrs after the dose on days 3-5 and 6 and 8 hrs after the last dose on day 5
Anti-Xa activity did not significantly decrease in either treatment groups after protamine sulfate administration on day 5.

Anti-IIa Activity

In group 1, significant increases were observed in anti-IIa activity 1 hr after the morning dose on days 1 and 5, 4 hrs after the morning and evening doses on each study day and 8 hrs after the evening dose on days 1, 3 and 4. The maximum drug effect was noted 4 hrs post dose.
In group 3, significant increases were observed in anti-IIa activity 4 hrs after the dose on each study day, 1 hr after the dose on days 3 and 6 and 8 hrs after the last dose on day 5. The maximum drug effect was noted 4 hrs post dose.
Anti-IIa activity only significantly decreased in group 3, but not group 1 after protamine sulfate administration on day 5.

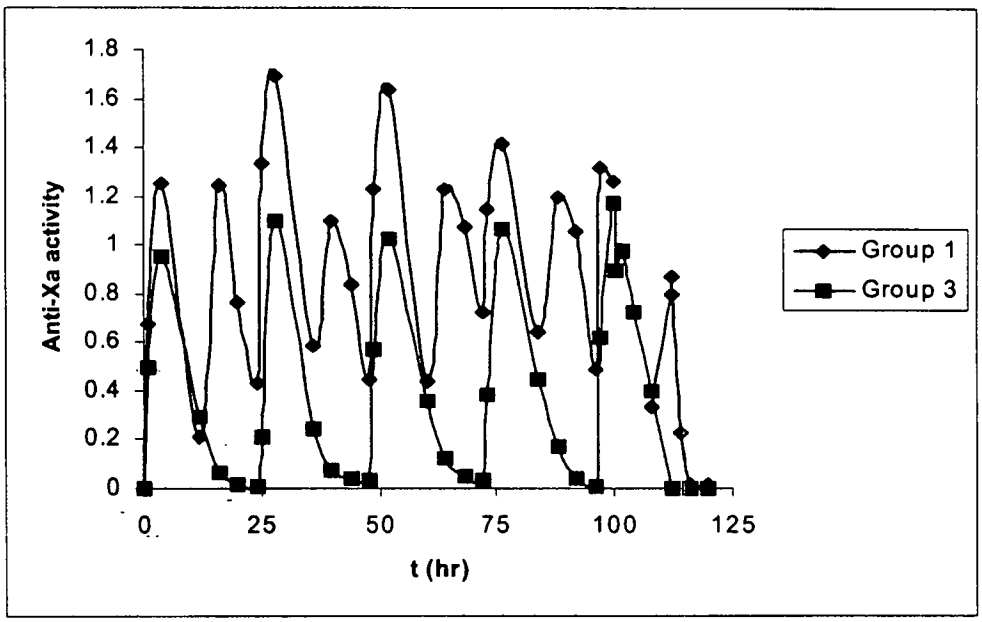


Fig. 3. Anti-Xa activity PK profiles for tinzaparin treatments after twice daily dosing in group 1 and once daily dosing in group 3