

CLr were comparable. The mean fraction of the dose excreted in urine derived from the anti-IIa activity remained similar to that after bolus administration. The anti-IIa activity based mean amounts excreted in urine were similar in the 2 periods whereas the corresponding CLr values on Day 4 tended to be smaller than on Day 1 of Period 1.

Safety

Three bleedings occurred in Period 1 and two bleedings in Period 2.

SGOPT and SGOT increases were observed on Day 5 of Period 2. Maximum observed values were 195 and 109 IU/mL. Fourteen and 29 values, respectively, were high abnormal values. Return to baseline occurred within 41 days.

Conclusions

After administration of a loading dose of 30 mg IV bolus followed by a SC maintenance dose of 1 mg/kg of enoxaparin bid, quasi-steady-state anti-Xa activities were attained rapidly. Mean Amin on Day 1 and Amax on Days 1 and 4 were outside of the sponsor postulated therapeutic range of 0.5- 1.1 IU anti-Xa activity/mL. The CV about the mean values ranged between 9 % and 25%. The mean Amax value after a single IV bolus of 30 mg was within the postulated therapeutic range.

The ratios of the AUC of the anti-Xa activity to the anti-IIa activity after the IV bolus only treatment were 8.1 and 9.4 by model independent and model dependent methods, respectively.

The ratios of the mean AUC_{0-τ} of the anti-Xa activity to the mean AUC_{0-τ} of the anti-IIa activities on Days 1 and 4 of the IV bolus loading dose plus SC maintenance dose treatment were 7.4 and 12.6, respectively. The anti-Xa and anti-IIa activities of enoxaparine display different kinetics so that their exoposure ratio changes over time.

The reported renal clearance and urinary excreted amounts of enoxaparin cannot be considered reliable. The anti-Xa- and anti-IIa activity based assays for enoxaparin in urine cannot be considered validated.

Comments

1. The sponsor used amidolytic assays for the measurement of the anti-Xa-activity and the anti-IIa- activity of enoxaparin to determine routine pharmacokinetic parameters including volume of distribution, intravenous clearance, renal clearance and the amounts excreted in urine. The dose is defined in terms of anti-Xa activity. It is unclear how meaningful values for oral clearance and colume of distribution for the moieties exhibiting anti-IIa activities can be obtained. Separate

doses corresponding to the respective anti-Xa- and anti-IIa activities of enoxaparin should be used in calculating the respective oral clearances and fractions of the dose excreted in urine.

2. Enoxaparin is not a single compound. It is composed of multiple active moieties with anti-Xa activities and anti-IIa activities. A precondition for obtaining meaningful pharmacokinetic parameters of a drug is the availability of a specific and sensitive method. Both the anti-Xa based and the anti-IIa based assays are measuring the respective activities of the moieties. The interpretability of the values of so obtained parameters is very limited.
3. Enoxaparin is comprised of different moieties exhibiting antithrombotic activities. Thus, it may not be realistic to expect a log linear terminal phase. Thus, the usual sensitivity criterion, i.e. an assay must allow minimally a follow-up for a period $\geq 3 \cdot t_{1/2\lambda z}$, may not be applicable for enoxaparin. With the more sensitive anti-Xa assay log-linear terminal phases are not reached after either IV or SC administration. With the less sensitive anti-IIa activity assay the plasma concentrations could be measured trough the dose interval at steady-state in the majority of the subjects. The $t_{1/2\lambda z}$ values must be interpreted with due caution.
4. Contrary to the stipulations of the protocol estimates for CL/F, V/F and $t_{1/2}$ for the anti-IIa and anti-Xa activities of enoxaparin were not reported for the regimen consisting of a 30 mg IV bolus followed by SC maintenance doses administered q 12 h.
5. A rationale for the proposed dose regimen for enoxaparin was not provided.
6. The apparent $t_{1/2\lambda z}$ for the anti-Xa- and anti-IIa activities of enoxaparin after IV or SC administration are more reliable if determined by compartment model dependent methods than by compartment-model dependent methods.
7. The anti-Xa activity assay in urine cannot be considered validated (see comments to Study Report _____ -Enoxaparin). Hence, the values for A_e , f_e and CL_r and the relationship between creatinine clearance and CL_r have to be interpreted with due caution. b(4)
8. In the Methods section (p.29) the report states that aPTT was to be determined on Day 1 (Period 2) 3 h after IV and SC administration. In the Results section (p. 42, Table 8) aPTT maximum and minimum values on Days 1 and Day 4 are reported. The report does not state whether citrated or CTAD blood was used. The study report RP54563Q-142 does not indicate whether the aPTT method used was the same as that described in report ' _____ Design and Evaluation of Analytical Methods Dedicated to the Pharmacokinetic Studies of Enoxaparin in Man: Clotting Methods, Amidolytic Methods (Anti-Xa Activity and Anti-IIa or Antithrombin Activity). b(4)

9. Table 6 indicates a mean Amax for the anti-Xa activity of enoxaparin of 1.164 IU/mL, whereas Table 15 gives a value of 1.148 IU anti-Xa/mL. An explanation for this discrepancy is not provided.
10. The mean age of the studied subjects was 57 (50-68) y, thus it is not appropriate to consider this population to be elderly as stated in the report.

Study RP54563Q-266: Pharmacokinetic Study in Patients undergoing Percutaneous Coronary Intervention (8-12 Hours after Subcutaneous Injection): The PEPCI Study

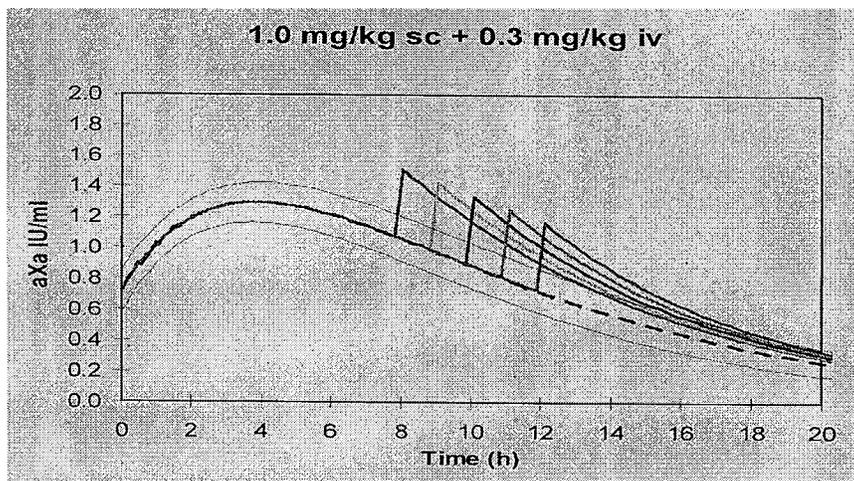
Study Investigators and Sites:

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Background

Mean plasma levels of 0.5 to 1.1 IU anti-Xa /mL were identified as the therapeutic window of enoxaparin in study TIMI 11A. Various models were tested to find an IV bolus “booster” dose of enoxaparin that would immediately give an anti-Xa level in the therapeutic range and would sustain this level for \geq 1-2 hours and could be safely administered during PCI to the patients who are already on treatment with enoxaparin 1 mg/kg SC every 12 h. Most of the PCI procedures last $<$ 1 h.

Based on simulations based on the data obtained in study RP54563-142 an IV bolus dose of 0.3 mg/kg given \geq 8 h after the last SC dose of 1 mg/kg to patients at steady-state is expected to result in plasma anti-Xa activity between 0.60 and 1.8 IU/mL for \geq 2 h after administration as shown in the below figure:



The present study was to evaluate the PK of enoxaparin after administration of a 0.3 mg/kg IV bolus dose in subjects with unstable coronary syndromes who were pretreated with enoxaparin (30 mg IV bolus plus 1 mg/kg SC injection or ≥ 5 SC injections of enoxaparin 1 mg/kg bid). The target level range for the 0-2 h time window after the PCI IV bolus was set at 0.6-1.8 IU anti-Xa/mL. This was despite the fact that the therapeutic range of enoxaparin was stated to range between 0.5 and 1.1 IU anti-Xa activity/mL. An explanation for this discrepancy was not provided.

Objectives

1. Validation of the proposed dose regimen in Percutaneous Coronary Intervention (PCI) by showing that an adequate anti-Xa level is achieved with enoxaparin
2. Characterization of the pharmacokinetic profile of enoxaparin administered subcutaneously (sc) followed by intravenous (IV) bolus injection in patients
3. Assessment of safety of proposed dose regimen

Formulations

100 mg/1 mL enoxaparin ampules Batch Nos. 199903006, 199905061

Design

This was an open-label, parallel group study with four groups of patients depending on timing of PCI after SC injection (Group 1: PCI 8-9 h after last SC administration, Group 2: PCI 9-10 h after last SC administration, Group 3: PCI:10-11 h after last SC administration, Group 4: PCI 11-12 h after last SC administration). Patients were admitted to the unit on Day 1 (day before PCI). The subjects received a 30 mg IV bolus followed by a SC dose of 1 mg/kg or \geq five SC doses (1 mg/kg) bid to attain steady-state prior to the PCI procedure. Subsequently, patients received an IV bolus of 0.3 mg/kg enoxaparin at start of PCI or at end of the diagnostic catheterization if PCI was not performed. The investigators decided which of the alternative regimens to use. Enoxaparin PK were determined prior to and following the PCI IV bolus. Fifty (50) patients were enrolled.

The scheduled activities are shown in the below flow chart:

Table 2: Overall flow chart

| Day of study | Day 1 | Day 1 or 2 or 3 | | | | | | | | | | | | | | | Day 30 ± 2 (Follow Up) |
|--------------------------|-------|--------------------|-----------|-----------|-----------|-----------|----------|-----------|-------|---------------------|-------|-------|-------|-------|--------|-----------|---------------------------|
| | | T ₀ (3) | 2 hrs (4) | 4 hrs (4) | 6 hrs (4) | 8 hrs (4) | pre-dose | PCI Start | 5 min | End of intervention | 2 hrs | 4 hrs | 6 hrs | 8 hrs | 12 hrs | 20-24 hrs | |
| Prior medications | X | | | | | | | | | | | | | | | | |
| Hospitalization | | X | | | | | | | | | | | | | | | |
| Phone call | | | | | | | | | | | | | | | | | X |
| Informed consent | X | | | | | | | | | | | | | | | | |
| Concomitant medications | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Serum Creatinine | X | | | | | | | | | | | | | | | | |
| Hematology | X | | | | | | | | | | | | | | | | X |
| Coagulation | X | | | | | | | | | | | | | | | | |
| ACT | | | | | | | | | | X | | | | | | | |
| Cardiac Markers (1) | X | | | | | | | | | | | | | | | | X |
| Pregnancy test | X | | | | | | | | | | | | | | | | |
| Medical History | X | | | | | | | | | | | | | | | | |
| Physical Examination | X | | | | | | | | | | | | | | | | X |
| Height/Weight | X | | | | | | | | | | | | | | | | |
| Vital Signs | X | | | | | | | | | | | | | | | | X |
| 12-lead ECG | X | | | | | | | | | | | | | | | | X |
| Blood sampling PK | | X | X | X | X | X (5) | X | | X | X (7) | X | X | X | X | X | | |
| Study Drug | X (2) | | | | | | | X (Bolus) | | | | | | | | | |
| Intervention (PCI) | | | | | | | | X | | | | | (8) | | | | |
| Cardiac & Adverse Events | X | | | X | X | X | X | X | X | X | X | X | X | X | | X | X (6) |

(1) Either troponin or CK, (2) IV bolus + SC or 5 SC, (3) Time "zero" = 5 min post IV bolus or pre 5th SC dose depending on regimen, (4) After last SC injection
 (2) (5) For the 8-9 hour dose group this was the same sample as the Pre-dose, (6) Only SAEs: Hemorrhages, local complications at sheath site, thrombocytopenia, and ischemic episodes
 (3) (7) If PCI was not done, sample was not drawn, (8) Sheath had to be removed no sooner than 6 hours post PCI bolus.

Inclusion and exclusion criteria applied in selecting the study population are listed below:

5.2.1 Inclusion Criteria

Patients were required to meet each of the following criteria at the time of randomization:

- Males and non-pregnant females ≥ 18 years of age. Females of child-bearing potential must be using an acceptable method of contraception, and must be non-pregnant/non-lactating. A serum pregnancy test was to be drawn and must be negative.
- Patients clinically stable for 24 hours and scheduled for planned PCI
- Informed consent obtained from the patient before starting any study specific procedures.

5.2.2 Exclusion Criteria

Patients having any of the following conditions were excluded from the study population

- Obesity defined as BMI > 35
- Active internal bleeding or history of hemorrhagic diathesis
- Cerebrovascular accident within 2 years before enrollment, or any cerebrovascular accident with residual neurological deficit. Transient ischemic attack within 6 months of enrollment
- Intracranial neoplasm, arteriovenous malformation or aneurysm
- Allergy to pork, pork products, aspirin, heparin, low molecular weight heparin or history of heparin/LMWH associated thrombocytopenia
- Uncontrolled Hypertension (systolic blood pressure > 180 mmHg or diastolic blood pressure > 110 mmHg)
- Thrombocytopenia < 100,000 / mm³
- Anemia (Hgb <11g male, 9g female)
- Current treatment with anticoagulants (oral, IV, or SC) other than enoxaparin
- Prior treatment with antiplatelet drug not permitted (e.g. Reopro® within 14 days or other marketed Gp IIb/IIIa antagonists (ie Aggrastat™, Integrilin™) within 12 hours

- Renal insufficiency (defined as creatinine clearance < 50 ml/min)

$$\text{Males: } \text{Cl (creat)} = \frac{(140 - \text{age [yrs]}) (\text{body wt [Kg]})}{(72) (\text{serum creatinine [mg/dl]})}$$

$$\text{Females: } \text{Cl (creat)} = \frac{(140 - \text{age [yrs]}) (\text{body wt [Kg]})}{(72) (\text{serum creatinine [mg/dl]})} \times 0.85$$

- Acute Q-wave myocardial infarction with onset of chest pain within previous 24 hours; or thrombolysis within 72 hours
- PCI with larger than a 8F sheath access site
- PCI performed for acute closures, degenerated saphenous vein grafts or those in which a GP IIb/IIIa receptor antagonist will be administered; need for intra-aortic balloon pump
- Greater than 50% stenosis of the left main coronary artery, unless the left coronary system is protected with at least one patent bypass graft
- Planned rotational atherectomy or directional coronary atherectomy
- Participation in other investigational drug clinical research study within 30 days of enrollment, or previous involvement in the current trial

5.2.3 Removal of Patients from Therapy or Assessments

Study drug administration was discontinued if:

- The patient wished to withdraw from the study.
- The patient developed a significant, unexpected, concurrent illness or adverse event.
- The investigator felt it is necessary to terminate the patient's participation for any other reasons.

All patients who received study drug were followed for safety. Therefore, no patient was considered discontinued from the study unless the patient withdrew his/her consent to participate. If a patient was prematurely discontinued, every effort was made to complete all post-infusion assessments.

5.2.4 Prior and Concomitant Treatments

All patients were treated concomitantly with aspirin.

Eptifibatide (Integrilin) could be used if clinically indicated during the PCI. The start of Eptifibatide (Integrilin) should be after the PCI bolus and after the 5 minutes post PCI bolus sample was drawn.

No other GP IIb/IIIa receptor antagonist was used unless clinically indicated for complications requiring their use during the PCI.

Pharmacokinetic Profiling

Blood samples for the determination of anti-Xa and anti-IIa levels were collected following SC administration of enoxaparin at quasi steady-state and after the PCI bolus as follows:

Either 5 minutes after the initial IV bolus or before the 5th SC dose (depending on the type of regimen used), 2, 4, 6, 8 h after the last SC dose, pre-IV bolus (start of PCI or end of diagnostic cardiac catheterization), 5 min, end of intervention (if PCI was not done this sample was not drawn), and 2, 4, 6, 8, 12 h post IV dose.

Bioassay

The plasma samples were analyzed as described in the reports DMPK/2032,

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Plasma anti-Xa and anti-IIa activities were measured using validated chromogenic assays and the results were expressed in IU/mL.

The anti-Xa assay calibration curve was from 0.010 to 0.401 IU anti-Xa/mL with a postulated LLOQ of 0.023 IU anti-Xa/mL. The quantitation range was extended to 2.00 IU anti-Xa/mL with a high diluted quality control sample (1:6). The r^2 values of the calibration curves were ≥ 0.993 . Accuracy determined from the QC samples ranged between - 0.30% and 8.7%. Precision was $\leq 7.3\%$.

$$C = C_0 * \text{EXP}(-K_a * T1) + \text{Dose}_{sc} * K_e / V / (K_e - K_a) * (\text{EXP}(-K_a * T1) - \text{EXP}(-K_e * T1)) + \text{Dose}_{iv} / V * \text{EXP}(-K_e * T2)$$

where:

- C_0 is the concentration of anti-Xa at the start of the last subcutaneous dose during which PCI occurred.
- V is the volume of distribution.
- K_e and K_a are the elimination and absorption rate constants, respectively
- $T1$ and $T2$ are the times elapsed after the SC dose and IV bolus, respectively

The time post PCI during which anti-Xa activity is higher than 0.4 IU/ml was estimated from the data predicted by the model or - if not evaluable- by visual inspection.

Statistical Analysis

A sample size of 12 subjects per group was estimated to be sufficient based on the data from previous studies. Only descriptive statistics were performed.

RESULTS

Fifty five (55) patients were enrolled and all were treated with enoxaparin. Five patients were not given the PCI bolus. Patient 10002 was discontinued because absence of coronary disease was diagnosed. Patients 30001 and 30006 did not receive the PCI bolus, because they were diagnosed to need CABG instead of PCI. Patient 20003 was withdrawn from the study after missing a SC dose and consequently the patient did not receive the PCI bolus. Patient 20020 discontinued consent. Fifteen patients received at least 5 SC injections (1 mg/kg q12 h) and 40 patients received an initial IV bolus of 30 mg followed by at least 1 SC injection of 1 mg/kg enoxaparin.

Two patients were enrolled even though their CL_{cr} was < 50 mL/min violating the exclusion criteria. The characteristics of the patients enrolled are shown in Table 4:

Table 4: Patient Characteristics

| | ALL (N=55) |
|--|---------------|
| Age (years): | |
| MEAN | 62.8 |
| SD | 10.66 |
| MEDIAN | 61 |
| MINIMUM | 38 |
| MAXIMUM | 84 |
| Age Category: | |
| <65 years | 34 (61.8%) |
| ≥65 years | 21 (38.2%) |
| Sex: | |
| Male | 38 (69.1%) |
| Female | 17 (30.9%) |
| Race: | |
| Caucasian | 42 (76.4%) |
| Black | 13 (23.6%) |
| Height (cm): | |
| MEAN | 172.2 |
| SD | 9.88 |
| MEDIAN | 175 |
| MINIMUM | 147 |
| MAXIMUM | 187 |
| Weight (kg): | |
| MEAN | 84.8 |
| SD | 14.24 |
| MEDIAN | 84 |
| MINIMUM | 52 |
| MAXIMUM | 123 |
| BMI: | |
| MEAN | 28.5 |
| SD | 3.63 |
| MEDIAN | 28.4 |
| MINIMUM | 21.5 |
| MAXIMUM | 35.8 |
| Obesity:* | |
| No | 35 (63.6%) |
| Yes | 20 (36.4%) |
| Blood creatinine results (mg/dl): | |
| MEAN | 92.1 |
| SD | 18.14 |
| MEDIAN | 88.4 |
| MINIMUM | 44.2 |
| MAXIMUM | 141.4 |
| Creatinine Clearance (ml/min): | |
| MEAN | 87.05 |
| SD | 26.35 |
| MEDIAN | 88.69 |
| MINIMUM | 28.7 |
| MAXIMUM | 143.5 |
| Creatinine Clearance: | |
| Cl<30ml/min | 1 (1.8%) |
| 30<=Cl<=60ml/min | 9 (16.4%) |
| Cl>60ml/min | 45 (81.8%) |

Extracted from Table 2.02 *APPENDIX II.F.*

Obesity defined as BMI ≥ 30 in male and BMI ≥ 28.6 in female. 2 patients had BMI > 35.

The distribution of the 50 patients treated with PCI IV bolus into the 4 groups based on time elapsed between PCI bolus and last SC dose of enoxaparin is shown in Table 3:

Table 3: Time from SC enoxaparin to PCI bolus

| | 8-9 hrs (N=12) | 9-10 hrs (N=12) | 10-11 hrs (N=11) | 11-12 hrs (N=15) |
|--|-------------------|--------------------|---------------------|---------------------|
| Time from last SC to PCI IV Bolus (hrs) | | | | |
| MEAN | 8.6 | 9.6 | 10.5 | 11.6 |
| SD | 0.3 | 0.3 | 0.3 | 0.5 |
| MEDIAN | 8.6 | 9.7 | 10.3 | 11.5 |
| MINIMUM | 8.02 | 9.08 | 10.05 | 11.00 |
| MAXIMUM | 8.98 | 9.97 | 10.98 | 12.78 |

Forty (40) patients underwent PCI and 15 patients underwent cardiac catheterization only. Median duration of PCI - available for 38 of the patients- was 62 min. With 4 patients the procedure lasted longer than 2 h.

Concomitant Medication

Fifty two (52) patients received aspirin on the day of the PCI procedure or diagnostic cardiac catheterization and 51 continued aspirin after discharge. Thirty nine (39) patients received clopidogrel on the day of PCI and 38 at discharge. For PCI, additional heparin was given to 4 patients (3 in Group 11-12 h and 1 in the group not treated with PCI bolus). Abciximab was administered to 6 patients and eptifibatide to 16 patients. Nitrates, beta-blockers and ACE/ angiotensin II inhibitors were also common concomitant medications.

PK Data

Patients 40002, 40012 and 40014 received heparin and were not evaluable for PK analysis after the PCI bolus. Patients 30002 (Group 1), 30003 and 30004 (Group 2) and 40009 (Group 4) exhibited time profiles of anti-Xa and anti-IIa activities indicating that they had not received the PCI bolus, even though according to the CRF the bolus was administered.

Anti-Xa activity

Compartment model independent methods

The individual anti-Xa activity time profiles for enoxaparin for the 4 groups are shown in Figures 1-4 below:

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Draft Labeling (b5)

Deliberative Process (b5)

The mean PK parameters derived from the anti-Xa activities of enoxaparin are shown in Table 5:

Table 5: Anti-Xa pharmacokinetic parameters: Mean (CV%)

| Parameter | Group 8-9 hrs post SC dose n=12 | Group 9-10 hrs post SC dose n=12 | Group 10-11 hrs post SC dose n=11 | Group 11-12 hrs post SC dose n=15 |
|----------------------------------|------------------------------------|-------------------------------------|--------------------------------------|--------------------------------------|
| Amax1* (IU/ml) | 1.178 (18) | 1.157 (25) | 1.245 (25) | 1.095 (17) |
| Partial AUC1* (h.IU/ml) | 8.81 (25) | 9.38 (24) | 10.88 (28) | 10.30 (14) |
| Aaverage [2-8h] (IU/ml) | 1.08 (21) | 1.06 (24) | 1.14 (27) | 0.97 (14) |
| Pre-IV PCI bolus (IU/ml) | 0.870 (31) | 0.799 (20) | 0.749 (33) | 0.530 (30) |
| Amax2* (IU/ml) | 1.421 (24) | 1.190 (26) | 1.227 (25) | 0.999 (31) |
| End of PCI (IU/ml) | 1.211 (29) | 0.932 (21) | 0.927 (18) | 0.856 (37) |
| 2h post PCI (IU/ml) | 1.101 (34) | 0.954 (25) | 0.935 (28) | 0.750 (33) |
| Partial AUC2* (h.IU/ml) | 9.43 (35) | 7.56 (30) | 8.01 (39) | 6.00 (33) |
| Aaverage [0-2h] post PCI (IU/ml) | 1.24 (26) | 1.02 (24) | 1.07 (27) | 0.85 (32) |
| Ratio Amax2/Amax1 | 1.20 (16) | 1.04 (22) | 1.00 (17) | 0.90 (28) |
| Ratio | 1.15 (16) | 0.97 (17) | 0.96 (17) | 0.85 (26) |
| Aaverage [0-2h]/[2-8h] | | | | |
| Time over 0.4 IU/ml (h) | 11.3 (42) | 9.2 (33) | 10.5 (68) | 7.0 (33) |

*Amax1: maximum value after last SC dose before IV bolus.

Amax2: maximum value post IV bolus.

AUC1: partial AUC between T0 and pre-IV PCI bolus.

AUC2: partial AUC between pre-IV PCI bolus and 12h after PCI bolus.

The mean Amax values after the last SC injection before the PCI bolus IV injection were similar among the 4 groups and ranged between 1.095 and 1.245 IU anti-Xa/mL. The mean anti-Xa activity in the period 2-8 h after the last SC injection among the 4 groups was also comparable. However, the mean pre-PCI IV bolus anti-Xa activities were 0.870, 0.799, 0.749 and 0.530

IU /mL in Group 1, 2, 3 and 4, respectively indicating that the anti-Xa activity in Group 4 was below the lower limit of the therapeutic range. The mean Amax values after the PCI IV bolus ranged between 0.999 and 1.421 IU/mL in the four groups and the mean anti-Xa activity in the period of 0-2 h after the PCI IV bolus ranged between 0.85 and 1.24 IU anti-Xa/mL with Group 4 displaying the smallest values. The anti-Xa activity levels 2 h after the PCI IV bolus were 1.101, 0.954, 0.935 and 0.750 IU/mL in Group 1, 2, 3 and 4, respectively, and in the target range. The CV about the above mean values ranged between 18 % and 37 %.

Figure 5 displays the individual minimum and maximum anti-Xa levels (IU mL) in the subjects of the 4 groups in the period 2-8 h after the last SC dose:

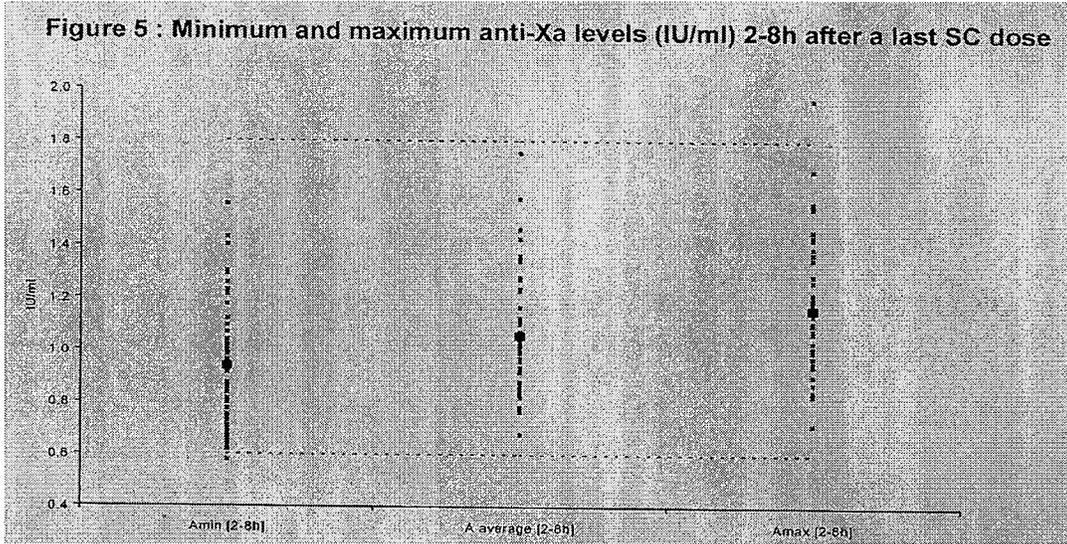
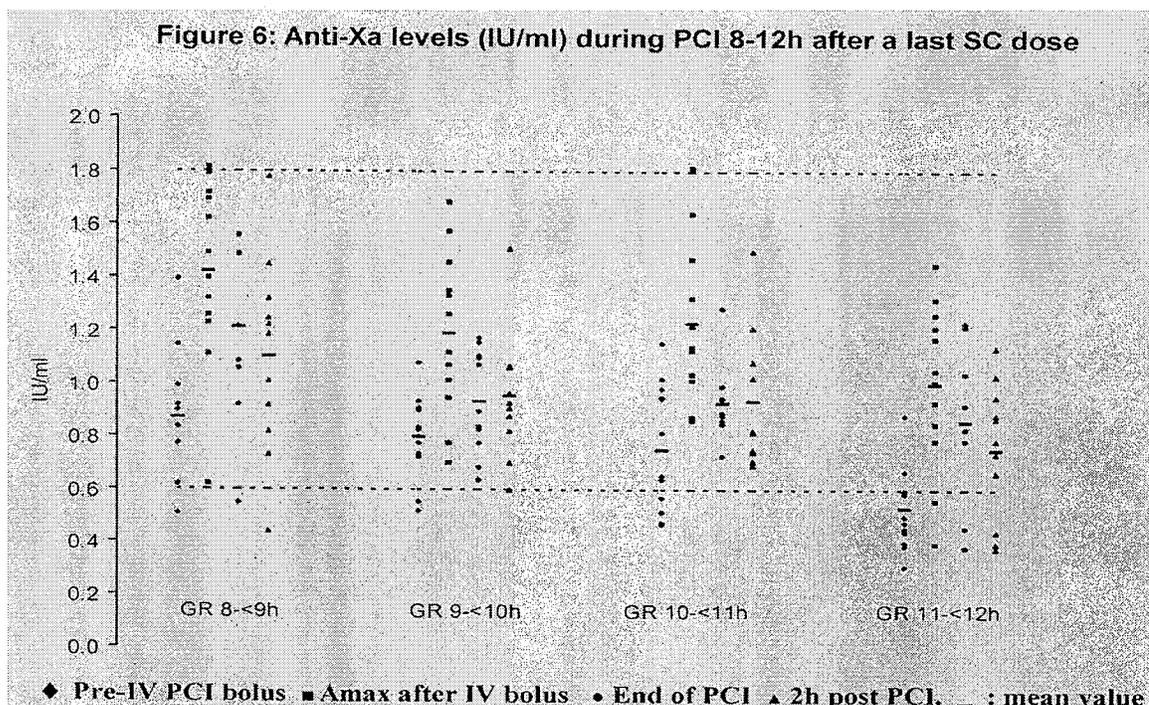


Figure 6 shows the individual anti-Xa levels (IU/mL) during the PCI procedure 8-12 h after the last SC dose in the different groups:

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Figure 6: Anti-Xa levels (IU/ml) during PCI 8-12h after a last SC dose



The plot shows that the large majority of the values measured were within the target range, however a significant number of subjects experienced Amax values exceeding the upper limit of the sponsor's postulated therapeutic range at various times of the regimen.

The profiles of the anti-Xa activity in patients 30004 (Group 1), 30002 and 30003 (Group 2) and 40009 (Group 4) did not indicate that a PCI bolus was administered.

Anti-Xa levels during the period of 6-8 h after the last SC dose were within the target range (0.60-1.8 IU/mL) in 49 of the 50 evaluable patients for Amin and Amax.

Group 1 receiving the PCI bolus between 8-9 h after the last sc dose:

The Amax values were within the target range (0.6-1.80 anti-Xa IU/mL) in 11 of the 12 patients after administration of the PCI bolus. Amax in one patient was 1.813 anti-Xa IU/mL. Mean anti-Xa levels remained in the target range in 10 of 11 evaluable patients. Patient 30004 with a value of 0.44 anti-Xa IU/mL 2 h post PCI had the IV bolus incorrectly administered.

Group 2 receiving the PCI bolus between 9-10 h after the last sc dose:

The Amax values were within the target range for all subjects in the group and remained in the target range for all 11 evaluable patients for 2 h after the PCI bolus.

Group 3 receiving the PCI bolus between 10-11 h after the last sc dose:

The Amax values were within the target range for 10 of the 11 patients. In one patient Amax was 1.81 anti-Xa IU/mL. The levels remained in the target range for all 10 evaluable patients for 2 h after administration of the PCI bolus.

Group 4 receiving the PCI bolus between 11-12 h after the last sc dose:

Amax was in the target range for 11 of the 12 evaluable patients and the anti-Xa activities remained within the target range for an additional 2 h after PCI bolus administration in 9 of the 12 evaluable patients. Two patients had levels below 0.6 anti-Xa IU/mL at Amax and 2 h following PCI bolus. Patient 20011 received the PCI bolus too late (12 h 47 min after the last sc administration) and patient 40009 received the PCI bolus incorrectly. Patient 40005 who had a value < 0.60 anti-Xa IU/mL 2 h after the PCI bolus, received the intravenous bolus injection too late (12 h 22 min after the last sc administration).

In summary the Amax values for the anti-Xa activity following the PCI intravenous bolus were found to be in the target range in 43 of the evaluable 47 patients. The 2 h anti-Xa levels were in the target range in 41 of the 45 evaluable patients. Two of the 4 patients with an anti-Xa level below 0.6 IU/mL received the PCI IV bolus 22 and 47 minutes later than prescribed by the protocol and in a third patient the PCI bolus was not administered correctly. As expected, the anti-Xa levels pre-IV PCI bolus, Amax2, end of PCI and 2 h post PCI decreased as the interval between last SC dose and PCI bolus increased.

Compartment model dependent methods

Table 6 show a comparison of the compartment model predicted and experimentally observed Amax values and anti-Xa levels 2 h following PCI IV bolus:

Table 6 : Predicted and observed anti-Xa plasma activity (IU/ml, mean ± SD) after a 0.3 mg/kg IV bolus given during a 1 mg/kg SC q12h regimen.

| PCI | Maximum value (end of bolus) | | | | | Minimum value (2-hour post-bolus) | | | | |
|---------|------------------------------|-----------|-----------|-------|-------------|-----------------------------------|-----------|-----------|-------|-------------|
| | N | Predicted | Observed | Ratio | % in target | N | Predicted | Observed | Ratio | % in Target |
| 8-<9h | 12 | 1.51±0.16 | 1.42±0.35 | 94% | 92% | 11 | 1.16±0.16 | 1.10±0.37 | 95% | 91% |
| 9-<10h | 12 | 1.42±0.16 | 1.19±0.31 | 84% | 100% | 11 | 1.08±0.16 | 0.95±0.24 | 88% | 100% |
| 10-<11h | 11 | 1.33±0.15 | 1.23±0.31 | 92% | 91% | 10 | 0.99±0.16 | 0.94±0.27 | 95% | 100% |
| 11-<12h | 12 | 1.25±0.15 | 1.00±0.31 | 80% | 83% | 12 | 0.92±0.15 | 0.75±0.25 | 82% | 75% |
| 2-8h* | 50 | 1.29±0.13 | 1.16±0.24 | 90% | 98% | 50 | 0.90±0.15 | 0.94±0.24 | 104% | 98% |

Ratio Observed/Predicted. *maximum and minimum values 2-8h after last sc dose before IV bolus

The predicted values systematically underestimated the experimentally observed values, but the extent of the bias is ≤ 12%.

Fits of the individual plasma anti-Xa activity of enoxaparin are shown for 2 representative subjects in the 4 groups:

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Table 11 : Anti-Xa activity – pharmacokinetic parameter after modeling – Mean (CV%)

| Parameter | Group 8-9 hrs post SC dose | Group 9-10 hrs post SC dose | Group 10-11 hrs post SC dose | Group 11-12 hrs post SC dose | All patients |
|-----------------------|----------------------------|-----------------------------|------------------------------|------------------------------|--------------|
| V (l) | 5.50 (23) | 6.91 (38) | 5.70 (35) | 4.15 (22) | 5.56 (33) |
| Ke (h ⁻¹) | 0.255 (41) | 0.252 (27) | 0.251 (45) | 0.200 (17) | 0.244 (37) |
| Ka (h ⁻¹) | 0.121 (38) | 0.132 (26) | 0.152 (43) | 0.185 (37) | 0.142 (39) |
| CL/F (l/h) | 1.49 (58) | 1.85 (63) | 1.47 (62) | 0.82 (22) | 1.43 (61) |

The CL values were greater than those observed in other studies. There was a high negative correlation between Ka and V and the Ka and Ke values were close with overlapping CI. Therefore the results should be interpreted with due caution.

Impact of Co-administration of Eptifibatide and Abciximab

The anti-Xa levels of enoxaparin in the presence and absence of eptifibatide (and abciximab) are shown in Table 7:

Table 7: Influence of eptifibatide (Integrilin) on anti-Xa profile post PCI

| Parameter | With Integrilin (n=16) | Without Integrilin and without Reopro (n=29) |
|----------------------------------|------------------------|--|
| Amax1* (IU/ml) | 1.022 (17) | 1.216 (21) |
| Aaverage [2-8h] pre-PCI (IU/ml) | 0.934 (16) | 1.107 (22) |
| Amax2* (IU/ml) | 1.058 (22) | 1.308 (28) |
| End of PCI (IU/ml) | 0.918 (22) | 1.067 (32) |
| 2h post PCI (IU/ml) | 0.828 (22) | 1.009 (34) |
| Aaverage [0-2h] post PCI (IU/ml) | 0.905 (22) | 1.137 (29) |
| Ratio Amax2/Amax1 | 1.035 (17) | 1.067 (24) |
| Ratio Aaverage [0-2h]/[0-8h] | 0.966 (17) | 1.020 (22) |

Amax1: maximum value after last SC dose before IV bolus.
Amax2: maximum value post IV bolus.

The reports states that in the presence of eptifibatide the anti-Xa levels of enoxaparin were about 20% smaller than in the absence of eptifibatide (Integrilin®) and abciximab (Reopro®). It is not clear how the data presented for the anti-Xa activity of enoxaparin in Table 7 were obtained. Was enoxaparin in the presence of eptifibatide (and abciximab) compared to enoxaparin in the absence of eptifibatide and abciximab or was enoxaparin in the presence of eptifibatide (and absence of abciximab) compared to enoxaparin in the absence of eptifibatide and abciximab?

Also one must assume that the patients were on other drugs that could have impacted the results.

Anti-IIa Activity

Compartment model independent methods

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Table 8: Anti-IIa pharmacokinetic parameters : Mean (CV%)

| Parameter | Group 8-9 hrs post SC dose n=12 | Group 9-10 hrs post SC dose N=12 | Group 10-11 hrs post SC dose n=11 | Group 11-12 hrs post SC dose n=15 |
|--------------------------|------------------------------------|-------------------------------------|--------------------------------------|--------------------------------------|
| Amax1* (IU/ml)* | 0.126 (47) | 0.114 (28) | 0.139 (25) | 0.132 (22) |
| Partial AUC1* (h.IU/ml) | 0.88 (51) | 0.84 (43) | 1.11 (31) | 0.87 (31) |
| PRE-IV PCI BOLUS (IU/ml) | 0.092 (68) | 0.079 (77) | 0.078 (77) | 0.032 (87) |
| Amax2* (IU/ml) | 0.266 (34) | 0.206 (48) | 0.231 (52) | 0.188 (74) |
| End of PCI (IU/ml) | 0.225 (47) | 0.144 (37) | 0.160 (55) | 0.127 (63) |
| 2h post PCI (IU/ml) | 0.145 (46) | 0.132 (45) | 0.116 (55) | 0.121 (113) |
| Partial AUC2* (h.IU/ml) | 0.98 (47) | 0.72 (61) | 0.72 (65) | 0.46 (66) |
| Ratio Amax2/Amax1 | 2.20 (37) | 1.82 (39) | 1.74 (53) | 1.56 (103) |

*Amax1: maximum value after last SC dose before IV bolus.
Amax2: maximum value post IV bolus.
AUC1: partial AUC between T0 and PRE-IV PCI bolus.
AUC2: partial AUC between PRE-IV PCI bolus and 12h after PCI bolus.

The Amax values for the anti-IIa activity of enoxaparin following the PCI IV bolus were by a factor 1.4 to 2.1 greater than those after the last SC administration.

Table 9: Ratios anti-Xa/anti-IIa – Mean (range)

| Parameter | Group 8-9 hrs post SC dose n=12 | Group 9-10 hrs post SC dose n=12 | Group 10-11 hrs post SC dose n=11 | Group 11-12 hrs post SC dose n=15 |
|------------------|------------------------------------|-------------------------------------|--------------------------------------|--------------------------------------|
| Amax1 | 10.5 (5.7-18.4) | 10.7 (6.9-17.5) | 9.1 (6.5-12.9) | 8.6 (6.3-12.9) |
| Partial AUC1 | 11.8 (6.8-26.6) | 13.4 (7.2-27.5) | 10.1 (7.0-13.9) | 12.9 (7.7-25.1) |
| Pre-IV PCI bolus | 11.0 (5.8-23.8) | 11.0 (3.2-18.0) | 12.1 (5.8-25.2) | 11.9 (6.7-18.6) |
| Amax2 | 6.9 (4.3-24.8) | 6.5 (4.2-10.8) | 6.4 (3.6-12.1) | 6.1 (1.9-11.7) |
| End of PCI | 5.3 (3.3-6.9) | 6.9 (4.3-10.8) | 7.2 (3.2-15.1) | 9.0 (4.2-19.0) |
| 2h post PCI | 7.5 (5.7-10.0) | 8.4 (4.7-16.1) | 9.5 (5.9-16.7) | 8.4 (1.7-14.2) |
| Partial AUC2 | 9.5 (7.7-13.2) | 13.5 (7.4-28.0) | 14.0 (8.0-26.2) | 14.8 (7.3-24.8) |

The mean ratio of the partial AUC1 (from time zero to the time of PCI IV bolus administration) of the anti-Xa activity to the partial AUC1 of the anti-IIa activity ranged between 11.8 and 12.9 among Groups 1-4. The mean ratio of the partial AUC2 (from pre-PCI bolus administration to 12 h after PCI bolus administration) ranged between 7.5 and 9.5 in the 4 Groups.

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The mean parameters derived from enoxaparin's effect on aPTT are listed in Table 10:

Table 10: APTT clotting time pharmacodynamic parameters. Mean (CV%)

| Parameter | Group 8-9 hrs post SC dose n=12 | Group 9-10 hrs post SC dose n=12 | Group 10-11 hrs post SC dose n=11 | Group 11-12 hrs post SC dose n=15 |
|----------------------|---------------------------------------|--|---|---|
| T0 (s) | 37.2 (18) | 39.0 (19) | 38.2 (21) | 47.4 (27) |
| Amax1* (s) | 48.3 (25) | 45.1 (12) | 49.4 (17) | 49.8 (23) |
| Pre-IV PCI bolus (s) | 42.4 (31) | 42.6 (21) | 46.0 (26) | 36.7 (17) |
| Amax2* (s) | 63.5 (29) | 65.0 (42) | 69.0 (32) | 57.9 (39) |
| End of PCI (s) | 55.4 (29) | 55.2 (42) | 54.6 (12) | 44.5 (15) |
| 2h post PCI (s) | 45.7 (21) | 46.5 (23) | 45.3 (19) | 48.2 (52) |

*Amax1: maximum value after last SC dose before IV bolus.
*Amax2: maximum value post IV bolus.

The mean Amax values (not corrected for baseline) after the last SC administration and after the PCI bolus in Groups 1- 4 ranged from 45 s to 50 s, and 58 s to 69 s, respectively.

Safety

No death and no major hemorrhages were observed. Four patients experienced a minor hemorrhage by Day 30 (one in each group receiving the PCI bolus). Five patients experienced a serious adverse event by Day 30. Two of the events occurred during the PCI procedure in Group 4 (PCI bolus 11-12 h after last SC injection) and included 2 incidences of thrombosis on the wire, one complicated by myocardial infarction. Anti-Xa levels were not available in these 2 patients. A third patient experienced a myocardial infarction the day following PCI. A fourth patient experienced a recurrent angina on Day 7 after PCI and a fifth patient who did not receive a PCI bolus displayed a thrombocytopenia post CABG.

Efficacy

By Day 30, 3 of the 55 patients, i.e. 3 of 40 patients undergoing the PCI procedure experienced an endpoint, i.e. recurrent angina, MI or urgent re-intervention.

Conclusions

The mean Amax values after the last SC injection before the PCI bolus IV injection were similar among the 4 groups and ranged between 1.095 and 1.245 IU anti-Xa/mL and exceeded the therapeutic range. The mean pre-PCI IV bolus levels attained with the tested dose regimen were- with the exception of Group 4 (0.530 IU anti-Xa/mL)- within the target range of 0.6-1.8 IU anti-Xa/mL. The Amax values after the PCI bolus ranged between 0.999 and 1.421 IU anti-Xa/mL and were within the target range, but not in the therapeutic range. The mean levels of enoxaprin 2 h after administration of the PCI bolus ranged between 1.101 and 0.750 IU anti-Xa activity/mL and were in the target and therapeutic range. The mean ratios of the anti-Xa to the anti-IIa activities with the tested dose regimen ranged between 9.5 and 14.8.

An analysis of the anti-Xa activity in the individual patients in Groups 1-4 performed by the Reviewer shows that Amax after the last SC injection exceeded in 24 (55 %) of the evaluated 44 patients the upper limit of the postulated therapeutic range of 1.1 IU/mL. The anti-Xa activity in the time interval 2-8 h after the last SC dose exceeded the upper therapeutic range in 13 (30%) of the patients. The anti-Xa activity measured prior to the PCI IV bolus exceeded in 1 patient in Group 3 the upper therapeutic limit and in 9 (20%) of the patients the activity was below the lower bound of the therapeutic range (0.5 IU/anti-Xa/mL). Five of the 9 patients were from Group 4 and 3 from Group 3. Maximum anti-Xa activity after the PCI IV bolus exceeded the upper therapeutic range in 27 patients (61%) with only 1 patient from Group 4. At the end of the PCI procedure and 2 h after the PCI procedure the anti-Xa activities exceeded the upper therapeutic range still in 10 (23%) and 8 (18%) patients, respectively, with none of the patients being from Group 4. Two (2) h after termination of the PCI procedure 2 patients from Group 4 and 1 patient from Group 3 displayed anti-Xa activities that were below the lower limit of the therapeutic range.

The tested dose regimen for patients undergoing PCI did provide anti-Xa activities in the target range but the anti-Xa activities were not consistently in the therapeutic range postulated by the sponsor for enoxaparin. The maximum anti-Xa activities attained with the dose regimen result in supra-therapeutic concentrations of enoxaparin possibly associated with a greater risk of bleeding. A POPPK/PD analysis of the data obtained in larger clinical trials with patients exhibiting the target disease with identification of the major covariates may help optimizing the dose regimen in STEMI patients whether or not they undergo PCI or diagnostic cardiac catheterization.

The present study showed that the PCI procedures can last in excess of 2 h. This fact should also be taken into account in defining the final dosage regimen for patients undergoing a PCI procedure.

Comments

1. The compartment model dependent approach yielded unreliable estimates for CL/F, V/F and t1/2. With the non-compartment dependent approach CL/F, V/F and t1/2 were not computed.
2. It is unclear why the sponsor claims that the anti-Xa activities of enoxaparin in a scenario in which a patient undergoes PCI > 8 h after the last SC maintenance dose should be greater than those when a patient undergoes PCI < 8 h after the last SC maintenance dose of enoxaparin.
3. The assumption that most of the PCI procedures last <1 h was not confirmed by the findings of the study. The procedures lasted > 1 h and in a few cases >2 h.
4. It is not clear how the data presented for the anti-Xa activity of enoxaparin in Table 7 were obtained. Was enoxaparin in the presence of eptifibatide (and abciximab) compared to enoxaparin in the absence of eptifibatide and abciximab or was enoxaparin in the presence of eptifibatide (and absence of abciximab) compared to enoxaparin in the absence of eptifibatide and abciximab? Also, the report does not comment on whether the decreased anti-Xa activity of enoxaparin noted in the presence of eptifibatide is considered clinically relevant. The impact of eptifibatide on the anti-IIa levels and the effect on aPTT was not tested. No conclusion regarding a possible interaction between enoxaparin and eptifibatide should be drawn.

Upon Request from the Reviewer the Sponsor provided the following additional Information regarding Study RP56453Q (PEPCI)

1. Data Base for Sponsor Postulated Therapeutic Range for Enoxaparin of 0.5-1.1 IU anti-Xa/mL for STEMI Patients and Sponsor Postulated Target Range of 0.6-1.8 IU anti-Xa/mL for STEMI patients undergoing PCI

The sponsor's answer indicates that the upper and lower bounds of the therapeutic range were the mean peak and trough plasma anti-X activities observed after an effective and safe regimen consisting of a 30 mg IV bolus and 1 mg/kg SC q 12 h in TIMI 1A. The target range for the anti-Xa activity of 0.6-1.8 IU/mL during PCI defined in study 545463Q-266 was based on prior studies and to achieve anticoagulation during the short period of intervention. The target range was to be higher than that observed in unstable angina patients. The upper value of 1.8 IU anti-Xa/mL corresponds to the 75th percentile of the peak anti-Xa activities in patients treated with 1.25 mg/kg q 12 h who did not experience major hemorrhage in the TIMI 1A study. The lower bound of 0.6 IU anti-Xa/mL corresponds to the median trough value obtained with a SC maintenance of 1 mg/kg q 12 and to the lower limit of anti-Xa activity during PCI in 2 prior studies.

2. Adequacy of Database to assess possible Impact of Eptifibatide on the PK of Enoxaparin

The sponsor agrees that the column in Table 7, p.42, of study report PR54563Q-266 on the the possible impact of of eptifibatide co-administration on enoxaparins's anti-Xa activities is only descriptive and no conclusion on the presence or absence of an interaction can be drawn.

3. Rationale for a Threshold of 100 mg for the First Two SC 1 mg/kg Maintenance Doses of the Proposed Regimen for STEMI Patients

The sponsor states that injections of > 100 mg enoxaparin would require 2 injections resulting in an increased risk for bruising and bleeding.

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5. Validation of Assays

Anti-Xa Activity of Enoxaparin in Plasma

Study DMPK/FR/2032: Complementary Validation of Anti-Xa Chromogenic Assay for the Determination of Enoxaparin in Human Plasma

Study Investigator and Study Site:

b(6)

High pressure liquid chromatography, electrophoresis and iso-electric focusing ¹³C-NMR are powerful tools for analysis, characterization and purification of enoxaparin. However, at present, none of these methods can be applied for a routine quantitative determination of enoxaparin in biological fluids. Thus, assay methods based on anticoagulant properties

of the compound, originally developed for UFH are used including global clotting tests and chromogenic assays to measure enoxaparin levels.

Objectives

This report describes additional aspects of the validation of the specific amidolytic anti-Xa activity based assay for enoxaparin that has been previously described

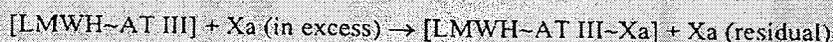
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This report describes the results of dilution experiments aimed at increasing the range of the assay in plasma, the results of stability tests after one and two freeze/thaw cycles and the results demonstrating ruggedness of the assay.

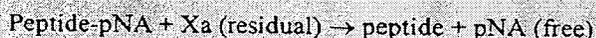
Methods

The amidolytic assay is based on an enzymic reaction in which the target enzyme Factor Xa interacts with a specific substrate. The substrate is an oligopeptide, CBS 3119 which contains a specific binding site for Factor Xa releasing a chromophoric group, paranitroanilin (pNA). pNA can be quantified spectrophotometrically at 405 nm.

- incubation of plasma with a known excess of purified serine protease (factor Xa) :



- hydrolysis of the chromogenic substrate (peptide-pNA) by the residual factor Xa and released of paranitroaniline (pNA) :



The quantity of paranitroaniline (pNA free) released, measured spectrophotometrically at 405 nm during a fixed time (initial rate method) is inversely proportional to the amount of LMWH present in the plasma.

The plot of the logarithm of the absorbance versus the anti-Xa activity of standards produces a linear dose-response curve in the 0.000- 0.401 IU anti-Xa/ml activity range.

The anti-Xa assay was validated by assessing the range of linearity of the calibration curve, the LLOQ, the impact of dilution, the precision and accuracy of quality control samples, the ruggedness of the method and the stability of the plasma samples after one to two freeze-thaw cycles.

Results

The calibration curves (plot of the logarithm of ΔA_{405}) is linear over the range of anti-Xa activities between 0.000 and 0.401 IU/mL. The LLOQ is 0.025 IU anti-Xa/mL.

The results on accuracy and precision of QC samples are shown in Tables 5 and 10:

Table 5 : Within-assay reproducibility of quality controls
Computed activities of each Δ A405nm related to each calibration curve
(March 07, 1996)

| File name | Nominal activity (IU anti-Xa/ml) | | | | |
|------------------|----------------------------------|--------------|--------------|--------------|--------------|
| | QC1a : 0.025 | QC2a : 0.050 | QC3a : 0.100 | QC4a : 0.200 | QC5a : 0.401 |
| VXawit1a,b | 0.021 | 0.050 | 0.113 | 0.226 | 0.387 |
| VXawit2a,b | 0.021 | 0.048 | 0.108 | 0.220 | 0.378 |
| VXawit3a,b | 0.022 | 0.047 | 0.110 | 0.220 | 0.386 |
| VXawit4a,b | 0.023 | 0.047 | 0.107 | 0.215 | 0.372 |
| VXawit5a,b | 0.022 | 0.045 | 0.107 | 0.224 | 0.376 |
| VXawit6a,b | 0.023 | 0.044 | 0.109 | 0.220 | 0.380 |
| Mean | 0.022 | 0.047 | 0.109 | 0.221 | 0.380 |
| SD | 0.001 | 0.002 | 0.002 | 0.004 | 0.006 |
| % CV | 4 | 5 | 2 | 2 | 2 |
| n | 6 | 6 | 6 | 6 | 6 |
| Min | 0.021 | 0.044 | 0.107 | 0.215 | 0.372 |
| Max | 0.023 | 0.050 | 0.113 | 0.226 | 0.387 |
| % relative error | -12 | -6 | 9 | 10 | -5 |

Table 10 : Between-assay reproducibility of quality controls
Computed activities of each Δ A405nm related to each calibration curve

| Date of assay | File name | Nominal activity (IU anti-Xa/ml) | | | | |
|---------------|------------------|----------------------------------|------------|------------|------------|------------|
| | | QC1: 0.025 | QC2: 0.050 | QC3: 0.100 | QC4: 0.200 | QC5: 0.401 |
| Jan.29.96 | VBL21A,B | 0.024 | 0.044 | 0.099 | 0.210 | 0.389 |
| Jan.29.96 | VBL21C,D | 0.020 | 0.052 | 0.100 | 0.185 | 0.353 |
| Feb.19.96 | VDQXa3a,b | 0.027 | 0.055 | 0.111 | 0.211 | 0.400 |
| Feb.22.96 | VDQXa4a,b | 0.026 | 0.059 | 0.111 | 0.192 | 0.366 |
| Mar.06.96 | VXabet1a,b | 0.022 | 0.048 | 0.108 | 0.195 | 0.368 |
| Mar.06.96 | VXabet2a,b | 0.021 | 0.045 | 0.106 | 0.224 | 0.397 |
| | Mean | 0.023 | 0.051 | 0.106 | 0.203 | 0.379 |
| | SD | 0.003 | 0.006 | 0.005 | 0.015 | 0.019 |
| | % CV | 12 | 12 | 5 | 7 | 5 |
| | n | 6 | 6 | 6 | 6 | 6 |
| | Min | 0.020 | 0.044 | 0.099 | 0.185 | 0.353 |
| | Max | 0.027 | 0.059 | 0.111 | 0.224 | 0.400 |
| | % relative error | -7 | 1 | 6 | 1 | -6 |

The within-assay and between-assay accuracies ranged between -12% and 10% and -7% and 6%, respectively. The within-assay and between-assay precision was $\leq 5\%$ and $\leq 12\%$, respectively.

The impact of diluting control samples (1.002 and 9.848 IU anti-Xa/mL) by factors ranging from 1/3 to 1/30 showed that dilutions within this range are appropriate as shown in Table 14:

**Table 14 : Effect of dilution factor on quality controls
Within-assay reproducibility (March 07, 1996)**

| Nominal activity (IU anti-Xa/ml) | Dilution factor | Mean | SD | % CV | n | % relative error | Test of linearity |
|----------------------------------|-----------------|--------|-------|------|---|------------------|--------------------------------------|
| 1.002 | 1/10 | 1.040 | 0.014 | 1 | 6 | 4 | Pr>F = 0.8520 range : 1/5 - 1/3 |
| | 1/5 | 1.121 | 0.011 | 1 | 6 | 12 | |
| | 1/4 | 1.078 | 0.020 | 2 | 6 | 8 | |
| | 1/3 | 1.038 | 0.015 | 1 | 6 | 4 | |
| 2.010 | 1/20 | 2.134 | 0.084 | 4 | 6 | 6 | Pr>F = 0.9658 range : 1/10 - 1/6 |
| | 1/10 | 2.196 | 0.081 | 4 | 6 | 9 | |
| | 1/8 | 2.123 | 0.038 | 2 | 6 | 6 | |
| | 1/6 | 2.050 | 0.038 | 2 | 6 | 2 | |
| 3.007 | 1/30 | 3.279 | 0.152 | 5 | 6 | 9 | Pr>F = 0.1834 range : 1/10 - 1/30 |
| | 1/20 | 3.340 | 0.173 | 5 | 6 | 11 | |
| | 1/15 | 3.384 | 0.060 | 2 | 6 | 13 | |
| | 1/10 | 3.270 | 0.047 | 1 | 6 | 9 | |
| | 1/8 | 3.022 | 0.043 | 1 | 6 | 0 | |
| 5.000 | 1/30 | 5.474 | 0.060 | 1 | 6 | 9 | Pr>F = 0.0551 range : 1/15 - 1/25 |
| | 1/25 | 5.622 | 0.114 | 2 | 6 | 12 | |
| | 1/20 | 5.551 | 0.125 | 2 | 6 | 11 | |
| | 1/15 | 5.228 | 0.126 | 2 | 6 | 5 | |
| 9.848 | 1/60 | 11.145 | 0.824 | 7 | 3 | 13 | Pr>F = 0.5047 range : 1/30 - 1/60 |
| | 1/50 | 11.639 | 1.007 | 9 | 3 | 18 | |
| | 1/40 | 11.026 | 0.816 | 7 | 3 | 12 | |
| | 1/30 | 10.209 | 0.844 | 8 | 3 | 4 | |

The precision and accuracy of the anti-Xa activity of enoxaparin in quality control samples left for 3 h at room temperature relative to freshly thawed samples is impaired as shown in Table 15:

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Table 15 : Rudgeness assay on computed activity of quality controls

Assays performed extemporaneously (t = 0)

| Assay number | File name | Nominal activity (IU anti-Xa/ml) | | | | |
|--------------|------------------|----------------------------------|-------|-------|-------|-------|
| | | 0.025 | 0.050 | 0.100 | 0.200 | 0.401 |
| 1 | Vxabet1a | 0.018 | 0.051 | 0.119 | 0.208 | 0.403 |
| 2 | Vxabet1b | 0.023 | 0.046 | 0.103 | 0.215 | 0.361 |
| 3 | Vxabet2a | 0.021 | 0.045 | 0.107 | 0.226 | 0.406 |
| 4 | Vxabet2b | 0.021 | 0.045 | 0.106 | 0.223 | 0.392 |
| 5 | Vxawit1a | 0.020 | 0.050 | 0.111 | 0.224 | 0.387 |
| 6 | Vxawit1b | 0.023 | 0.050 | 0.114 | 0.229 | 0.388 |
| | Mean | 0.021 | 0.048 | 0.110 | 0.221 | 0.390 |
| | SD | 0.002 | 0.003 | 0.006 | 0.008 | 0.016 |
| | % CV | 9 | 6 | 5 | 4 | 4 |
| | n | 6 | 6 | 6 | 6 | 6 |
| | Min | 0.018 | 0.045 | 0.103 | 0.208 | 0.361 |
| | Max | 0.023 | 0.051 | 0.119 | 0.229 | 0.406 |
| | % relative error | -16 | -4 | 10 | 10 | -3 |

Assays performed after 3.0 hours at the laboratory temperature (t = 3.0)

| Assay number | File name | Nominal activity (IU anti-Xa/ml) | | | | |
|--------------|------------------|----------------------------------|-------|-------|-------|-------|
| | | 0.025 | 0.050 | 0.100 | 0.200 | 0.401 |
| 1 | Vxabet4a | 0.017 | 0.053 | 0.125 | 0.243 | 0.437 |
| 2 | Vxabet4b | 0.015 | 0.052 | 0.115 | 0.231 | 0.407 |
| 3 | Vxabet4c | 0.027 | 0.062 | 0.128 | 0.266 | 0.440 |
| 4 | Vxabet4d | 0.029 | 0.063 | 0.130 | 0.260 | 0.420 |
| 5 | Vxabet4e | 0.018 | 0.042 | 0.109 | 0.233 | 0.397 |
| 6 | Vxabet4f | 0.026 | 0.042 | 0.113 | 0.230 | 0.395 |
| | Mean | 0.022 | 0.052 | 0.120 | 0.244 | 0.416 |
| | SD | 0.006 | 0.009 | 0.009 | 0.016 | 0.020 |
| | % CV | 27 | 18 | 7 | 6 | 5 |
| | n | 6 | 6 | 6 | 6 | 6 |
| | Min | 0.015 | 0.042 | 0.109 | 0.230 | 0.395 |
| | Max | 0.029 | 0.063 | 0.130 | 0.266 | 0.440 |
| | % relative error | -12 | 5 | 20 | 22 | 4 |

Therefore, exposure of plasma samples to room temperature > 3 h should be avoided and the samples should be stored before during and after analysis at 4 ° C.

The impact of freeze-thaw cycles on the performance of the anti-Xa activity assay is shown in Table 16:

Table 16 : Stability assay performed on cycle of freezing - defreezing
Computed activity of fresh quality controls

| Assay number | File name | Nominal activity (IU anti-Xa/ml) | | | | |
|--------------|------------------|----------------------------------|-------|-------|-------|-------|
| | | 0.025 | 0.050 | 0.100 | 0.200 | 0.401 |
| 1 | Vxabet1a,b | 0.022 | 0.048 | 0.108 | 0.195 | 0.368 |
| 2 | Vxabet2a,b | 0.021 | 0.045 | 0.106 | 0.224 | 0.397 |
| 3 | Vxabet2a,b | 0.024 | 0.048 | 0.105 | 0.220 | 0.389 |
| | Mean | 0.022 | 0.047 | 0.106 | 0.213 | 0.355 |
| | SD | 0.002 | 0.002 | 0.002 | 0.016 | 0.015 |
| | % CV | 7 | 4 | 1 | 7 | 4 |
| | n | 3 | 3 | 3 | 3 | 3 |
| | Min | 0.021 | 0.045 | 0.105 | 0.195 | 0.368 |
| | Max | 0.024 | 0.048 | 0.108 | 0.224 | 0.397 |
| | % relative error | -11 | -6 | 6 | 6 | -4 |

Computed activity of quality controls after one cycle

| Assay number | File name | Nominal activity (IU anti-Xa/ml) | | | | |
|--------------|------------------|----------------------------------|-------|-------|-------|-------|
| | | 0.025 | 0.050 | 0.100 | 0.200 | 0.401 |
| 1 | Vxacon1a,b | 0.024 | 0.055 | 0.102 | 0.218 | 0.389 |
| 2 | Vxacon1a,b | 0.013 | 0.040 | 0.100 | 0.211 | 0.362 |
| 3 | Vxacon1a,b | 0.012 | 0.051 | 0.099 | 0.213 | 0.380 |
| | Mean | 0.016 | 0.049 | 0.100 | 0.214 | 0.377 |
| | SD | 0.007 | 0.008 | 0.002 | 0.004 | 0.014 |
| | % CV | 41 | 16 | 2 | 2 | 4 |
| | n | 3 | 3 | 3 | 3 | 3 |
| | Min | 0.012 | 0.040 | 0.099 | 0.211 | 0.362 |
| | Max | 0.024 | 0.055 | 0.102 | 0.218 | 0.389 |
| | % relative error | -35 | -3 | 0 | 7 | -6 |

Computed activity of quality controls after two cycles

| Assay number | File name | Nominal activity (IU anti-Xa/ml) | | | | |
|--------------|------------------|----------------------------------|-------|-------|-------|-------|
| | | 0.025 | 0.050 | 0.100 | 0.200 | 0.401 |
| 1 | Vxacon2a,b | 0.024 | 0.056 | 0.100 | 0.213 | 0.376 |
| 2 | Vxacon2a,b | 0.016 | 0.052 | 0.094 | 0.200 | 0.351 |
| 3 | Vxacon2a,b | 0.005 | 0.056 | 0.092 | 0.203 | 0.373 |
| | Mean | 0.015 | 0.055 | 0.095 | 0.205 | 0.367 |
| | SD | 0.010 | 0.002 | 0.004 | 0.007 | 0.014 |
| | % CV | 64 | 4 | 4 | 3 | 4 |
| | n | 3 | 3 | 3 | 3 | 3 |
| | Min | 0.005 | 0.052 | 0.092 | 0.200 | 0.351 |
| | Max | 0.024 | 0.056 | 0.100 | 0.213 | 0.376 |
| | % relative error | -40 | 9 | -5 | 3 | -9 |

Precision and accuracy of the anti-Xa assay are outside of the allowable range at the 0.025 IU anti-Xa/mL level after one and two freeze-thaw cycles.

Conclusions

The validation of the assay measuring anti-Xa activity of enoxaparin did not include a determination of a safe time period for exposure of plasma samples to room temperature. The study did only determine that samples exposed for a period > 3 h to room temperature are not stable.

Comments

1. The storage time and temperature in the freeze/thaw experiments are not indicated.

Study DMPK/FR/2320: 6 Month Stability Data of the Enoxaparin Anti-Xa Activity in Human Plasma Stored at -20° C and -80° C. Addendum #1 to [REDACTED]
"Complementary Validation of Anti-Xa Chromogenic Assay for the Determination of Enoxaparin in Human Plasma

b(4)

Study Investigator and Study Site:]
[REDACTED] [REDACTED]

b(6)

Objectives

To evaluate the stability of the anti-Xa activity in plasma samples stored at -80°C and -20°C

Methods

The stability of the anti-Xa activity was investigated in plasma samples stored for 1 week, 2 weeks, 4 weeks, 6 weeks, 2 months, 3 months and 6 months of storage. Quality control samples with nominal activities of 0.050, 0.200 and 0.300 IU anti Xa/mL prepared at the start of the study were used. A fresh standard calibration curve was performed with each run of stability QC samples. Accuracy was calculated from the difference between the nominal and calculated mean activities.

Results

Accuracy and precision calculated from the freshly prepared quality control samples (nominal activities 0.050, 0.200 and 0.300 IU anti-Xa/mL) are shown in Table 3:

Table 3 - Back-calculated activities (IU anti-Xa/mL) of quality controls

| Date of assay | Nominal activity (IU anti-Xa/mL) | | |
|---------------|----------------------------------|-------|-------|
| | 0.050 | 0.200 | 0.300 |
| 30-Mar-99 | 0.050 | 0.219 | 0.322 |
| 6-Apr-99 | 0.052 | 0.202 | 0.298 |
| 13-Apr-99 | 0.052 | 0.209 | 0.297 |
| 27-Apr-99 | 0.051 | 0.201 | 0.296 |
| 11-May-99 | 0.046 | 0.211 | 0.318 |
| 1-Jun-99 | 0.057 | 0.211 | 0.309 |
| 29-Jun-99 | 0.052 | 0.213 | 0.299 |
| 28-Sep-99 | 0.056 | 0.204 | 0.294 |
| Mean | 0.052 | 0.209 | 0.304 |
| SD | 0.003 | 0.006 | 0.011 |
| CV% | 6.5 | 3.0 | 3.6 |
| n | 8 | 8 | 8 |
| Min | 0.046 | 0.201 | 0.294 |
| Max | 0.057 | 0.219 | 0.322 |
| % Diff | 3.6 | 4.3 | 1.3 |

Accuracy ranged between 1.3% and 4.3% and precision was < 6.5% in the extemporaneously measured samples.

The stability data of the QC samples at -20 ° C (nominal concentrations 0.051, 0.102 and 0.204 IU anti-Xa/mL) are shown in Table 4:

Table 4 - Stability data at -20°C

| date of assay | Time of storage | Nominal activities (IU anti-Xa/mL) | | |
|---------------|-----------------|------------------------------------|-------|-------|
| | | 0.051 | 0.102 | 0.204 |
| 30-Mar-99 | T0 | 0.045 | 0.094 | 0.214 |
| | | 0.046 | 0.104 | 0.211 |
| | | 0.047 | 0.106 | 0.221 |
| | Mean | 0.046 | 0.101 | 0.215 |
| % Diff | -9.8 | -6.6 | 5.6 | |
| 06-Apr-99 | 1 week | 0.056 | 0.112 | 0.234 |
| | | 0.054 | 0.111 | 0.232 |
| | | 0.054 | 0.115 | 0.241 |
| | Mean | 0.055 | 0.113 | 0.236 |
| % Diff | 7.2 | 10 | 16 | |
| 13-Apr-99 | 2 weeks | 0.056 | 0.115 | 0.238 |
| | | 0.061 | 0.122 | 0.247 |
| | | 0.053 | 0.118 | 0.250 |
| | Mean | 0.057 | 0.118 | 0.245 |
| % Diff | 11 | 16 | 20 | |
| 27-Apr-99 | 1 month | 0.041 | 0.087 | 0.195 |
| | | 0.039 | 0.089 | 0.205 |
| | | 0.040 | 0.096 | 0.211 |
| | Mean | 0.040 | 0.091 | 0.204 |
| % Diff | -22 | -11 | -16 | |
| 11-May-99 | 5 weeks | 0.045 | 0.104 | 0.222 |
| | | 0.045 | 0.094 | 0.229 |
| | | 0.046 | 0.105 | 0.231 |
| | Mean | 0.045 | 0.101 | 0.227 |
| % Diff | -11 | -1.0 | 11 | |
| 01-Jun-99 | 2 months | 0.050 | 0.106 | 0.206 |
| | | 0.051 | 0.103 | 0.212 |
| | | 0.047 | 0.100 | 0.205 |
| | Mean | 0.049 | 0.103 | 0.208 |
| % Diff | -3.3 | 1.0 | 2.0 | |
| 29-Jun-99 | 3 months | 0.048 | 0.102 | 0.211 |
| | | 0.047 | 0.103 | 0.216 |
| | | 0.054 | 0.114 | 0.243 |
| | Mean | 0.050 | 0.106 | 0.223 |
| % Diff | -2.6 | 4.2 | 9.5 | |
| 28-Sep-99 | 6 months | 0.050 | 0.108 | 0.219 |
| | | 0.058 | 0.109 | 0.209 |
| | | 0.052 | 0.109 | 0.217 |
| | Mean | 0.056 | 0.109 | 0.215 |
| % Diff | 11 | 6.5 | 5.4 | |

At time zero the accuracy of the QC samples ranged from -9.8% to 5.6%. After 4 weeks of storage the accuracy ranged from -22% to -0.16% and after 6 months from 5.4 to 11%.

The stability data of the QC samples (same nominal concentrations) at -80° C are shown in Table 5:

Table 5. Stability data at -80°C

| date of assav | Time of storage | Nominal activities (IU anti-Xa/mL) | | |
|---------------|-----------------|------------------------------------|--------------|--------------|
| | | 0.051 | 0.102 | 0.204 |
| 30-Mar-99 | T0 | 0.045 | 0.094 | 0.214 |
| | | 0.046 | 0.104 | 0.211 |
| | | 0.047 | 0.106 | 0.221 |
| | Mean | 0.046 | 0.101 | 0.215 |
| | % Diff | -9.8 | -0.65 | 5.6 |
| 06-Apr-99 | 1 week | 0.059 | 0.121 | 0.240 |
| | | 0.052 | 0.120 | 0.238 |
| | | 0.061 | 0.107 | 0.238 |
| | Mean | 0.057 | 0.116 | 0.239 |
| | % Diff | 12 | 14 | 17 |
| 13-Apr-99 | 2 weeks | 0.060 | 0.118 | 0.245 |
| | | 0.057 | 0.118 | 0.241 |
| | | 0.057 | 0.114 | 0.214 |
| | Mean | 0.058 | 0.117 | 0.233 |
| | % Diff | 14 | 14 | 14 |
| 27-Apr-99 | 1 month | 0.042 | 0.094 | 0.200 |
| | | 0.038 | 0.094 | 0.200 |
| | | 0.045 | 0.097 | 0.200 |
| | Mean | 0.042 | 0.095 | 0.200 |
| | % Diff | -18 | -6.9 | -2.0 |
| 11-May-99 | 6 weeks | 0.051 | 0.111 | 0.228 |
| | | 0.045 | 0.106 | 0.229 |
| | | 0.032 | 0.097 | 0.181 |
| | Mean | 0.043 | 0.105 | 0.213 |
| | % Diff | -16 | 2.6 | 4.2 |
| 01-Jun-99 | 2 months | 0.056 | 0.109 | 0.213 |
| | | 0.060 | 0.111 | 0.211 |
| | | 0.060 | 0.111 | 0.212 |
| | Mean | 0.059 | 0.110 | 0.212 |
| | % Diff | 15 | 8.2 | 3.9 |
| 29-Jun-99 | 3 months | 0.052 | 0.112 | 0.230 |
| | | 0.053 | 0.107 | 0.223 |
| | | 0.053 | 0.102 | 0.215 |
| | Mean | 0.053 | 0.107 | 0.223 |
| | % Diff | 3.3 | 4.9 | 9.2 |
| 28-Sep-99 | 6 months | 0.055 | 0.111 | 0.229 |
| | | 0.061 | 0.111 | 0.218 |
| | | 0.059 | 0.109 | 0.219 |
| | Mean | 0.058 | 0.110 | 0.222 |
| | % Diff | 14 | 8.2 | 8.8 |

The accuracy of the QC samples stored at -80 ° ranged from - 18% to 17% within the 6 month period tested without any apparent dependence on the length of storage.

Conclusion

The results indicate that plasma samples to be assayed for anti-Xa activity of enoxaparin can be stored for 6 months at -20°C or -80°C.

Comments

None

Anti-Xa Activity of Enoxaparin in Urine Samples

Study _____ **Enoxaparin: An Amidolytic Anti-Xa Activity Assay for the Determination of Low Molecular Weight Heparin in Biological Active Components in Urine**

b(4)

Study Investigator and Study Site: _____

b(6)

Normal urine contains a wide spectrum of mucopolysaccharides with predominance of chondroitin sulfates and heparan sulfates. The average amount of endogenous mucopolysaccharides excreted daily in urine of healthy male volunteers has been estimated to be about 5 mg. It is important to differentiate between endogenous and exogenous mucopolysaccharides.

Objectives

The report describes the amidolytic assay procedure developed to measure the anti-Xa activity of enoxaparin in urine. The urine samples were obtained from healthy volunteers receiving 20 or 40 mg enoxaparin by the SC route.

Methods

The assay is a two-step procedure with pretreatment of the urine samples with alcohol followed by measurement of the anti-Xa activity by an amidolytic method with an adequate supply of antithrombin III. The amidolytic method uses an enzymic reaction in