Study PK91006: RP 54563 Enoxaparin Open Single Ascending Dose Pharmacokinetic and Tolerance Study of Enoxaparin After SC Administration of 1.0 mg/kg, 1.25 mg/kg, 1.5 mg/kg, and 2.0 mg/kg in Sixteen Normal Healthy Volunteers. *Pharmacokinetic Study Report*. 3 May 1994. **[Vol. 6, pages 6-1-74 to 77, 84, 85, 87]**

$Cl_{s/F}$ [1.25 mg/kg] = 2.50 ± 0.40 l/h (CV = 16 %)
[range = 1.96 - 3.27 l/h]

$Cl_{s/F}$ [1.5 mg/kg] = 2.56 ± 0.59 l/h (CV = 23 %)
[range = 1.65 - 3.84 l/h]

$Cl_{s/F}$ [2.0 mg/kg] = 2.05 ± 0.37 l/h (CV = 18 %)
[range = 1.48 - 2.74 l/h]

The statistically significant "dose" effect as evidenced on individual values [GLM procedure Table A171] is due to the difference ($p < 0.05$, Table A172) between the 1.0 mg/kg dose clearance (4.04 l/h) and the clearances calculated for the 1.25 mg/kg, 1.5 mg/kg and 2.0 mg/kg doses [2.50 l/h, 2.56 l/h and 2.05 l/h respectively]. Due to the rather low level of anti-IIa activity at the 1.0 mg/kg dose it can be assumed that the plasma clearance of anti-IIa activity is then over estimated.

Clearance values of individual subject were not significantly correlated to the dose injected [Tables A183 - A184].

5.2.4.2 - Activated partial thromboplastin time : APTT

The APTT baseline values (see individual raw data Tables A29 to A33) were 29.59 ± 3.37 sec (CV = 11%) , 30.25 ± 3.87 sec (C.V. = 13 %) , 29.20 ± 3.15 sec (CV = 11 %) and 29.56 ± 2.59 sec (CV = 9 %) for 1.0 mg/kg , 1.25 mg/kg , 1.5 mg/kg and 2.0 mg/kg treatment groups, respectively. Following one single subcutaneous injection of enoxaparin whatever the dose level APTT values increases slightly but significantly ($p<0.05$) (Tables 13, A25 to A38, Figures 5 - 6 and A215 to A244) indicating that these enoxaparin doses slightly prolonge APTT, ie. A(Δt)max ~ + 11.5 to + 22.0 sec (mean values - range + 8.3 to + 30.1 sec).

A (Δt) max and ratios (M/C)

The average (n = 16 or 11) values of the individual maximum clotting time prolongation [A (Δt) max] are [Table 13] :

A (Δt) max [1.0 mg/kg] = + 11.46 ± 2.20 sec (CV = 19 %)
[range = + 8.30 - + 16.90 sec]

A (Δt) max [1.25 mg/kg] = + 13.35 ± 2.36 sec (CV = 18 %)
[range = + 9.00 - + 16.30 sec]

A (Δt) max [1.5 mg/kg] = + 15.64 ± 3.93 sec (CV = 25 %)
[range = + 9.00 - + 21.60 sec]

A (Δt) max [2.0 mg/kg] = + 21.86 ± 5.13 sec (CV = 23 %)
[range = + 14.50 - + 30.10 sec]

A "dose" and a "subject" effect are statistically significant on APTT clotting time prolongation [GLM procedure, Tukeys multicomparaison test, Table A243]. The APTT maximum clotting time prolongations A (Δt) max are linearly correlated to the dose injected [Table A244] :

A (Δt) max (sec) = 0.765 + 10.448 x dose (mg/kg) (r = 0.744)
n = 59 ($p < 0.001$)

The corresponding mean maximum APTT clotting time (Amax) and ratios (individual values of ratios are reported tables A34 to A38) are as follows :

	Amax (sec)	M/C ratios
1.0 mg/kg	41.04 ± 4.58	1.48 ± 0.16
1.25 mg/kg	43.49 ± 5.56	1.56 ± 0.21
1.5 mg/kg	44.84 ± 6.55	1.62 ± 0.24
2.0 mg/kg	51.41 ± 7.42	1.85 ± 0.26

Tmax

The maximum clotting time prolongations were observed 3.0 to 3.5 hours post dosing [Table 13] :

1.0 mg/kg

Median Tmax : 3.0 h

Tmax [mean] : 3.1 ± 1.1 h (CV = 36 %)

[range = 2.0 - 5.0 h]

1.25 mg/kg

Median Tmax : 3.5 h

Tmax [mean] : 3.5 ± 1.3 h (CV = 37 %)

[range = 2.0 - 6.0 h]

1.5 mg/kg

Median Tmax : 3.3 h

Tmax [mean] : 3.5 ± 1.2 h (CV = 35 %)

[range = 2.0 - 7.0 h]

2.0 mg/kg

Median Tmax : 3.0 h

Tmax [mean] : 3.5 ± 0.9 h (CV = 26 %)

[range = 2.5 - 5.0 h]

No statistically significant difference was observed between Tmax values of the treatment groups [Tables A246].

5.2.4.3 - Prothrombin time : PT

In the four treatment groups, the prothrombin clotting time (PT) values [see individual raw data, Tables A43 to A47] were : 13.2 ± 0.80 sec, CV = 6 % [1.0 mg/Kg], 13.2 ± 0.89 sec; CV = 7 % [1.25 mg/kg], 12.99 ± 0.72 sec, CV = 6 % [1.50 mg/kg] and 13.18 ± 0.77 sec ; CV = 6 % [2.0 mg/kg] prior to enoxaparin , respectively. After subcutaneous injection the effect of the drug on maximum PT clotting time prolongation is weak the average value of the individual maximum A(Δt)max prolongation are seen 2.5 to 3.3 hours post dosing (median Tmax values) [Table 14].

A (Δt) max [1.0 mg/kg]	=	+ 1.63 ± 0.27 sec [range = + 1.2 - + 2.10 sec]	(CV = 17 %)
A (Δt) max [1.25 mg/kg]	=	+ 1.62 ± 0.21 sec [range = + 1.2 - + 1.90 sec]	(CV = 13 %)
A (Δt) max [1.50 mg/kg]	=	+ 2.03 ± 0.39 sec [range = + 1.4 - + 2.7 sec]	(CV = 19 %)
A (Δt) max [2.0 mg/kg]	=	+ 2.38 ± 0.37 sec [range = + 1.90 - + 2.9 sec]	(CV = 15 %)

The corresponding mean PT clotting time maximum international normalized ratio (INR) values are as follows :

	PT (absolute value, sec)	INR
1.0 mg	14.83 ± 0.81	1.30 ± 0.11
12.5 mg/kg	14.84 ± 0.87	1.30 ± 0.12
1.5 mg/kg	15.01 ± 0.74	1.32 ± 0.10
2.0 mg/kg	15.55 ± 0.86	1.42 ± 0.12

All these data may be considered as only extremely weakly influenced by the injected dose.

6 - DISCUSSION

6.1 - Clinical tolerance

The study conditions (clinical monitoring, compliance with inclusion conditions, compliance with evaluation procedures) did not demonstrate any anomalies likely to invalidate any finding or interfere with the interpretation of results for particular subjects or for the study as a whole. With regards to the general and biological tolerance, they were excellent under the conditions of the study.

criteria 24 hours post dosing, is associated with a statistically significant ($p < 0.01$) longer half-life for [redacted] [$t_{1/2\beta}$ range = 6.84 h - 9.99 h, mean values] than that observed for anti-Xa amidolytic activity [$t_{1/2\beta}$ range = 4.96 h - 5.85 h, mean values]

These characteristic features of Heptest® and the differences existing between [redacted] and anti-Xa activity were already observed following the sc injection of the enoxaparin 40 mg dose (14, 29). They are the probable consequence of the multienzymatic potentiation effects of enoxaparin on the coagulation process which are taken into account in this complex (see the [redacted] data sheet) clot-based assay and not evidenced by the specific anti-Xa amidolytic activity measurement.

The peak plasma [redacted] and clotting time prolongation [A (Δt) max] increases linearly with the dose ($p < 0.001$) as are the AUC (0-36 h) and AUC (0-∞). No dose-dependency changes was observed on MRT and t1/2β. Nevertheless, a statistically significance difference was found between dosage regimen. As for the anti-Xa activity, the 1.0 mg/kg dose exhibits lower MRT and t1/2β. mean values than the 1.25 mg/kg, 1.5 mg/kg and 2.0 mg/kg doses.

6.3 - Global anticoagulant activities APTT and PT clotting times

APTT

Regarding the effect of enoxaparin on the activated partial thromboplastin time, the observed clotting times are moderatly prolonged : ranging from + 11.5 sec to + 22.0 sec (mean values) when compared to the baseline value of subjects, and increase with the dose. The APTT ratio is around 1.5 - 1.9 over the 1.0 mg/kg - 2.0 mg/kg dose range. These results suggest that the APTT laboratory test is not an appropriate method for an enoxaparin dose adjusted monitoring during established DVT when this monitoring is necessary.

PT

No statistically significant differences of the prothrombin time prolongation could be established between dosage regimen. The effect of enoxaparin on PT is extremely weak. The INR is about 1.3 - 1.4 over the 1.0 mg/kg - 2.0 mg/kg dose range as compared to the INR baseline value 1.05 - 1.10.

6.4 - Effect of the anticoagulant mixture on biological activities measured

During the course of the study, blood was collected into tubes containing a citrate solution (0.109 M) as anticoagulant (samples for pharmacokinetic profile evaluation) and into [redacted] containing the so-called CTAD mixture consisting of a citrate (0.109 M) and inhibitors of platelet activation : theophylline, adenosine and dipyridamole solution (blood collected before injection and 3 h, 4 h, 12 h and 24 h post dosing, respectively for comparison).

Samples collected into [redacted] exhibited a statistically significante higher anti-Xa and anti-IIa activities [Tables A255 to A267, Appendix X]. As a mean, an increase corresponding to 3 to 10 % of the total anti-Xa activity values equivalent to 0.04 - 0.13 IU anti-Xa/ml for the high anti-Xa activity measured 3 and 4 hours post dosing or to 0.01 - 0.08 IU anti-Xa/ml for the lower ones measured 12 h and 24 h post-dosing were observed. Comparatively, anti-IIa activity differences calculated on mean anti-IIa activities are higher : 15 to 41 % of total activity equivalent to 0.02 - 0.04 IU anti-IIa/ml 3 and 4 hours post injection and 0.01 - 0.02 IU anti-IIa/ml 12 and 24 hours post injection. Thus, the sampling of blood on [redacted] allows a better estimate of anti-Xa and anti-IIa activities in man following injection of heparin and derivatives.

The incidence of the CTAD mixture on clot-based assays is less important. Statistically significant differences were observed between citrated tubes and [redacted] on APTT clotting times but not on [redacted] clotting times [Tables A268 to A281]. The APTT effect varies between 1 to 11 % (mean values) corresponding to a 1 to 5 sec global variation. Such variation cannot be considered as biologically relevant.

As published earlier (30, 31), the release of platelet factor 4 (PF4) modifies the experimental conditions of anti-Xa clot based assays. The PF4 binds with high affinity to heparins which results in an efficient neutralization of anticoagulant activities and prevents the detection of these compounds in the incubation medium. The CTAD mixture was claimed to have the potential advantage to avoid the platelet activation "in vitro" and the artefactual release of PF4 in the test tube (32). If no statistically significant effect was obtained on [redacted] clotting times, results showed a statistically significant increase of the anti-Xa and anti-IIa activities and APTT clotting times measured following blood collection into [redacted] It

likely due to the lack of accuracy of the measurement of the low circulating anti-IIa activity levels mainly following the injection of the 1.0 mg/kg dose. The clearance process remains stable over the dose range (1.0 to 2.0 mg/kg).

The dose proportionality relationship was also demonstrated for the [redacted] biological activity [A(Δt)max and AUC]. Neither the distribution (MRT) nor the elimination (t1/2β) processes were influenced by the dose increase.

The APTT peak plasma activity [A(Δt)max] is linearly correlated with the dose. The effect of enoxaparin on the conventional laboratory methods APTT and PT is weak as generally observed. Their usefulness for enoxaparin monitoring during curative therapy for dose adjustment may be challenged.

On the basis of four biological criteria : specific amidolytic anti-Xa activity, specific amidolytic anti-IIa activity, [redacted] and APTT, enoxaparin exhibits a homogenous, linear and dose independent disposition profile in the high 1.0 - 2.0 mg/kg dose range as previous by described or lower doses (20 mg or ~ 0.3 mg/kg up to 80 mg or ~ 1.1 mg/kg dose range).

The general and local clinical tolerance were good. A slight and tolerable pain has been reported during and shortly after the injection. Minor hematomas at the injection site, well tolerated and which disappeared spontaneously were also observed.

At the 1.5 mg/kg dose level, the mean anti-Xa Amax activity is 1.47 ± 0.17 IU anti-Xa/ml (CV = 12 %) [with a $C_{average}$ value ($AUC_{(0\text{-}24\ h)}$/24 h) of 0.70 IU anti-Xa/ml] while the mean anti-IIa Amax activity is 0.18 ± 0.05 IU anti-IIa/ml [with a $C_{average}$ value of 0.055 IU anti-IIa/ ml], the corresponding apparent elimination half-life values being close to 5.5 (anti-Xa activity) and to 2.8 h (anti-IIa activity).

Overall, this study leads to the conclusion that, contrary to unfractionated heparin used at high doses for the cure of thromboembolic events, the disposition profile of enoxaparin is not modified by the dose level in a very large range (0.3 up to 2.0 mg/kg), then remains predictable without any need of monitoring of hemostasis parameters. This specificity of enoxaparin will contribute to facilitate the use of enoxaparin in patients treated for the cure of DVT because the relationship between effectiveness and pharmacokinetics should be simpler than for unfractionated heparin.

Study PK91107: Efficacy and Safety of Enoxaparin Administered as a Single, Daily Subcutaneous Injection for the Treatment of Venous Thromboembolic Disease. *Pharmacokinetic Study Report.* 24 January 1997. **[Vol. 13, pages 6-1-31 to 34]**

Achievement of steady-state (Day 2 to Day 8 or Day 10)

In patients with DVT and/or PE treated once daily with enoxaparin 1.5 mg/kg, a 1.3 fold increase is observed on baseline [redacted] activity values [the Amin increased from 33.6 s to 44.3 s in absolute value from Day 2 to Day 7] with a statistically significant difference ($p<0.05$) between baseline values of Days 2-4 and Days 4-8 [Tukey's studentized range test - Appendix II.5]. The activities recorded on Day 1 where not taken into account in the assessment of Amin due to the interference with the standard heparin treatment. The A(3h) circulating activities are not statistically significantly different from Day 3 to Day 10 [Tukey's studentized range test - Appendix II.5].

Accordingly, the steady-state is achieved by Day 4 in terms of [redacted]

8.3.4. Clot based assay : aPTT

8.3.4.1. Mean plasma aPTT activity

The individual aPTT activity plasma levels recorded following repeated injection of enoxaparin at 1.5 mg/kg (SC, OD) are reported Table 5 (Appendix II.3). The mean profile of aPTT plasma levels versus time is illustrated in Figure 5 page 40 and individual profiles portrayed in Appendix II.4.

Due to standard heparin treatment given to patients, the aPTT activity recorded on Day 1 before enoxaparin injection ranged from 23.1s to 176.7s depending on patients. As for [redacted] all data were expressed as absolute values as no aPTT baseline activity was recorded before standard heparin or LMWH treatment. The time-course of aPTT profiles are similar on Day 2 and Day 7 (Figure 5 p.40).

8.3.4.2. aPTT activity parameters

Individual parameters are listed Table 5 (Appendix II.2) and the statistical analysis reported in Appendix II.5.

The mean values of aPTT parameters on Day 2 and Day 7, are summarized Table 8 (p.31) :

TABLE 8. *Summary of aPTT parameters on Day 2 and on Day 7*

	Day 2	Day 7
At=0 (s)	30.3 ± 6.6	30.4 ± 4.7
Amax (s)	46.3 ± 12.8	42.5 ± 6.8
t max (h) *	5.0 [3.0-12.0]	4.0 [3.0-24.0]
Ratio max	1.60 ± 0.41	1.48 ± 0.24

Mean ± SD or *median and range (n = 16 patients) - Extracted from Table 5 - Appendix II.2

The effect of a 1.5 mg/kg dose of enoxaparin on aPTT is weak with a slight increase of A max values : + 13s - 16s as a mean when compared to a control (baseline of a normal control in the aPTT assay : 30s) of maximum aPTT. No significant increase of aPTT is observed following repeated administration.

8.3.5. Clot based assay : PT

8.3.5.1. Mean plasma PT activity

The individual PT activity plasma levels and INR values recorded following repeated injection of enoxaparin at 1.5 mg/kg (SC, OD) are reported Tables 6 and 7 (Appendix II.3). The mean profile of PT activity plasma levels versus time is illustrated in Figure 6 page 41 and individual profiles portrayed in Appendix II.4.

On Day 1 baseline activity ranged from 11.5s to 18.1s. As for [redacted] and aPTT, all data were expressed as absolute values as the baseline activity was not recorded before standard heparin or LMWH treatment. The PT activity recorded from Day 2 to Day 8 are only slightly influenced by enoxaparin injections. The antivitamin K treatment increase PT values on Days 10 and 11. (Figure 6 p.41).

8.3.5.2. PT activity parameters

Individual parameters are listed Table 6 (Appendix II.2) and the statistical analysis reported in Appendix II.5.

The mean values of aPTT parameters on Day 2 and Day 7, are summarized Table 9 (p.32).

TABLE 9. *Summary of PT parameters on Day 2 and on Day 7*

	Day 2	Day 7
At=0 (s)	12.5 ± 0.8	12.6 ± 0.8
Amax (s)	15.2 ± 4.6	16.9 ± 6.6
INR *	1.45 ± 0.78	1.68 ± 1.18

Mean ± SD or *median and range (n = 16 patients) - Extracted from Tables 6 and 7- Appendix II.2
* INR = International normalized ratio

The PT assay was not influenced by enoxaparin treatment. Repeated administration of enoxaparin did not lead to an increase of the maximum of activity with Amin and A(3h) circulating activities close to baseline activity recorded on Day 1 [Appendix II.5].

9. DISCUSSION

On Day 1 of enoxaparin administration the activities measured reflect both the effect of heparin (initial anticoagulant treatment given to patients before inclusion in the study) and of enoxaparin. The presence of heparin explained Day 1 baseline activities and may influence the activities recorded 3 hours post enoxaparin injection. The anti-IIa assay and the [redacted] are the most sensitive tests to the presence of unfractionated heparin. This confirms that the [redacted] is not a pure anti-Xa assay but is a global test sensitive to anti-IIa activity.

In patients with DVT and/or pulmonary embolism given enoxaparin 1.5 mg/kg (SC, OD) the biodisposition profiles of anti-Xa on Day 2 are in accordance with previously published data. Comparison of anti-Xa data of the present study and those obtained in 16 healthy volunteers given a single 1.5 mg/kg enoxaparin dose (study K 91006) indicates that the maximum plasma anti-Xa activity recorded in patients (Amax = 1.26 ± 0.21 IU anti-Xa/ml - Day 2), achieved 3.1 hours post SC injection, is close to the maximum plasma anti-Xa activity recorded in healthy young volunteers (Amax = 1.47 ± 0.17 IU anti-Xa/ml).[13] The AUC (0-24h), the clearance and the elimination half-life are in the same rank of order [AUC (0-24h) = 15.21 h.IU/ml, CL/F = 0.73 l/h and t1/2 = 5.0 h - Day 2 - in patients versus AUC (0-24h) = 16.47 h.IU/ml, CL/F = 0.65 l/h and t1/2 = 5.4 h in the dose ranging study healthy volunteers]. Repeated administration of enoxaparin in patients lead to an increase of plasma anti-Xa activities with a 30 - 35 % difference on parameters Amax and AUC(0-24h) between Day 2 and Day 7. Nevertheless, the elimination parameters : apparent elimination half-life and clearance even though slightly modified by the repeated administration remain in the same range as those estimated in healthy volunteers following a single dose administration [t1/2 range = 4.4 - 6.9 h and CL/F range = 0.39 - 0.89 l/h in patients on Day 7 versus t1/2 range = 4.4 - 6.3 h and CL/F range = 0.59 - 0.81 l/h in healthy volunteers given a single dose].

At the 1.5 mg/kg dose, the anti-thrombin activity remains weak as generally observed with maximum circulating activity in the 0.04 - 0.39 IU anti-IIa/ml range on Day 2 and 0.08 - 0.28 IU anti-IIa/ml on Day 7. These activities are similar to the circulating anti-IIa activity measured in healthy volunteers [0.10 - 0.26 IU anti-IIa/ml] given a 1.5 mg/kg single dose. The anti-thrombin activity in plasma is cleared more rapidly from the blood stream than the anti-Xa activity. [13] As generally assumed, the anti-IIa activity is selectively potentiated by oligosaccharides with a chain length longer than 18 monosaccharides while the anti-Xa activity is potentiated by all chains. In patients the anti plasma anti-Xa activity/anti-IIa activity ratios. were found in the 10 - 17 mean range 3 to 4 hours post injection (table 3 - Appendix II. 3].

The anticoagulant activity (aPTT) was only slightly influenced by enoxaparin injections with a maximum clotting time in the 30.7 - 74.0 s range on Day 2 and Day 7(control + 30 s) and a ratio of aPTT maximum over control in the 1.07 to 2.49 range. The PT is not influenced by enoxaparin treatment. No comparison with healthy volunteers data could be made in terms of [redacted] as in the present study only absolute values were measured taking into account the normal baseline activity.

Repeated administration of enoxaparin induced an increase of anti-Xa and [redacted] activity in plasma. The steady-state is achieved on Day 3 and 4 in terms of anti-Xa and [redacted] activities, respectively.

10. CONCLUSION

The anti-Xa activity, anti-IIa activity, [redacted] and aPTT and PT disposition profiles of repeated 1.5 mg/kg once daily administration of the 200 mg/ml enoxaparin formulation was defined on the second and the seventh days after the morning injection in patients with deep vein thrombosis and/or pulmonary embolism. High plasma levels of anti-Xa and [redacted] activities were recorded during the study. Repeated administration of enoxaparin induced an increase of 30% to 35% of anti-Xa and [redacted] Amax and AUC(0-24h) parameters and a slight increase by 0.7 hour of the apparent elimination half-life (anti-Xa activity). The steady state is achieved on Day 3 and 4 in terms of anti-Xa activity and [redacted] respectively. The effects of high dose of enoxaparin on anti-IIa activity, aPTT and PT are weak, as generally observed, and no variations of plasma baseline and A(3h) activities were recorded following repeated administration. Parameters estimated in the present study are similar to previously published data in healthy volunteers given a single 1.5 mg/kg dose and the same formulation.