Pharmacokinetics of the low molecular weight heparin enoxaparin during 48 h after bolus administration as an anticoagulant in haemodialysis

Benjamin Guillet1, Nicolas Simon2, Jérôme José Sampol1, Anne-Marie Lorec-Penet3, Henri Portugal3, Yvon Berland1, Bertrand Dussol1 and Philippe Brunet1

1Service de Néphrologie, Hôpital de la Conception, Marseille, 2Laboratoire de Pharmacologie Médicale et Clinique, UFR de Médecine, Marseille and 3Laboratoire Central, Hôpital Sainte-Marguerite, Marseille, France

Abstract

Background. The interest of low molecular weight heparins (LMWH) regarding bleeding risk is controversial in renal failure patients. In haemodialysis patients, there are very few data on the pharmacokinetics of LMWH after the end of the session. The aim of the study was to evaluate the duration of anticoagulation after bolus administration of the LMWH enoxaparin at the start of haemodialysis.

Methods. The pharmacokinetics of enoxaparin were studied during the 48 h following a single bolus injection at the start of the dialysis session in 30 chronic haemodialysis patients. Pharmacokinetics were determined using a population approach (Non Linear Mixed Effects Modelling).

Results. A single injection of enoxaparin at 60 U IU/kg (4000 ± 455 IU) led to an anti-Xa activity higher than 1.2 IU/ml during the first 2 h of the session, and between 0.4 and 1.2 IU during the third and fourth hours. After the end of the session, anti-Xa activity remained higher than 0.4 IU/ml up to 10 h after injection, and higher than 0.1 IU/ml up to 24 h. The pharmacokinetic model showed that only weight improved the predicted vs observed anti-Xa activity plot. The model was used to simulate single and multiple dosing with decreased enoxaparin doses. Whatever the procedure, anti-Xa activity remained high (>0.22 ± 0.99 UI/ml) up to 12 h after the start of the dialysis session.

Conclusions. These results suggest that haemodialysis patients receiving the LMWH enoxaparin during dialysis are at risk of bleeding up to 10 h after the injection.

Keywords: anticoagulation; anti-Xa; enoxaparin; haemodialysis; nonmem; population pharmacokinetics

Haemodialysis requires extracorporeal circulation of blood. Therefore, a steady state has to be reached between hypo-coagulability leading to haemorrhagic risk and hyper-coagulability leading to thrombosis of the extracorporeal circuit. Unfractionated heparin (UFH) has over the years proven to be a reliable anticoagulant. More recently, low molecular weight heparins (LMWH) have been introduced for haemodialysis anticoagulation [1]. LMWH act longer than UFH [2] and can be given as a single bolus injection at the start of the dialysis session. LMWH are as effective as UFH in preventing thrombosis in animal models and cause less blood loss than UFH. However, these findings have not been confirmed in clinical studies [2,3]. For haemodialysis patients, some studies reported a shorter venous compression time and a lesser need for blood transfusions with LMWH than with UFH [1,4,5]. However LMWH have not always been found to be superior to other anticoagulation regimens in terms of dialysis-related bleeding: a randomized trial found no benefit with LMWH compared with citrate in haemodialysis patients at risk of bleeding [6]. Another randomized study showed that dialysis with the LMWH enoxaparin was associated with an increased frequency of minor haemorrhage between sessions [7]. No randomized prospective study demonstrated that LMWH could be used more safely than UFH in haemodialysis patients at risk of bleeding.

Because LMWH are cleared principally by the renal route, they should be monitored with caution in patients with renal failure. In these patients, several studies reported a significant increase in the half-life of LMWH [3,8–11]. Half-life values were 1.36–2.77 times longer in patients with renal failure than in healthy subjects [8–11]. These modifications of the pharmacokinetics may be clinically relevant as bleeding frequency
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is increased in patients with renal failure receiving LMWH for treatment of venous thrombosis or cardiac diseases [12,13].

Haemodialysis treatment has little or no influence on the pharmacokinetics of LMWH [11,14]. Low-permeability membranes do not modify plasma anti-Xa activities [11,14] and high-permeability membranes induce either no effect [14] or a slight elimination of LMWH [11].

In haemodialysed patients, most studies have investigated the effect of LMWH during dialysis sessions and there are very few data on their kinetic profile after the end of sessions. The anticoagulant effect of the LMWH reviparin was prolonged at least 10 h after its injection [11]. In another study on the LMWH tinzaparin, anti-Xa activity was measured up to 24 h after the injection: anti-Xa activity returned to baseline values only after 12 h [15].

The aim of this study was to determine the pharmacokinetic parameters of the LMWH enoxaparin during the 48 h following a single bolus injection at the beginning of the haemodialysis session. This study could provide insight on the haemorrhagic risk of haemodialysis patients and help to determine the duration of the anticoagulant effect of enoxaparin.

Enoxaparin pharmacokinetic data were assessed through the anti-Xa activity. This is the established method for assessing systemic exposure to LMWH because heparin concentrations are not directly measurable. Moreover, this method has the advantage of providing a measure of the pharmacodynamic response to the drug [3].

Subjects and methods

Patients

Thirty adult patients (20 male, 10 female) in end-stage renal failure requiring chronic haemodialysis were enrolled (Table 1). The sample size was determined by the necessities of the pharmacokinetic modelling. The inclusion criterion was age older than 18 years, chronic haemodialysis started for at least 3 months, routine use of enoxaparin as anticoagulant, lack of residual renal function (anuria), and native arteriovenous fistula or synthetic graft as vascular access. Exclusion criteria were anaemia with haemoglobin level inferior to 10 g/dl, recent trauma, surgery, infectious disease or haemorrhagic disorder (<1 month), current administration of heparin between dialysis sessions, and dialysis through a central venous catheter. The study was approved by the local ethics committee and written informed consent was obtained from all participants.

Haemodialysis technique

The mean enoxaparin doses were 60 IU/kg (4000 ± 4551U). These doses were adjusted to the actual body weight and were those routinely administered in the unit.

Blood sampling and laboratory measurements

The pharmacokinetic follow up was carried out with repetitive measures of plasma anti-Xa activity during the 48 h after the beginning of the dialysis. The sampling scheme designed for the population pharmacokinetic analysis used sparse sample time points covering the full anti-Xa activity profile. Three samples were retrieved per patient, with different sampling times between patients. Two samples were retrieved during the dialysis session (t = 0–4 h) and a third sample after the end of the session up to 48 h following the enoxaparin injection (t = 4–48 h). The first two samples were collected from the afferent line and the last sample was obtained from venipuncture. Separate experiments performed during dialysis sessions showed similar values for anti-Xa activities obtained from the afferent line or venipuncture. Blood samples were collected into citrated tubes (BD Vacutainer, 9 NC 0.129 M). Anti-Xa activity was determined by a chromogenic assay (Rotachrom® heparin, STA). The lower limit of detection was 0.1 IU/ml; the standard curve was linear between 0.1 and 21 IU/ml; the intra-assay variance and the day-to-day variation coefficient were 3.8 and 3.5% respectively.

Clotting and bleeding were routinely evaluated from the time to haemostasis after needle removal, and from the clotting of the dialyser and blood lines according to the following scale: 1, no clotting; 2, partial clotting of the dialyser or the blood lines; 3, complete clotting of the dialyser; 4, complete clotting of the blood lines.
Data analysis

Anti-Xa activity-time data analysis was performed using a Non-linear Mixed Effects Modelling approach, implemented in the MP2 program (Micropharm population, INSERM, Paris, France) [16] and in the NONMEM software (version V, level 1.0) [17]. One- and two-compartment pharmacokinetic models with bolus administration and first-order elimination were tested to fit the data. The following models were used to describe the inter-subject variability of the pharmacokinetic parameters:

\[ P_j = P_{\text{pop}} \times (1 + \eta_{pj}) \]  
proportional model

\[ P_j = P_{\text{pop}} + \eta_{pj} \]  
additive model

in which, \( P_j \) is a kinetic parameter of the \( j \)th individual, \( P_{\text{pop}} \) is the population mean value of the parameter and \( \eta_{pj} \) is the inter-individual error, distributed normally with zero mean and variance equal to \( \omega^2 \).

Residual error was modelled in two ways:

\[ C_{\text{obs}} \]  
proportional error

\[ C_{\text{obs}} = C_{\text{pred}} + \epsilon_{ij} \]  
additive error

in which, \( C_{\text{obs}} \) and \( C_{\text{pred}} \) are the \( i \)th observed and the model-predicted Anti-Xa activity in the \( j \)th patient, and \( \epsilon_{ij} \) is the residual error, distributed normally with zero mean and variance \( \sigma^2 \).

Model building procedure

A first analysis was done to find the base model that best defined the data. The models were described in terms of pharmacokinetic parameters such as clearance and volume of distribution. The assumption was made that the pharmacokinetics were linear and thus that the parameters were constant. The model selection was based on plots (observed vs predicted anti-Xa activity, residuals vs predicted) and on predictive performance assessed in terms of bias (mean prediction error, ME) and precision (root mean square prediction error, RMSE) as follows:

\[ ME = 1/n \cdot \sum PE_i \]
\[ RMSE = \sqrt{(1/n \cdot \sum PE_i^2)} \]

where \( PE_i \) stands for the difference between the \( i \)th measured and predicted anti-Xa activity pair taken at a given time, \( n \) being the number of pairs. The base model estimated the pharmacokinetic parameters without any covariates. Once it was defined, the influence of each covariate on the pharmacokinetic parameters was tested. These covariates were gender, age and body weight. Plot of observed vs predicted anti-Xa activity, the change in objective functions and the change in parameter variability were noted. A decrease in the objective function value of at least 6.61 (\( \chi^2 \) distribution with one degree of freedom for \( P < 0.01 \)) relative to the base pharmacokinetic model was required for the addition of a single parameter in the model. Covariates that significantly reduced the objective function were then combined in a stepwise fashion until no further reduction of the objective function occurred (full model). An intermediate multivariate model was then obtained including all significant covariates. Finally, to keep only the covariates with the largest contribution to predict anti-Xa activity in a final model, a change in the objective function of at least 10.82 (\( P < 0.001 \)) was required for a parameter to be retained during backward stepwise multiple regression analysis. The terminal half-life was determined by the following equation: \( T_{1/2} = \log 2/\beta \).

Anti-Xa activity simulation

The final model (including inter- and intra-individual variances) and the population database were used to perform a Monte-Carlo simulation as implemented in NONMEM. Three schemes of administration and dosing were simulated: (i) one i.v. bolus of 4000 IU at the start of the dialysis session; (ii) one i.v. bolus of 2000 IU at the start and a second bolus of 1000 IU after 2 h; and (iii) one i.v. bolus of 1000 IU at the start followed by a 2-h infusion of 1000 IU/h.

Results

All 30 dialysis sessions were performed with no bleeding at a mean enoxaparin dose of 4000 ± 455 IU. One case of partial clotting of bloodlines was recorded. Figure 1 shows anti-Xa activity vs time for blood samples obtained during the 48 h after the single bolus injection of the LMWH enoxaparin at the beginning of the dialysis session.

Anti-Xa activity after enoxaparin administration for dialysis anticoagulation followed a bi-exponential elimination curve with a rapid phase followed by a slow phase. A pharmacokinetic model with two compartments and first-order elimination best fitted the data (Figure 2). However, the highest anti-Xa activities corresponding to values observed immediately after infusion were less described by the model. The models were parameterized in terms of central volume of distribution (\( V_s \)), clearance (CL), peripheral volume of distribution (\( V_p \)) and intercompartmental clearance (\( Q \)). After several analyses, it was found that the proportional error model was the most appropriate for inter-subject and residual variabilities. Among the covariates tested (age, weight, gender), only weight
significantly decreased the objective function and improved the predicted versus observed anti-Xa activity plot; weight was therefore kept in the final model. Whatever the parameter tested, the inclusion of age never improved the model. The pharmacokinetic parameter estimates for the base and final model are shown in Table 2. The terminal half-life ($t_{1/2b}$) based on microconstant pharmacokinetic parameters was $13.9\text{ h}$.

The central volume of distribution was best described by the following equation: $V_c = (\theta_1 \times \text{weight})$, which decreased the corresponding inter-individual variability from 38 to 26% (Table 2). The addition of an intercept term did not further decrease the objective function and thus was not kept in the model. Furthermore, the intercept value was extremely small ($1.99 \times 10^{-6}$). A gender influence was also found on the central volume of distribution ($P < 0.01$). However, when we tested the combination of gender and weight, the influence of gender became insignificant, suggesting an interaction between gender and weight. The influence of weight on the clearance was modelled as follows: $\text{CL} = (\theta_3 \times \text{weight} + \theta_4)$, leading to a smaller decrease in inter-individual variability (30–28%). In the final model, however, the presence of intercept did not modify the fit or the objective function and it was thus removed (its value was 11.6). None of the covariates tested on inter-compartmental clearance ($Q$) or peripheral volume of distribution ($V_p$) were kept in the final model. When the covariates were tested separately, a gender effect was found on inter-compartmental clearance; the objective function fell from −170 to −177 and the inter-individual variability from 89.5 to 50%. However, in the backward elimination phase, the deletion of this covariate did not change the objective function and thus it was not retained in the final model. Final bias and prediction were 0.05 anti-Xa IU/ml [confidence interval (CI) −0.01–0.11] and 0.30 anti-Xa IU/ml (CI 0.15–0.45), respectively.

The population pharmacokinetic parameter estimates of the final model were used to simulate different administration schemes. The results of the simulations are shown in Figure 3.

**Discussion**

This study describes for the first time the anti-Xa activity during 48 h after a single bolus injection of the LMWH enoxaparin at the beginning of a haemodialysis session. This study was performed with enoxaparin and any generalization to other LMWH should be inferred with caution as the pharmacology of each LMWH is unique. The main result is that an injection of enoxaparin at 60 IU/kg (4000 ± 455 IU) led to an anti-Xa activity higher than 1.2 IU/ml during the first 2 h of the dialysis session, and between 0.4 and 1.2 IU/
In the present study, haemodialysis was performed with a standard procedure including a 4000 IU bolus at start of the dialysis. The first simulated procedure included a 2000 IU bolus at the start followed by a 1000 IU bolus after 2 h. This led to lower peak values than with the standard procedure. However, anti-Xa values after 4 h were similar to those observed with the standard procedure. Anti-Xa activity was faster than in the present study. This could be due to differences in the type of LMWH, and in the dose regimen.

The Non-linear Mixed Effects Modelling approach we used allows population pharmacokinetic analysis with a limited number of blood samples per patient. This method is particularly relevant for patients with a weak venous state or patients with anaemia, in whom large blood sampling should be avoided [16]. The analysis of the whole population dataset simultaneously, instead of an individual analysis, contributes to a powerful estimation of pharmacokinetic parameters [20]. Furthermore, the NonMEM method makes it possible to separate the inter- and intra-individual variabilities.

The NonMEM method serves to test if covariates (age, body weight, gender) interfere with the pharmacokinetic parameters. The effect of age was tested on all pharmacokinetic parameters but was never able to improve the objective function significantly. This could be partly explained by the low range of age in this study (Table 1). In contrast, body weight decreased the objective function, improved the fit and decreased the inter-individual variability of central volume of distribution and clearance. Gender also improved the objective function when tested on central volume of distribution. These results are not surprising as body weight and gender are commonly correlated with the volume of distribution. However, the effect of gender was likely due to an interaction with body weight and was not retained in the final model.

According to the population pharmacokinetic parameters, we have simulated single and multiple dosing with decreased enoxaparin doses (Figure 3). The standard procedure was a 4000 IU bolus at start of the dialysis. The first simulated procedure included a 2000 IU bolus at the start followed by a 1000 IU bolus after 2 h. This led to lower peak values than with the standard procedure. However, anti-Xa values after 4 h were similar to those observed with the standard procedure, suggesting a decrease in the bleeding risk during the dialysis session but not during the post-dialysis period. The second procedure included a 1000 IU bolus at the start followed by a continuous infusion of 1000 IU/h during 2 h. Compared with the standard procedure, this procedure led to lower peak values and to lower residual values at the end of the session. Mean anti-Xa activity 12 h after the beginning of the session was $0.40 \pm 0.15$ IU with the standard procedure, but only $0.22 \pm 0.09$ IU/ml with the second procedure. These simulations suggest that procedures of enoxaparin administration including continuous infusion lead to a lower bleeding risk than procedures based on bolus infusion. Note that initial studies...
showing a benefit of LMWH as anticoagulant in haemodialysis were performed with continuous infusions [1]. Whatever the procedure, anti-Xa activity remained high up to 12 h after the beginning of the dialysis session.

In summary, this study shows that enoxaparin at the dose of 60 U/kg led to an effective anti-Xa activity during the dialysis session for every patient. A decrease in this dose would lead to an excessive risk of clotting. On the other hand, this dose led to unnecessarily high anti-Xa activities up to 12 h after the bolus injection. These results confirm that a bolus injection of enoxaparin at the start of the haemodialysis session is a simple and effective mode of dialysis anticoagulation that could be used in most patients. In patients at risk of bleeding, however, other anticoagulation procedures should be used. They include haemodialysis without heparin or minimal continuous heparinization. Simulations obtained from the population pharmacokinetic modelling suggest that continuous administration of enoxaparin could reduce anti-Xa activity. Unfortunately, no formulation for enoxaparin continuous infusion is available at the moment. Thus, it would be easier to avoid this drug in patients at risk of bleeding.

Conflict of interest statement. None declared.

References


Received for publication: 26.7.02
Accepted in revised form: 4.6.03