Increased Potency and Decreased Elimination of Lamifiban, a GPIIb-IIIa Antagonist, in Patients with Severe Renal Dysfunction

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Summary

Activation of the platelet membrane receptor glycoprotein (GP) IIb-IIIa is essential for thrombus formation. The novel nonpeptide GPIIb-IIIa antagonist, lamifiban, represents a promising approach for antiplatelet therapy in patients with cardiovascular disease. Since renal impairment frequently occurs in these patients, we designed a phase I study to assess the tolerability, pharmacodynamics and pharmacokinetics of lamifiban in patients with renal impairment. Four healthy volunteers (Group 1) with creatinine clearance (CLCR) >75 ml/min, eight patients (Group 2) with mild to moderately impaired renal function (CLCR 30-74 ml/min) and eight patients (Group 3) with severe renal impairment (CLCR 10-29 ml/min) were studied. They received stepwise increased doses of lamifiban intravenously (IV). There was a linear relationship between the systemic clearance of the drug and renal function (R² = 0.86). The mean plasma concentration required for half-maximal inhibition of thrombin-receptor agonist peptide (TRAP) induced platelet aggregation (EC₅₀) ex vivo was 21, 28 and 11 ng/ml in Groups 1, 2 and 3. The patients in Group 3 were sensitized to the antiplatelet effect allowing an 18-fold dosage reduction without compromising the pharmacodynamics. In conclusion, the decreased clearance of lamifiban may act in concert with increased potency of the drug in patients with severe renal impairment, and the drug dosage should be reduced accordingly.

Introduction

Vascular disease is the major cause of death in the industrialized world, with thrombotic occlusion frequently occurring as the terminal event in the manifestation of a vascular problem (1). The platelet membrane receptor GPIIb-IIIa for the adhesive proteins fibrinogen and von Willebrand factor plays a central role in thrombus formation at sites of vascular damage and high shear rates as in injured coronary arteries (2-4). Although secondary prevention with acetylsalicylic acid (ASA) and heparin reduces the risk of myocardial infarction, stroke and vascular death (5, 6), vascular thrombi are frequently resistant to the anti-thrombotic action of conventional therapy with these agents (7-9). More efficacious anti-thrombotic agents are needed to overcome this clinical problem. GPIIb-IIIa antagonism represents a strategy to counteract platelet aggregation induced by all physiological agonists, and thus prevents the final common pathway of thrombus formation (10).

GPIIb-IIIa antagonists belong to a class of potential agents including monoclonal antibodies, snake venom polypeptides (disintegrins), and synthetic nonpeptides. The prototype GPIIb-IIIa antagonist is a chimeric Fab fragment of the anti-GPIIb-IIIa monoclonal antibody, c7E3 Fab, which has been demonstrated to reduce by 35% the risk of acute complications associated with high-risk angioplasty compared with ASA and heparin (11). The low molecular weight nonpeptide (0.468 kD) lamifiban (Ro 44-9883) is one of the most potent and selective GPIIb-IIIa antagonist yet available (12-15). Recently, a dose-ranging randomized double-blind trial showed that lamifiban administered for 3-5 days reduced the incidence of death and infarction significantly at 30 days in 365 patients with unstable angina (16).

Studies in healthy volunteers have shown that lamifiban is not metabolized, but is eliminated almost exclusively by the renal route, with 90% of the drug being recovered unchanged in the urine (Unpublished data on file, F. Hoffmann La Roche Ltd., Basel, Switzerland). Consequently, the elimination of lamifiban will be compromised in patients with impaired renal function as frequently seen with atheromatous vascular disease and end organ kidney damage. Decreased drug elimination may jeopardize the therapy, because blockage of GPIIb-IIIa carries a substantial risk of hemorrhage which is dose-dependent (8, 17). In addition, it is known that patients with uremia have an acquired defect of primary hemostasis (18). Uremic platelets have been shown to have an impaired aggregation response (19, 20), and in end-stage renal disease impaired function of the platelet membrane GPIIb-IIIa has been demonstrated (21). Thus, it is likely that the pharmacodynamic response to lamifiban will change with progressive renal dysfunction. We therefore performed a dose escalating study to evaluate the pharmacokinetics and pharmacodynamics of lamifiban in patients with different degrees of renal impairment in comparison with healthy subjects.

Subjects, Materials and Methods

Subjects. The study included 4 healthy subjects and 16 patients with a varying degree of renal impairment (Table 1). Screening of subjects was based on medical history, physical examination, electrocardiogram recording and laboratory tests. Renal function was assessed as glomerular filtration rate (GFR) and creatinine clearance (CLCR). The subjects were stratified according to renal function: Group 1 (n = 4) with normal renal function (CLCR >75 ml/min), Group 2 (n = 8) with mild to moderate renal impairment (CLCR 30-74 ml/min), Group 3 (n = 8) with severe renal impairment (CLCR 10-29 ml/min).

Study design. The study was carried out in agreement with the Helsinki declaration, and approval was obtained from the Regional Ethics Committee. Groups 1, 2 and 3 received the test drug in sequence, and the drug dose was escalated separately for each group in three steps. Lamifiban was administered intravenously (IV) three times separated by one week washout period. Group 1 received loading doses of 200, 400 and 700 μg IV followed by 4 h infusions of...
1, 2 and 5 μg/min, respectively. Group 2 was divided into two equal subgroups. Group 2a received loading doses of 40, 200 and 750 μg IV followed by infusions of 0.2, 1.0 and 4.5 μg/min. Based on pharmacodynamic measurements in Group 2a dose adjustments were made, and patients in Group 2b received loading doses of 200, 400 and 750 μg IV followed by infusions of 0.9, 1.8 and 4.5 μg/min. In Group 3, the patients received loading doses of 150, 250 and 500 μg followed by infusions of 0.09, 0.15 and 0.3 μg/min, respectively.

**Pharmacokinetics.** Following each treatment blood samples were drawn at intervals for 28-72 h, and platelet poor plasma was separated and stored in polystyrene tubes at -20°C pending HPLC analysis with electrochemical detection of lamifiban. Briefly, the method employed automated solid phase extraction on C2 columns followed by reversed phase ion pair chromatography. The HPLC equipment consisted of pump (L-600 Merck/Hitachi Darmstadt, Germany) and LC-6A Shimadzu Duisburg, Germany), autosampler (Model 232 Gibson/Abimed, Langenfeld, Germany), and electrochemical detector (Coulochen 5100A Bischoff-Analysentechnik, Leonburg, Germany). Separation was achieved with Nucleosil 100 C 18 (5 μm) column and isocratic elution. The mobile phase consisted of 78% v/v 0.05 M phosphoric acid adjusted to pH 2.20 and 22% v/v acetonitrile to which 1.0 g/l sodium dodecyl sulphate was added. Only analytical grade chemicals were used. The pharmacokinetic analysis was based on an open one-compartment model using the computer program NONMEM version IV, level 2.0 (Scientific Consulting Inc., Durham, NC). Due to baseline correction Emax (maximal effect) was fixed to 100. Consequently, the model estimated 2 parameters: the EC50 (the concentration to achieve 50% of the effect) and the (shape parameter). The model is defined by the following equation:

\[
E = 100 \times \left[ 1 - \left( \frac{C}{C + EC_{50}} \right) \right]
\]

In the case of ADP-induced platelet aggregation the shape parameter was different from 1, however when the TRAP data were fit the shape parameter was not required since a simple inhibitory effect model gave an excellent fit. This model is defined by the following equation:

\[
E = 100 \times \left[ 1 - \left( \frac{C}{C + EC_{50}} \right) \right]
\]

The mean parameter estimates together with their CV% and 95% confidence intervals are reported. A weighting of 1/y² was used.

**Tolerability assessment.** Routine clinical laboratory tests were determined at screening, at baseline and at discharge from the clinic. In addition, whole blood platelet counts were recorded 2 and 4 h after onset of treatment in case of lamifiban-induced acute thrombocytopenia. Records of adverse events were based on spontaneous reports and intermittent interviews. Blood pressure, pulse rate and body temperature was assessed at intervals and recorded as vital signs. Summary tables of vital signs and clinical laboratory data were prepared by calculating means over time and these were screened for trends. In addition, the magnitude of any changes from baseline data were calculated.

**Results.**

The study included 16 patients with variable degree of renal impairment due to various kinds of renal disease, and with considerable concurrent medication (Table 2). Lamifiban was generally well tolerated.
Drug-related adverse events occurred in two patients who developed hematomas at the site of a bleeding time incision and at the venipuncture site used for blood sampling, respectively. Thrombocytopenia did not occur during or after any of the treatment courses, and no serious adverse event appeared. There was no change in laboratory values or deviations from baseline observations during the study.

Lamifiban was titrated according to pharmacodynamic measurements. The third titration step aimed at a 60% reduction of TRAP induced platelet aggregation which was obtained with similar infusion rates in Groups 1 and 2, whereas patients in Group 3 required 18-19-fold reduction in infusion rate to maintain the same effect (P < 0.0001). Table 3 summarizes the pharmacodynamics at two separate timepoints (2 and 4 h) during infusion and the actual infusion rates on the third treatment day. The need for substantially lower infusion rates in Group 3 possibly reflects the increased potency of lamifiban in patients with poor renal function (Fig. 1). The estimated mean plasma concentrations to obtain half-maximal effect (EC\textsubscript{50}) of TRAP-induced platelet aggregation were 21 ng/ml (95% confidence interval: 19-23) in Group 1, 28 ng/ml (95% confidence interval: 26-30) in Group 2, and 11 ng/ml (95% confidence interval: 10-12) in Group 3. The corresponding EC\textsubscript{50} values for ADP were 4 ng/ml (95% confidence interval: 3.8-4.7) in Group 1, 6 ng/ml (95% confidence interval: 5.1-6.7) in Group 2, and 3 ng/ml (95% confidence interval: 2.0-3.5) in Group 3.

The dose-response curves in terms of infusion rate contra effect on TRAP-induced platelet aggregation were steeper for patients in Group 3 than for the other subjects irrespective of renal function (Fig. 2). In Group 3 the bleeding times were >20 min in all subjects at concentrations in the range of 11-27 ng/ml. For subjects in Groups 1 and 2 the concentration of lamifiban required to achieve similar bleeding time prolongation was 31-44 ng/ml and 28-64 ng/ml, respectively. The bleeding time results are summarized in Fig. 3.

The platelet recovery time was estimated using ADP induced platelet aggregation, which was the more sensitive measure of platelet function in this study. The effect of lamifiban was rapidly reversible in subjects with normal renal function, but recovery became progressively slower in patients with mild, moderate and severe renal impairment (Fig. 4). The median time to recover from lamifiban-induced inhibition of ADP-stimulated platelets (ADP\textsubscript{T50rec}) was 12 h (range: 4-24) in Group 1, 24 h (range: 16-24) in Group 2 and 50 h (range: 24-68) in Group 3.

There was a linear correlation between the clearance of lamifiban and GFR on one hand (Fig. 5) and CLCR on the other hand (data not shown). The median of lamifiban clearence was 7.3 l/h (range: 6.8-9.6) in Group 1, 3.7 l/h (range: 2.5-5.0) in Group 2, and 1.4 l/h (range: 0.9-2.4) in Group 3. There was apparently no correlation between the pharmacokinetic parameters of lamifiban and other potential co-variates such as age and body weight, although the study population is too

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**Table 2** Individual disease characteristics of patients with different grades of renal impairment

<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>CLCR</th>
<th>Renal disease</th>
<th>Concurrent medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>53</td>
<td>F</td>
<td>72</td>
<td>Chronic pyelonephritis, Tx reis</td>
<td>Prednisolone, CsA, azathioprine, metoprolol, nifedipine</td>
</tr>
<tr>
<td>24</td>
<td>M</td>
<td>71</td>
<td>Alport syndrome, Tx reis</td>
<td>Prednisolone, CsA, azathioprine, furosemide, nifedipine</td>
</tr>
<tr>
<td>48</td>
<td>F</td>
<td>62</td>
<td>Polycystic kidney disease</td>
<td>Metoprolol, enalapril, furosemide, nifedipine</td>
</tr>
<tr>
<td>71</td>
<td>F</td>
<td>54</td>
<td>Chronic pyelonephritis, Tx reis</td>
<td>Prednisolone, CsA, azathioprine, metoprolol, nifedipine</td>
</tr>
<tr>
<td>28</td>
<td>M</td>
<td>49</td>
<td>Chronic glomerulonephritis, Tx reis</td>
<td>Prednisolone, CsA, azathioprine, furosemide, nifedipine</td>
</tr>
<tr>
<td>73</td>
<td>F</td>
<td>42</td>
<td>Chronic glomerulonephritis, amaurosis, Tx reis</td>
<td>Prednisolone, CsA, azathioprine, enalapril, clofibrate</td>
</tr>
<tr>
<td>59</td>
<td>F</td>
<td>38</td>
<td>Chronic glomerulonephritis</td>
<td>Prednisolone, CsA, azathioprine, furosemide, clofibrate</td>
</tr>
<tr>
<td>29</td>
<td>M</td>
<td>34</td>
<td>Polycystic kidney disease, Tx reis</td>
<td>Prednisolone, CsA, azathioprine, furosemide, nifedipine</td>
</tr>
<tr>
<td>22</td>
<td>M</td>
<td>29</td>
<td>Kidney hypoplasia and agenesis</td>
<td>Calcitriol</td>
</tr>
<tr>
<td>72</td>
<td>M</td>
<td>22</td>
<td>Chronic glomerulonephritis</td>
<td>Enalapril, calciotin, furosomide</td>
</tr>
<tr>
<td>48</td>
<td>F</td>
<td>21</td>
<td>Polycystic kidney disease</td>
<td>Calcitriol</td>
</tr>
<tr>
<td>25</td>
<td>M</td>
<td>20</td>
<td>Polycystic kidney disease</td>
<td>Furosomide, calcitriol, allopurinol, captopril</td>
</tr>
<tr>
<td>66</td>
<td>M</td>
<td>19</td>
<td>Chronic glomerulonephritis, nephrotic syndrome</td>
<td>Metoprolol, CsA, furosomide, captopril, nifedipine, calcitriol</td>
</tr>
<tr>
<td>24</td>
<td>F</td>
<td>14</td>
<td>Polycystic kidney disease</td>
<td>AsA, metoprolol, ironatrin, furosomide, captopril, enalapril, leuoalpinol</td>
</tr>
<tr>
<td>31</td>
<td>M</td>
<td>14</td>
<td>Nephroclerosis</td>
<td>Furosomide, captopril, calcitriol, oxamoxin, allopurinol</td>
</tr>
<tr>
<td>35</td>
<td>F</td>
<td>11</td>
<td>Polycystic kidney disease</td>
<td>Furosomide, captopril, calcitriol, potassium carbonate, sodium hyosgen carbonate, etyphotoxinct</td>
</tr>
</tbody>
</table>

CLCR = creatinin clearance; CsA = cyclosporin A; Tx reis = kidney transplant; ASA = acetylsalicylic acid

**Table 3** Infusion rates and antiplatelet effect at steady state (2 and 4 h) in the study groups

<table>
<thead>
<tr>
<th>Group (n=4)</th>
<th>Infusion Rate µg/min</th>
<th>TRAP % activity</th>
<th>Group (n=8)</th>
<th>Infusion Rate µg/min</th>
<th>TRAP % activity</th>
<th>Group (n=12)</th>
<th>Infusion Rate µg/ml</th>
<th>TRAP % activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>5.15</td>
<td>38.6</td>
<td>5.49</td>
<td>37.0</td>
<td>0.09</td>
<td>4.07</td>
<td>34.4</td>
<td>0.09</td>
</tr>
<tr>
<td>95% CI</td>
<td>4.96-5.44</td>
<td>36.4-44.5</td>
<td>4.96-6.27</td>
<td>33.6-40.5</td>
<td>0.09-0.30</td>
<td>34.0-50.8</td>
<td>0.09-0.30</td>
<td>34.0-50.8</td>
</tr>
</tbody>
</table>

* p < 0.0001 for comparison of infusion rates by ANOVA

**Fig. 1** Concentration (lamifiban ng/ml) versus effect (% inhibition TRAP-induced platelet aggregation relative to baseline) in each of the treatment groups. Actual values and predicted curves based on a simple inhibitory effect model are shown.
Fig. 2  Combined data of platelet inhibition showing individual dose (actual lamifiban infusion rate μg/min) versus mean effect (% inhibition TRAP-induced platelet aggregation relative to baseline) for all subjects after 2 and 4 h of treatment.

Fig. 3  Bleeding time (y-axis) in relation to plasma concentration of lamifiban (x-axis) for healthy volunteers (■), patients with moderate renal impairment (+), and patients with severe renal impairment (○). Note that bleeding time >20 min appeared at lower concentrations in the latter group.

Fig. 4  Mean effect (% inhibition ADP-induced platelet aggregation relative to baseline) versus scheduled sampling times (h) in each of the treatment groups. The error bars represent ± one standard deviation.
small to draw firm conclusions with respect to co-variation. Therefore, renal function, as measured by GFR or CLCR, has a dominant influence on the pharmacokinetics of lamifiban suggesting that dose adjustment based on renal function is necessary.

**Discussion**

In the present study a bolus dose of 750 μg followed by infusion at 5 μg/min lamifiban was regarded as reference dose for relevant and safe platelet inhibition in healthy volunteers (Group 1). The subsequent dose escalations in Groups 2 and 3 were performed to determine equipotent doses in patients with renal dysfunction. The study, therefore, had an adaptive design, and the required dose adjustments were based on the pharmacodynamic effects measured, provided acceptable tolerability. However, it should be kept in mind that the doses were evaluated by ex vivo tests which do not necessarily parallel in vivo efficacy. The choice of reference dose was supported by the results of a preliminary study in patients with unstable angina (16). On the other hand, the results of the first phase of the multicenter trial of Platelet IIb-IIIa Antagonist for the reduction of Acute Coronary Syndrome events in a Global Organization Network (PARAGON A) (22) lead to the selection of a lower bolus dose (500 μg) followed by a lower infusion rate (1-2 μg/min) in the pivotal study, PARAGON B. Hence, the results of the present study need to be interpreted in light of the lower dose used in PARAGON B.

The pharmacokinetics of lamifiban was significantly altered in patients with renal impairment due to reduced systemic clearance of the drug. However, lamifiban CL, t1/2, β, Vd, and AUC could not be determined accurately due to interfering peaks probably resulting from concomitant medications and reduced sensitivity of the HPLC assay. Therefore, it was not possible to evaluate the pharmacokinetics of lamifiban using standard non-compartmental methods and a population approach using NONMEM computerized models was used instead. While the EC50 values probably reflect the difference between the groups, the accuracy and the precision may have been affected by the problems encountered in measuring the concentrations of lamifiban. Since lamifiban is excreted unchanged by the renal route, a need for dose adjustments in patients with severe renal impairment was expected. However, the dosage reduction required in this study (on average by a factor of 18) to obtain the same pharmacodynamic effect in patients with severe renal impairment (CLCR or GFR reduced on average by a factor of 7; see Table 1) cannot be explained entirely by changes in the pharmacokinetics of the drug.

In the present study bleeding time was included as in vivo measure of platelet inhibition. The normal range for bleeding time using the Monject diabetic gun, has not been formally established. However, since each individual served as his own control, the increased bleeding duration revealed by this method, was considered relevant and thus reflected the influence of lamifiban on platelet function. The Monject method was preferred to the conventional determination of bleeding time according to Ivy due to the great number of tests (n = 16) being performed in each individual. The scarring of the skin which is frequently seen with the Ivy method, was considered undesirable in this study.

Impaired function of platelet membrane GPIIb-IIIa has been demonstrated in end stage renal disease (21) and it is possible, that patients with severe renal impairment, but not yet in end stage renal disease, manifest such a dysfunction. However, uremic platelets may also exhibit a reduction in several of the biochemical responses necessary for aggregation, including reduction of platelet granular content of ADP and serotonin (23, 24), reduced platelet thromboxane production (20, 25), and rise in cytoplasmic free calcium and cAMP levels (26, 27). The results of the present study suggest that lamifiban has a lower EC50 for both ADP and TRAP induced platelet aggregation ex vivo in patients with CLCR in the range 10-30 ml/min. In addition, the concentrations required to attain bleeding times >20 min in vivo were also lower in these patients. The higher sensitivity to the pharmacodynamic effect of lamifiban in these patients may be explained by altered binding to the platelet GPIIb-IIIa, which is likely to be true for other GPIIb-IIIa antagonists as well. Whether this also reflects impaired function of GPIIb-IIIa, warrants further study. The difference in EC50, which was observed in all groups, comparing TRAP and ADP, may be explained by the fact that TRAP, a strong agonist, stimulates the platelets to a much greater extent than ADP, causing activation of more GPIIb-IIIa receptors. Because of the resulting increase in the number of receptors on the cell surface, a higher concentration of lamifiban is required to inhibit the response to TRAP. Interestingly, the increase in the bleeding time to values >20 min appeared at concentrations in the range of the EC50 for TRAP, whereas the bleeding times were comparable to baseline values at the EC50 for ADP. We chose to estimate recovery by ADP stimulation as there is still a measurable effect with ADP when there is no
measurable effect with TRAP, which is the more powerful agonist of platelet aggregation.

The sustained platelet recovery time in patients with severely impaired renal function is an important finding considering the clinical use of lamifiban. Recovery as defined in this study, was 4-fold extended in these patients compared to subjects with moderate or no renal impairment. The delay in platelet recovery is a serious threat to the patient if a major bleeding should occur, and strategies for assisted clearance of lamifiban by hemodialysis or charcoal hemofiltration should be addressed in further studies with this drug. However, the problem of sustained platelet inhibition may be expected only in patients with severe renal impairment (CLCR <30 ml/min). For this reason severe renal impairment should be considered as a relative exclusion criteria in clinical trials with lamifiban until improved control of platelet recovery has been reached.

The present study showed that dose adjustments of lamifiban were not necessary for patients with CLCR >30 ml/min, but patients with lower CLCR required substantial reduction in the infusion rate of lamifiban. By dose adjustments according to renal function treatment with lamifiban should be safe even in the case of renal dysfunction. Lamifiban was generally well tolerated in this study. However, our patients were carefully screened to avoid bleeding diathesis, and the study situation was not comparable to that of a therapeutic approach including heparin and ASA in acute cardiac events. Previously, a few cases of severe thrombocytopenia have been reported with GPIIb-IIIa antagonists even at low dosages (28). In our study no incident of thrombocytopenia occurred, but the study population was small to discover rare adverse events.

Considering the results of this study, some open questions do, however, remain to be addressed. A means to speed up the recovery following cessation of a lamifiban infusion in patients with severely impaired renal function would greatly enhance the safe use of the drug in such patients. Studies in patients with end stage renal disease that requires dialysis will show whether lamifiban is easily removed from circulation by hemodialysis. Therefore a study of lamifiban administration to patients prior to, during and after hemodialysis, seems warranted.

The introduction of the GPIIb/IIIa antagonists has opened a new therapeutic era in the management of cardiovascular disease because these agents are far more potent platelet inhibitors than the conventional antithrombotics (29). In particular, the nonpeptide lamifiban represents a promising therapeutic approach being highly potent and selective with respect to GPIIb/IIIa antagonism. We demonstrated a complete inhibition of platelet aggregation at tolerable plasma concentrations of lamifiban. However, the changes in pharmacokinetics and pharmacodynamics that we also demonstrated in patients with severe renal impairment, requires circumspection in drug dosing. Because lamifiban has increased potency and decreased clearance in patients with severe renal impairment, the drug dosage should be reduced accordingly.

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References


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