Safety, Pharmacokinetics and Pharmacodynamics of Batifiban Injection Following Single- and Multiple-Dose Administration to Healthy Chinese Subjects

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Abstract
Batifiban, a synthetic cyclic peptide, is a potent platelet glycoprotein GPIIb/IIIa antagonist that may be useful in treating and preventing acute coronary syndromes. The pharmacokinetics and pharmacodynamic (inhibition of platelet aggregation) effects, and tolerability of batifiban were investigated in healthy subjects following single bolus doses of 55 µg·kg⁻¹, 110 µg·kg⁻¹, or 220 µg·kg⁻¹, or multiple doses of a bolus followed infusion for 24 h (180 µg·kg⁻¹ plus 2.0 µg/min·kg⁻¹, and 220 µg·kg⁻¹ plus 2.5 µg/min·kg⁻¹ ) in this phase I clinical trial. Plasma levels of batifiban and areas under the curve were found to be proportional to doses. Batifiban was rapidly eliminated with a half-life of approximately 2.5 h. Significant differences were noted for plasma levels of batifiban and areas under the curve between male and female. No significant differences in the terminal half-life were found between males and females. Batifiban reversibly inhibits ex vivo platelet aggregation in a dose- and concentration-dependent manner, consistent with its mechanism as a GP IIb/IIIa antagonist. Single and multiple intravenous doses of batifiban were found to be safe and well tolerated in healthy subjects. These results support a bolus plus infusion regimen of batifiban in treating and preventing acute coronary syndromes.

Key words Batifiban; GP IIb/IIIa integrin receptor; platelet aggregation; pharmacokinetics; pharmacodynamics; safety

Introduction
Platelet glycoprotein (GP) IIb/IIIa antagonists has been extensively applied in the treatment of patients with acute coronary syndromes and in those undergoing percutaneous coronary intervention (PCI), and consistently presented the improved clinical outcomes[1-4]. Until now, a new class of platelet aggregation inhibitors has been developed in the past years and three compounds have been approved for clinical use[5]. Batifiban, a cyclic heptapeptide containing six amino acids and one mercaptopropionyl (des-amino cysteinyl) residue, is an intravenous antagonist of the GP IIb/IIIa integrin receptor. An interchain disulfide bridge is formed between the cysteine amide and the mercaptopropionyl moieties.
Chemically it is N5-(aminoiminomethyl)-N2-(3-mercaptop-1-oxopropyl)-L-arginylglcy-L-α-aspartyl-L-α-trypotophyl-L-α-prolyl-L-α-cysteinamide,cyclic(1→6)-disulfide (Fig 1).

The biological and pharmacodynamic effect of batifiban was believed to be similar to those obtained with Abciximab, as batifiban can also block circulating vitronectin binding to integrin ανβ3, in addition to blocking fibrinogen binding to glycoprotein GPIIbIIIa, thus not only preventing platelet aggregation induced thrombus formation, but also preventing integrin ανβ3 induced restenosis. The pharmacokinetic characteristics of batifiban was similar to eptifibatide in its rapid reversibility of inhibition\[6-9\]. Pharmacokinetic profiles of batifiban in dogs and monkeys showed that batifiban presented linear Pharmacokinetic characteristics, and renal was a major route of excretion, accounting for about 90% total body clearance, with the majority of the compound excreted in the urine as the parental compound and minor portion as deaminated metabolites\[10\]. These data together with its acute and long-term toxicity studies in primates provided the basis for the safety of this drug in clinical trials. Batifiban was well tolerated in healthy volunteers. We report on the the pharmacokinetics and pharmacodynamic effect and safety of single -dose and multiple-dose administration in healthy volunteers.

Methods

Approved Procedure

The study protocol was approved by the Medical Ethical Committee of Tongji Medical College, Huazhong University of Science and Technology, all subjects provided written informed consent by the medical supervisor of the trial about the aim, course, and possible risks of the study before entering the study. These studies were performed in accordance with good clinical practice (GCP) guidelines of the State Food and Drug Administration in China and the Declaration of Helsinki (as revised in Edinburgh 2000).

Study Population

All 60 healthy volunteers, including 30 women and 30 men were divided into 5 groups: 3 groups for the single-dose format studies and 2 groups for a multiple-dose format study. The subjects ranged in age from 19 to 27 years (22.45 ± 1.91) and weighed between 50.0 and 71 kg (58.29 ± 5.76).

All subjects had no allergy history and had stopped using any drugs 3 weeks before the study. Cigarette and alcohol were forbidden during the trial period. Physical examination and laboratory tests, including blood pressure, heart rate, body temperature, hematology, blood biochemistry, prothrombin time(PT), activated partial thromboplastin time (aPTT),
fibrinogen (Fbg), urinalysis, occult blood (OB) test, and 12-lead electrocardiograms (ECGs), and stemite showed no abnormal findings.

Subjects were excluded if they had a history of clinically significant bleeding within past 30 days; a history of known hemorrhagic stroke at any time, or stroke of unknown etiology within past 2 years; major surgery within weeks of treatment; participated in an experimental protocol within past 30 days; a history of alcohol or drug abuse; blood donation within the previous 3 months; a history of clinically significant cardiovascular, renal, hepatic, pulmonary, gastrointestinal, endocrine, hematological, vascular, or collagen diseases; a history of nervous system or muscle disease, seizure, or a psychiatric disorder that might hinder compliance with the study;

**Drug Administration**

Batifiban injection was supplied by Sinoasis Pharm. (Guangzhou, China) as 20 mg in 10-mL injections (lot: 20070201). It was stored at 2-4°C. Dilution with 0.9% normal sodium injection to a final concentration was performed before administration over 30 to 60 minutes.

**Study Design**

On the days of pharmacokinetic measurements, slight breakfast was allowed before administration of medication, and water was allowed as required.

In the single-dose format study, 3 groups of 12 volunteers received 55, 110, and 220 µg·kg⁻¹ batifiban injection, respectively, which was dissolved in 0.9% normal sodium injection, as an intravenous injection. After a 12-hour overnight fast in the hospital, the medication was given by intravenous injection within 1 minutes in one arm. An indwelling cannula was placed in the other arm for blood sampling. Before sampling, about 0.5 mL blood was discarded. Then, 5mL blood samples were collected in heparinized tubes at the following times on the days of the pharmacokinetic measurements: immediately prior to drug administration (0h), and at 0.033, 0.083, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, and 12 hours after drug administration. The urine samples were collected in 0-1h, 1-2h, 2-4h, 4-8h and 8-12h aliquots for the determination of culminative excreted percent of BAT in urine.

In the multiple-dose format study, one group of 12 volunteers were administered as a 180 µg·kg⁻¹ bolus, immediately followed by a 2.0 µg·kg⁻¹ min infusion for 24 hours. The other group of 12 volunteers were given as a 220 µg·kg⁻¹ bolus, immediately followed by a 2.5 µg·kg⁻¹ per min infusion for 24 hours. During the course, the medication injection was put in one arm and indwelling cannula was placed in the other arm. The blood samples were collected in heparinized tubes as the following times: immediately prior to drug administration (0h); at 0.0167, 0.083, 0.167, 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8, 12, and 24 hours after the bolus injection immediately followed infusion for 24h and at 0.083, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, and 12 hours after the termination of infusion. Meanwhile, 2ml blood samples were collected in citrated tubes at the following times: immediately prior to drug administration (0h); at 0.167, 1, 4, 8, and 24 hours during infusion and 4h after the termination of infusion for the assay on platelet aggregation. All blood for content assay was centrifugated within 30 minutes at 4000 rpm for 10 minutes, and plasma samples were collected and stored at –70°C until analyzed.

**Safety and Tolerance**

Subjects’ symptoms, objective signs, and vital signs, including blood pressure, heart rate, and body temperature, were checked in different time after drug administration, and ECGs and Routine laboratory tests, including hematology, blood biochemistry, PT, aPTT, Fbg, urinalysis, and OB test were performed before and 24h after the end of administration.

**Drug Assay**

For batifiban concentration assays of plasma and urine samples, analysis was performed with the use of high-performance liquid chromatography and electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS), and the analytical methods were validated in principle as described [11]. Briefly, The instrumentation system used in this work consisted of a Waters 2695 series HPLC (Milford, MA, USA), a syringe pump (Harvard Apparatus, South Natick, MA,
USA) and a QuattroMicro API triple quadrupole mass spectrometer by Waters (Milford, MA, USA). Data acquisition and peak integration were accomplished using the Micromass Masslynx software (version 4.1).

The Micromass QuattroMicro triple quadrupole mass spectrometer was operated under the positive electrospray ionization mode (ESI+). Ionization conditions were optimized as follows: cone and desolvation gas at 50 and 450 L·h⁻¹, capillary at 3.5 kV, RF lens at 0 V, skimmer at 3V, ion source and desolvation gas temperature at 100°C and 350°C, ion energy at 1.0 V, low- and high-mass resolution at 11 for quadrupole 1 and 3, dwell time at 0.1 s, and the inter-scan delay at 0.01s. The ionization parameters were the same as those described previously.

The mobile phase used was acetonitrile/water containing 0.1% formic acid (60:40, v/v) at a flow rate of 0.25 ml·min⁻¹. The stationary phase was a HyPURITY C18 column (150×2.1 mm, 5 μm, USA) thermostated at 40°C with a precolumn packed with C18 (5μm, Turner Science Instrument Co. Ltd., PR China). The detection was performed using multiple-reaction-monitoring (MRM) mode for batifiban (m/z 820→ m/z 623,159) and the I.S.( m/z 834→ m/z 645,159) after a injection volume of 10μL.

Eptifibatide has been chosen as the internal standard, and a solid-phase extraction has been developed for extraction of batifiban and I.S. from plasma samples. Briefly, the internal standard was spiked to 0.5 mL of plasma, and then the mixed plasma was transferred into Oasis HLB cartridges (100 mg, Milford, MA, USA). After washing the columns with 2 ml water, the analyte was eluted with 0.5 ml methanol, and then the elutes were evaporated at 40°C under a gentle stream of nitrogen. The residues was reconstituted in 150 μl mobile phase and 10 μl of the solution was injected into HPLC - MS/MS for analysis.

The linear relationship was obtained between the peak areas and batifiban plasma concentration from 2.45 to 5000 ng·ml⁻¹ (r = 0.9983) using a weighted (1/x²) linear regression, the lower limit of quantification (LOQ) was 2.45 ng/mL. The intraassay and interassay coefficients of variation (CV%) for the 3 quality control standards (4.9, 78.1, and 2500 ng/ml) were ≤9.09% and 11.53%, respectively. The mean absolute recovery varied from 71.79% to 88.56%, whereas accuracy ranged between 95.8% and 105.4% for the plasma samples. A stability study showed that BAT and the internal standard were stable in plasma at room temperature for at least 12 hours, as well as for 3 months at −70°C and after 3 freeze-thaw cycles. The retention times for BAT and I.S were 1.18 minutes and 1.19 minutes, respectively.

The limit of quantification was 2.45 ng·ml⁻¹ for eptifibatide in urine, with a linear range from 2.45 to 5000.0 ng·ml⁻¹ (r= 0.9960) using a weighted (1/x2) linear regression. The mean absolute recovery varied from 80.28% to 95.00%, whereas accuracy ranged between 92.2% and 101.4% for the plasma samples.

Pharmacokinetic Analysis

Single- and multiple-dose pharmacokinetic parameters were calculated from plasma concentration-time data by noncompartmental methods. The maximum observed serum concentration (Cmax) was obtained directly from experimental data, and time to maximum serum concentration (Tmax) was defined as the time of first occurrence of Cmax. Linear trapezoidal rule was used to estimate the area under the concentration-time curve (AUC) from 0 to the last quantifiable concentration of batifiban (AUC₀₋ₜ). The area under the plasma concentration versus time curve from 0 to infinity (AUC₀₋∞) was calculated as AUC₀₋ₜ + Ct/λz, where λz is the slope of the log-linear regression of the terminal concentration data points.

The terminal elimination half-life (t₁/₂) was calculated as (ln2)/λz. Apparent total intravenous clearance after single-dose administration (CL) was calculated as dose/AUC₀₋∞ and after multiple dosing as the infusion rate divided by Cₛₛ. Mean steady-state concentration (Cₛₛ) was determined as the average mean of the values obtained at 12 and 24 hours.

The amount of batifiban excreted during each collection interval were calculated by multiplying the concentration of batifiban by the urine volume voided during that interval. Cumulative amounts were calculated as the sum of the amounts excreted during all preceding collection intervals. For each collection interval, percentage of the dose recovered was calculated by dividing the amount excreted in urine
during that interval by the administered dose and multiplying the result by 100. Total percentage of the dose recovered was the sum of the individual percentages of dose recovered during each collection interval for each subject.

**Pharmacodynamic Analysis**

Blood samples were drawn at baseline (30 minutes before the study drug bolus) and during the study drug infusion at 10 min, 1, 4, 8, 24 and 4 h after the drug infusion finished, which were assayed for platelet aggregation. The platelet aggregation studies were performed at each investigational site and analyzed by the Institute of Hematology at the Union hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan. Blood samples were anticoagulated in Sodium Citrate. Platelet aggregation was measured by light transmission aggregometry in response to 20 mmol·L⁻¹ ADP per previously described methods [12]. Platelet aggregation was summarized by plotting medians.

**Statistical Analysis**

Comparisons of pharmacokinetic parameters were performed among dose levels using analysis of variance (ANOVA), and between the multiple dose data and sexual data in each group using analysis of Paired Student t tests. Prior to comparisons, AUC and $C_{\text{max}}$ should be divided by dose administration (D) and then $\text{AUC}/D$ was computed from log transformed data. Css should be divided by infusion rate. Data are presented as mean ± standard deviation (SD). The linearity of BAT pharmacokinetics was evaluated by examining $C_{\text{max}}/D$, $\text{AUC}/D$, $t_{1/2}$, and CL as a function of the single-dose administration, and $C_{\text{SS}}/R$, $C_{\text{max}}/D$, $\text{AUC}/D$, $t_{1/2}$, and CL as a function of the multiple-dose administration.

All statistical tests used a 5% level of significance to determine significance. All $P$ values cited were 2-tailed. The Drug and Statistics Software (DAS, Version 2.0; Mathematical Pharmacology Professional Committee of China, China) was used to calculate the pharmacokinetic parameters.

**Results**

All 60 subjects (30 women and 30 men) completed the trials. There were no major differences between the groups with respect to demographics.

**Safety and Tolerability Results**

Among single and multiple doses of batifiban, there were no serious adverse events, or AEs leading to withdrawals in this study, batifiban was well tolerated by all volunteers. Overall, 11 of the 60 subjects (18.3%) reported at least 1 adverse event (total number of events = 13), of which 3 samples of WBC in hematology and 3 samples of Fbg dropped a little, and 7 samples of RBC in urinalysis were observed. All of the adverse events were mild in intensity and required no intervention; Meanwhile, all these adverse reaction were not dose-dependent and released within several days without treatment.

**Pharmacokinetics**

The profiles of mean concentrations versus time of batifiban in plasma after Single- and multiple-dose administration to Healthy Chinese Subjects are illustrated in Figure 2 and 3. Mean pharmacokinetic variables for batifiban injection are summarized in Table 1 and 2.

For Single-Dose Pharmacokinetics, mean values of $t_{1/2}$, $V_d$, CL in plasma seemed to be independent of dose. No significant ($P>0.05$) differences were observed in $t_{1/2}$, $V_d$, CL, $C_{\text{max}}/D$ and logarithmically transformed $\text{AUC}/D$ among the 3 groups. The pharmacokinetics of the drug in the dosage range of 55 to 220µg·kg⁻¹ in Chinese subjects fit the linear dynamic feature.

Pharmacokinetic parameters derived in the multiple dose format study showed that mean values for Css and AUC also increased in proportion to dose, and mean values of $t_{1/2}$, $V_d$, CL in plasma was independent to dose. No significant ($P>0.05$) differences were also observed in $t_{1/2}$, $V_d$, CL, $C_{\text{SS}}/D$ and Logarithmically transformed $\text{AUC}/D$. The pharmacokinetics of the drug in the multiple dose in Chinese subjects also fit the linear dynamic feature. Although the $t_{1/2}$ prolonged a little compared with those in single bolus groups, there were no significant
differences (P>0.05) among all intravenous administration groups.

The effects of sex on the pharmacokinetics of BAT in Single and multiple dosing data (AUC, Cmax, Css, $V_d$ and CL) from all subjects were analysed as follows.

**Fig 2.** Time course of mean concentrations of batifiban in plasma after intravenous bolus administration of batifiban injection (55, 110 and 220 $\mu$g·kg$^{-1}$, respectively) to twelve healthy subjects (n=12).

**Fig 3.** Time course of mean concentrations of batifiban in plasma after a intravenous bolus followed by a continuous infusion of batifiban injection (180 $\mu$g·kg$^{-1}$ plus 2.0 $\mu$g/min·kg for 24h and 220 $\mu$g·kg$^{-1}$ plus 2.5 $\mu$g/min·kg$^{-1}$ for 24h, respectively) to twelve healthy subjects (n=12).
Table 1 Main pharmacokinetic properties of batifiban subjects receiving single intravenous bolus dose of batifiban (Values are expressed as mean ± SD, n=12)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>55µg·kg⁻¹</th>
<th>110µg·kg⁻¹</th>
<th>220µg·kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men</td>
<td>Women</td>
<td>Men</td>
</tr>
<tr>
<td><strong>AUC₀⁻¹²h, µg·h/L</strong></td>
<td>303.39±95.65</td>
<td>317.92±99.85</td>
<td>317.56±92.65</td>
</tr>
<tr>
<td><strong>AUC₀⁻∞, µg·h/L</strong></td>
<td>597.35±86.59</td>
<td>628.46±95.99</td>
<td>628.56±96.46</td>
</tr>
<tr>
<td><strong>t½, h</strong></td>
<td>2.554±1.698</td>
<td>2.368±0.844</td>
<td>2.379±0.857</td>
</tr>
<tr>
<td><strong>Tmax, h</strong></td>
<td>0.033±0.000</td>
<td>0.033±0.000</td>
<td>0.033±0.000</td>
</tr>
<tr>
<td><strong>CL, L/h/kg</strong></td>
<td>0.193±0.073</td>
<td>0.179±0.027</td>
<td>0.195±0.084</td>
</tr>
<tr>
<td><strong>V, L/kg</strong></td>
<td>0.649±0.424</td>
<td>0.607±0.226</td>
<td>0.674±0.264</td>
</tr>
<tr>
<td><strong>Cmax, µg/L</strong></td>
<td>282.6±26.91</td>
<td>664.6±70.26</td>
<td>664.7±70.57</td>
</tr>
</tbody>
</table>

Compared with the male subjects: **P<0.01
### Table 2. Main pharmacokinetic properties of batifiban receiving an intravenous bolus followed by a continuous infusion of batifiban injection (Values are expressed as mean±SD, n=12)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>180µg/kg plus 2.0µg/min·kg⁻¹</th>
<th>220µg/kg plus 2.5µg/min·kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men</td>
<td>Women</td>
</tr>
<tr>
<td>AUC₀-₃₆h, mg·h/L</td>
<td>22.883±3.34</td>
<td>20.43±1.04</td>
</tr>
<tr>
<td>AUC₀-∞, mg·h/L</td>
<td>22.989±3.36</td>
<td>20.52±1.03</td>
</tr>
<tr>
<td>t₁/₂, h</td>
<td>2.928±0.317</td>
<td>2.824±0.219</td>
</tr>
<tr>
<td>Tₘ₉, h</td>
<td>0.017±0.000</td>
<td>0.017±0.000</td>
</tr>
<tr>
<td>CL, L/h/kg</td>
<td>0.136±0.018</td>
<td>0.149±0.008</td>
</tr>
<tr>
<td>V, L/kg</td>
<td>0.57±0.082</td>
<td>0.611±0.075</td>
</tr>
<tr>
<td>Css, µg/L</td>
<td>896.12±138.93</td>
<td>800.2±30.78</td>
</tr>
<tr>
<td>Cmax, µg/L</td>
<td>1447±300.06</td>
<td>1190±138.8</td>
</tr>
</tbody>
</table>

Compared with the male subjects: * P<0.05, ** P<0.01

### Table 3. Cumulative excreted percent of Batifiban in urine receiving an intravenous bolus followed dose of batifiban (Values are expressed as mean±SD, n=12)

<table>
<thead>
<tr>
<th>Case</th>
<th>55 µg·kg⁻¹</th>
<th>110 µg·kg⁻¹</th>
<th>220 µg·kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men</td>
<td>Women</td>
<td>Men</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Cumulative excreted</td>
<td>45.88±15.70</td>
<td>48.27±16.13</td>
<td>43.49±16.39</td>
</tr>
</tbody>
</table>

### Table 4. Percentage of inhibition of platelet aggregation(%) of batifiban receiving an intravenous bolus followed by a continuous infusion of batifiban injection (Values are expressed as mean±SD, n=12)

<table>
<thead>
<tr>
<th>Time(h)</th>
<th>180µg/kg plus 2.0µg/min·kg⁻¹</th>
<th>220µg/kg plus 2.5µg/min·kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men</td>
<td>Women</td>
</tr>
<tr>
<td>0.167</td>
<td>76.32±12.36</td>
<td>78.36±11.42</td>
</tr>
<tr>
<td>1</td>
<td>68.43±20.25*</td>
<td>73.19±19.15</td>
</tr>
<tr>
<td>4</td>
<td>83.18±10.83</td>
<td>84.30±12.59</td>
</tr>
<tr>
<td>8</td>
<td>81.10±19.19</td>
<td>83.32±18.72</td>
</tr>
<tr>
<td>24</td>
<td>78.21±22.98</td>
<td>73.45±27.63</td>
</tr>
</tbody>
</table>

Compared with percentage of inhibition of platelet aggregation at 0.167h or 4h: * P<0.05
As shown in the 2, there were significant \( P < 0.01 \) differences in Logarithmically transformed AUC\(_{0,t}/D\) and AUC\(_{0-\infty}/D\), CL between the female and male subjects in three single-dose groups, and also there were significant \( P < 0.01 \) differences in \( V_d \), \( C_{max}/D \) between the female and male subjects in 220µg/kg bolus group. Also in two multiple dose groups, there were significant \( P < 0.01 \) or \( P < 0.05 \) differences in logarithmically transformed AUC\(_{0,t}/D\) and AUC\(_{0-\infty}/D\), \( C_{max}/D\), \( C_{ss}/D\), CL between the female and male subjects, and also there were significant \( P < 0.01 \) differences in \( V_d \) between the female and male subjects in 220 µg·kg\(^{-1}\) bolus plus 2.5µg/min·kg\(^{-1}\) infusion group. However, there were no significant \( P > 0.05 \) differences in \( t_{1/2} \) between the female and male subjects.

**Pharmacodynamics**

As shown in Table 3, The cumulative percents of batifiban excreted in urine in three single-dose groups 12h after bolus administration were \((45.88 \pm 15.70)\%\), \((45.14 \pm 16.76)\%\), \((36.71 \pm 11.74)\%\), respectively, there was no significant difference in the cumulative percents of batifiban excreted in urine among three single-dose groups, and also no significant difference presented between the male and the female subjects. Almost 50\% of parental batifiban excreted in urine indicates adjustment of dosage appears to be needed in subjects with impaired renal function or eldly subjects in the treatment.

As shown in Table 4 and Figure 4, Batifiban at both doses, 180 µg·kg\(^{-1}\) bolus plus 2.0 µg/min·kg\(^{-1}\) infusion and 220µg·kg\(^{-1}\) bolus plus 2.5 µg/min·kg\(^{-1}\) infusion, produced significantly inhibition \( (P < 0.01) \) of ex vivo platelet aggregation induced by ADP. In general, there was an immediate inhibition of platelet aggregation after the bolus and start of infusion, and a sustained inhibition of platelet aggregation detected between 4 and 24 hours. At steady state, >80% inhibition after ADP stimulation was seen in all groups. However, at the 1-hour sampling point, the low mutiple-dose subjects showed less inhibition of platelet aggregation than observed either 10min after the bolus or at steady state, which suggested that inhibition of
ADP-induced ex vivo platelet aggregation showed a concentration-dependent effect as the concentrations of Batifiban in citrated plasma were 1117±268.4 ng·ml⁻¹ at 10min, 728.8±144.3 ng·ml⁻¹ at 1h (P<0.05, compared with the concentration at 0.167 h or 4 h) and 894.8±171.9 ng·ml⁻¹ at 4 h. However, there was no significant difference in inhibition of platelet aggregation at steady state between the two multiple-dose groups. Also, there was no significant difference in inhibition of platelet aggregation at steady state between the male and the female subjects in each multiple-dose group. As Batifiban has short t₁/₂, this effect to inhibit platelet aggregation was rapidly lost after discontinuation of the infusion, declining towards baselines within 4 hours after termination.

Discussion

Batifiban is similar to eptifibatide, a synthetic cyclic heptapeptide which binds competitively to the glycoprotein (GP) IIb/IIIa receptor complex, thus inhibiting platelet aggregation by preventing the binding of fibrinogen and other ligands to GP IIb/IIIa. Batifiban will intravenously administrated adjunct to aspirin and heparin to prevent acute cardiac ischemia complications in patients undergoing Percutaneous Transluminal Coronary Angioplasty (PTCA). Although good safety and tolerability and linear pharmacokinetics presented in animals, such as dogs and monkeys, however, little information has been reported on the pharmacokinetics and pharmacodynamics of BAT in humans. The pharmacokinetic and pharmacodynamic characteristics and dose proportionality of BAT were investigated in healthy Chinese subjects following single bolus doses of 55 µg·kg⁻¹, 110 µg·kg⁻¹, or 220 µg·kg⁻¹, or multiple doses of an bolus followed infusion for 24 h (180 µg·kg⁻¹ plus 2.0 µg/min ·kg⁻¹, and 220 µg·kg⁻¹ plus 2.5 µg/min·kg⁻¹) in this phase I clinical trial. Cₘₐₓ, Cₙₐ, levels and AUC values of BAT increased in a linear and proportional manner with increasing intravenous doses.

Batifiban was rapidly metabolized following single bolus intravenous doses, with mean t₁/₂ ranged from 2.0 to 2.9 hours in subjects with an intravenous CL of approximately 0.19L/h·kg⁻¹. In comparing the single-dose with the multiple-dose profile, the pharmacokinetic analysis of BAT showed very similar properties. No significant difference in mean t₁/₂ estimated from the multiple doses compared with those from single doses indicated that no significant accumulation of BAT with infusion dosing appeared, and also constant infusion was needed to maintain a steady-state concentration of BAT in plasma during the treatment.

The estimated pharmacokinetic parameters showed relatively low interindividual variation. The linear pharmacokinetics and the low pharmacokinetic variability should facilitate the definition of dose regimens and the prediction of plasma levels after a bolus plus infusion administrations. All of pharmacokinetic characteristics presented by batifiban in humans were similar to those of eptifibatide[13-15].

Another main objective of the study is to examine the effects of sex on the pharmacokinetics of BAT. In both study parts, there were significant differences in AUC, Cₘₐₓ, Cₙₐ and CL, however no significant differences in t₁/₂ and the cumulative percents of batifiban excreted in urine between the female and male subjects. It suggested that probably there was the distribution difference or the others between the female and male subjects. Considering no marked effect of sex on the inhibition rate of platelet aggregation, hence, no adjustment of dosage on the basis of sex appears needed.

Inhibition of ex vivo platelet aggregation induced by ADP also showed a concentration-dependent effect from data from the ascending-dose tolerability study(unpublished). Also the same results was achieved in the 180 µg·kg⁻¹ plus 2.0 µg/min·kg⁻¹ group, there was an immediate inhibition of platelet aggregation after the bolus and start of infusion, and relatively less (P<0.05) inhibition of platelet aggregation at 1h than the observed values at 10min or 4h after the bolus, and a sustained >80% inhibition of platelet aggregation detected between 4 and 24 hours, in which administration of a single 180 µg·kg⁻¹ bolus combined with an infusion produced an early peak level, followed by a small decline (P<0.05, concentraton at 1h compared with that at 0.167 h or 4 h) prior to attaining steady state. Thus, a bolus plus infusion is the
optimal dose regimen to obtain good clinical effect. However, as the 100% inhibition of in vitro platelet aggregation was 817.94 ng·ml⁻¹, although there was significant difference in mean Css in the two multiple dose groups, and between the male and female subjects, however no significant difference in Inhibition of ex vivo platelet aggregation for them, in which the steady state concentrations ranged from 800.2 ng·ml⁻¹ to 1629 ng·ml⁻¹, close or above the concentration of BAT with 100% inhibition of In vitro platelet aggregation. It suggested that the 220 µg·kg⁻¹ plus 2.0 µg/min·kg⁻¹ probably was the optimal dose for the efficacy study.

The study has shown that BAT is well tolerated even the given multiple dose up to 220 µg·kg⁻¹ bolus plus 2.5µg/min/kg infusion for 24h. No adverse effects were observed with any of the safety parameters; no changes in platelet counts were found during or after the infusion, only slight drop in WBC(5%) and Fbg (5%), RBC in urine (11.67%) was observed. Of importance, all of the adverse events were mild in intensity and required no intervention, and not dose-dependent and released within several days without treatment.

In conclusion, the results of the present study indicate that BAT has predictable pharmacokinetics. Batifiban displays linear pharmacokinetics in the dose range of 55 µg·kg⁻¹ to 220 µg·kg⁻¹ after single bolus doses, and of 180 µg·kg⁻¹ bolus plus 2.0 µg/min·kg⁻¹ infusion to 220 µg·kg⁻¹ bolus plus 2.5 µg·min·kg⁻¹ infusion. The compound is rapidly cleared from plasma, which supports the use of BAT for the bolus followed infusion dose regimen in order to obtain the steady state concentration rapidly. Although marked effect of sex on the disposition of BAT was observed, no significant difference in inhibition of platelet aggregation at steady state between the male and the female subjects in each multiple-dose group. It suggested that more attention should be paid on female subjects on bleeding or other adverse reaction due to higher concentration of batifiban during infusion in clinical usage. However, adjustment of dosage appears to be needed in subjects with impaired renal function or elderly subjects in the treatment due to almost 50% of batifiban excreted in urine. Taken together, the excellent safety, tolerability, linear pharmacokinetics and the concentration–dependent inhibition of ex vivo platelet aggregation warrant further investigation of batifiban in the target patient population.

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References


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