Study on the Pharmacokinetics of Fasudil, a selective Rho kinase inhibitor

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Abstract

Aim To study the pharmacokinetics of Fasudil. **Design and Method** The HPLC methods with UV detection were used for measurement of Fasudil concentration in biological samples, respectively. **Results** After intravenous dose at 3, 6 and 12 mg·kg⁻¹ of Fasudil in rats, C-T data were fitted to tow-compartment models of intravenous, the pharmacokinetic parameters of Fasudil were as follows: the serum concentration at time 0 (C₀) were 3.30±1.05, 5.10±0.96 and 18.82±5.91 µg·ml⁻¹, and the areas under the serum concentration-time curves(AUC) were 52.54±7.97, 102.87±42.97 and 325.79±73.00 µg·min·ml⁻¹, the terminal half- life (t₁/₂) were 24.9±4.5, 41.1±27.5 and 41.3±21.1 min, respectively. After a single dose of 6 mg·kg⁻¹ Fasudil intravenous in rats, the drug concentration were observed higher in lung, kidney, spleen and intestine at 5 and 20 min after dosing. The drug concentration levels decreased at 60min after dosing in most tissues. After a single dose of 6 mg·kg⁻¹ Fasudil intravenous in rats, the excretion of Fasudil in urine, feces and bile amounted to 41.1%, 6.4% and 38.4% of dose, respectively. The plasma protein binding ratio of Fasudil with rat serum was 53.2±4.4%. **Conclusion** The HPLC methods used in this study is specific, sensitive, precise and accurate, completely meeting the requirements of pharmacokinetic study of Fasudil. Fasudil is distributed extracellularly and very quickly from central to peripheral compartment and eliminates from the body rapidly; the pharmacokinetic behavior of the drug in rats complies with linear kinetics.

Key words

Fasudil; high performance liquid chromatography; Pharmacokinetics; rat;

Introduction

Fasudil hexahydro-1-(5-isoquinolinesulfonyl)-1H-1,4-diazeoine), (Fig 1), a selective Rho-kinase inhibitor, which attenuates many vasoconstrictions, increases hemodynamic function, and regulates important features of inflammatory reactions. Fasudil is well- tolerated. An intravenous form of fasudil has been approved in Japan since 1995, and used in tens of thousands of patients with vascular spasms in the brain.

In China, Fasudil has been under preclinical phase of research and showed powerful effects in animals. The present study is designed to investigate...
the pharmacokinetic properties of Fasudil in rats in order to provide experiment basis for its development as a new drug.

![Chemical structure of Fasudil](Fig 1. Chemical structure of Fasudil)

Materials and methods

Chemicals and reagents

Fasudil was prepared by the chemical department of our institute, batch number: 970301. acetonitrile, HPLC grade, was produced by Tianjin Chemical Reagent Factory. Other chemicals used were of analytical grade. Distilled water, prepared from demineralized water, was used throughout the study.

Animals

Male Wistar rats (weighing 150~200g, Grade I, Certificate No 001) were produced by the animal department of our institute.

Apparatus and analytical condition for assay of Fasudil

The high performance liquid chromatographic (HPLC) analyses were used. It consisted of Waters 510 pump, 710B injector, Shimadzu SPD-6A UV-visible detector and a ODS column (250mm×4.6mm I.D., 5µm Particle size). The mobile phase was water- acetonitrile- ammonia (100:100:1, v/v) at flow rate of 1.0ml•min⁻¹. The UV detection was at 215nm.

Extraction procedure for assay of Fasudil

Biological samples (serum, tissue homogenate, urine, feces or bile) 0.5mL and 0.5mL trichloroactic acid were mixed for 20 seconds and centrifuged at 15000•min⁻¹ for 5min and 20µL of the upper layer were injected into the liquid chromatograph.

Pharmacokinetic study

According to the requisition on pharmacokinetic study design, three doses of 3, 6 and 12mg•kg⁻¹ in rats were used for pharmacokinetic studies. The drug was dissolved with 9% sodium chloride solution for injection. The animals were fasted for 15h and the blood samples were collected via eye vein at 0, 5, 10, 20, 40, 60 and 120min after intravenous in rats. After centrifugation, the serum was used for determination of Fasudil.

Distribution

The brain, heart, lung, liver, kidney, spleen, stomach, intestinal, muscle, fat, testis and uterus were taken from rats and prepared for tissue homogenate at 5, 20 and 60min after intravenous of 6mg•kg⁻¹.

Excretion

Urine and feces excretion 6 of male rats were placed into metabolic cages, respectively. The urine and feces were collected before injection and at 0~2, 2~4, 4~8, 8~12, 12~24, 24~36, and 36~48h after dosing Fasudil of 6 mg•kg⁻¹. The drug contents in urine and feces (treated with water) were assayed with the designed methods, respectively.

Bile excretion Rats were anaesthetized with uratan and fixed on a stage. A bile fistula was performed from the common bile duct with a thin plastic tube to collect bile. The bile was collected before dosing and at 0~2, 2~6, 6~8, 8~12 and 12~24h after injection Fasudil of 6 mg•kg⁻¹.

Plasma protein binding

Tree concentration of Fasudil (2.5, 5 and 10µg•mL⁻¹) were used for the plasma protein binding test using a filtering method.

Pharmacokinetic analysis

Using the 3P97 Program (version 1997) developed by Mathematical Pharmacological Committee, Chinese Pharmacological Society, the compartment model of plasma concentration – time curves was fitted and the pharmacokinetic parameters including K (first-order rate constant), t₁/₂β (half-life), V₅ (apparent volume of distribution), CL (total body clearance), AUC (area under drug plasma concentration-time curve) were estimated.
Statistical analysis
Data are presented as arithmetic mean ± standard deviation (SD).

Results

Analytical method validation
According to the requisition for pharmacokinetic studies, the analytical method was validated.[13-15]

Chromatographic behavior
In the conditions as described above, the retention time of Fasudile was about 5 min (Fig1). The endogenous substances in serum did not interfered separation.

Calibration curves
The linearity was evaluated by constructing a calibration curve in the various biological media. The linearity was tested by linear regression of the peak areas (Y) versus the concentration of the drug (X).

The calibration curve of Fasudil in rat serum (concentration at the ranges of 0-10 µg·mL⁻¹) was
\[ Y = 0.1862 + 0.00005634X \quad (r=0.9996) \]

The calibration curves of Fasudil in rat tissue homogenate were
- for heart \( Y = 0.23 + 0.00008X \quad (r=0.999) \)
- for liver \( Y = 0.23 + 0.00009X \quad (r=0.997) \)
- for kidney \( Y = 0.353 + 0.00008X \quad (r=0.999) \)
- for brain \( Y = 0.315 + 0.00008X \quad (r=0.999) \)
- for muscle \( Y = 0.194 + 0.00008X \quad (r=0.999) \)

The calibration curves of Fasudil in rat urine, feces and bile were
- for urine \( Y = 0.489 + 0.0001X \quad (r=0.999) \)
- for feces \( Y = 1.219 + 0.0001X \quad (r=0.994) \)
- for bile \( Y = 0.31 + 0.001X \quad (r=0.999) \)

Sensitivity, recovery and precision
The limit of detection was 0.2 µg·mL⁻¹, the recovery were 80.9%, 86.0% and 88.2%, the relative standard deviation (RSD) were 2.8%, 3.3% and 3.6% for within-day assay and 6.7%, 5.3% and 5.2% for between-day at Fasudil concentrations of 1, 2 and 5 µg·mL⁻¹, respectively.

Pharmacokinetic study
The concentration-time data of Fasudil after intravenous of three doses in rats were shown in Table 1 and Fig 2. The results indicated that the plasma concentration after intravenous administration decayed biexponentially with time with an extremely rapid initial phase and followed by a relatively slow elimination phase. The two-compartment model for Fasudil was further confirmed by Wss, r, R², AIC and F test. The main pharmacokinetic parameters were shown in Table 2 after intravenous Fasudil in rats. There were good correlation between \( C_0 \), AUC and doses.
Table 1. Comparison of Fasudil serum concentrations after intravenous of Fasudil 3, 6 and 12 mg·kg⁻¹ in rats (n=5, mean ± SD)

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>3 mg·kg⁻¹ Serum concentration(µ g·mL⁻¹)</th>
<th>6 mg·kg⁻¹</th>
<th>12 mg·kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.30±1.08</td>
<td>5.10±0.96</td>
<td>18.8±5.91</td>
</tr>
<tr>
<td>5</td>
<td>2.40±0.54</td>
<td>3.22±1.01</td>
<td>9.27±2.61</td>
</tr>
<tr>
<td>10</td>
<td>1.42±0.23</td>
<td>2.36±0.76</td>
<td>6.49±1.33</td>
</tr>
<tr>
<td>20</td>
<td>0.81±0.16</td>
<td>1.39±0.45</td>
<td>4.24±0.94</td>
</tr>
<tr>
<td>40</td>
<td>0.42±0.07</td>
<td>0.85±0.37</td>
<td>2.55±0.54</td>
</tr>
<tr>
<td>60</td>
<td>0.17±0.10</td>
<td>0.46±0.32</td>
<td>1.78±0.97</td>
</tr>
<tr>
<td>120</td>
<td>nd</td>
<td>0.15±0.22</td>
<td>0.31±0.26</td>
</tr>
</tbody>
</table>

![Graph of mean serum concentration-time curves of Fasudil](image1)

Table 2. The main pharmacokinetic parameters of Fasudil after intravenous Fasudil 3, 6 and 12 mg·kg⁻¹ in rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>3 mg·kg⁻¹</th>
<th>6 mg·kg⁻¹</th>
<th>12 mg·kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₀ (µ g·mL⁻¹)</td>
<td>3.30±1.05</td>
<td>5.10±0.96</td>
<td>18.8±5.91</td>
</tr>
<tr>
<td>T₁/₂ (min)</td>
<td>24.9±4.5</td>
<td>41.1±27.5</td>
<td>41.3±21.1</td>
</tr>
<tr>
<td>V(Є) (mL)</td>
<td>0.94±0.26</td>
<td>1.17±0.24</td>
<td>0.72±0.20</td>
</tr>
<tr>
<td>CL(Є) (mL·min⁻¹)</td>
<td>0.052±0.009</td>
<td>0.061±0.027</td>
<td>0.037±0.008</td>
</tr>
<tr>
<td>AUC₀-Tₙ (µ g·min⁻¹·mL⁻¹)</td>
<td>52.5±8.0</td>
<td>102.9±43.0</td>
<td>325.8±73.0</td>
</tr>
<tr>
<td>MRT (min)</td>
<td>15.8±2.6</td>
<td>23.5±9.8</td>
<td>26.3±6.0</td>
</tr>
</tbody>
</table>

Tissue distribution
The drug could be widely distributed into tissues after intravenous 6 mg·kg⁻¹ in rats (Table 3). As shown in Fig 3, the concentrations of Fasudil were observed in lung, kidney, spleen and intestine higher at 5 and 20min after dosing.

Excretion
Urine and feces excretion
41.1%, 6.4% of administered dose were
excreted. The results in the urine and the feces indicated that Fasudil in the urine and could distribute widely and rapidly to tissues and excreted gradually from tissues.

As shown in Fig 3, the concentrations of Fasudil in the urine and the feces during 0–48 h after intravenous dosing of Fasudil 6 mg•kg⁻¹ in rats. The results indicated that the drug was mainly excreted via urine.

Bile excretion 38.4% of administered dose was excreted in the bile during 0–24 h after intravenous dosing of Fasudil 6 mg•kg⁻¹ in rats.

Protein Binding
At the concentration of Fasudil 2.5, 5 and 10µg•mL⁻¹, the plasma protein binding ratios were 55.2±5.8%, 54.7±1.3% and 49.6±3.3%, respectively.

### Table 3  Tissue distribution of Fasudil

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Drug concentrations (µg•g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5min</td>
</tr>
<tr>
<td>Heart</td>
<td>2.80±1.80</td>
</tr>
<tr>
<td>Liver</td>
<td>4.44±3.60</td>
</tr>
<tr>
<td>Spleen</td>
<td>8.40±3.16</td>
</tr>
<tr>
<td>Lung</td>
<td>18.92±11.72</td>
</tr>
<tr>
<td>Kidney</td>
<td>12.56±11.84</td>
</tr>
<tr>
<td>Brain</td>
<td>3.80±2.29</td>
</tr>
<tr>
<td>Stomach</td>
<td>4.41±3.94</td>
</tr>
<tr>
<td>Intestine</td>
<td>6.21±5.05</td>
</tr>
<tr>
<td>Muscle</td>
<td>4.06±1.40</td>
</tr>
<tr>
<td>Fat</td>
<td>2.49±0.94</td>
</tr>
<tr>
<td>Uterus</td>
<td>1.17±0.81</td>
</tr>
<tr>
<td>Testis</td>
<td>4.82±6.16</td>
</tr>
<tr>
<td>Serum</td>
<td>3.22±1.01</td>
</tr>
</tbody>
</table>

Discussion and Conclusion
In this study pharmacokinetics in rats of Fasudil was investigated. The results showed that the assay method utilized by us is very sensitive, high specific, precise so as to completely meet the requirements of Fasudil pharmacokinetic study [13-15].

The data shows that fasudil is distributed extracellularly and transferred very quickly from central to peripheral compartment and eliminates from the body rapidly; the pharmacokinetic behavior of the drug in rats complies with linear kinetics.

References