PHARMACOKINETICS, TISSUE DISTRIBUTION, METABOLISM AND EXCRETION OF DEPSIDE SALTS FROM SALVIA MILTIORRHIZA IN RATS

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Running title: DISPOSITION OF DEPSIDE SALTS FROM SALVIA MILTIORRHIZA IN RATS

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ABBREVIATION: MLB, Magnesium lithospermate B; LSB, lithospermic acid B; RA, rosmarinic acid; LA, lithospermic acid; LC-MS/MS, liquid chromatography/tandem mass spectrometry
ABSTRACT:

Salviae miltiorrhiza, a traditional Chinese medical herb known as “Danshen”, has been widely used in clinics to improve blood circulation, relieve blood stasis and treat coronary heart disease. Depside salts from S. miltiorrhiza are a novel drug in which magnesium lithospermate B (MLB) and its analogues are the active components. The pharmacokinetics, tissue distribution, metabolism, and excretion of three of the major components, lithospermic acid B (LSB), rosmarinic acid (RA), and lithospermic acid (LA), were studied by liquid chromatography-tandem mass spectrometry following intravenous administration in Sprague-Dawley rats. The elimination half-lives for LSB, RA, and LA were 1.04, 0.75, 2.0 h, respectively, when 60 mg/kg of S. miltiorrhiza depside salts were administrated. The areas under the curve for LSB, RA, and LA were 51.6, 6.6, and 25.2 mg·h/l, respectively, and the values decreased in the individual tissues in the following order: kidney > lung > liver > heart > spleen > brain for LSB; kidney > lung > heart > liver > spleen > brain for RA; and heart > lung > kidney > liver > spleen > brain for LA. Following intravenous administration of 60 mg/kg of S. miltiorrhiza depside salts, 86% of the LSB was excreted in the bile within 6 h. The main metabolites M1 and M2 were found in the serum. Overall the results show that depside salts from S. miltiorrhiza are rapidly and widely distributed to tissues after intravenous administration in rats but that they are also rapidly cleared and excreted.
Introduction

Danshen, the dried roots of the medicinal plant *Salvia miltiorrhiza*, is a traditional Chinese medicine used for the treatment of coronary heart disease, hepatitis, menstrual disorders, menostasis, blood circulation diseases and other cardiovascular diseases (Li, 1998). Recent studies have examined the pharmacological activities of the water-soluble components of Danshen, which are the main active constituents.

Macropore resin separation has been used to isolate lithospermic acid (LA), magnesium lithospermate B (MLB), rosmarinic acid (RA), pro lithospermic acid, ammonium potassium lithospermate B and magnesium salvianolate E oligomers of caffenic acids from Danshen (Zhou et al., 1999). The depside salts from *S. miltiorrhiza*, in which MLB/LSB and its analogues RA and LA are active components, are used as a novel drug for the treatment of coronary heart disease.

The pharmacological activities of MLB (LSB), RA, and LA have been widely studied. MLB, the most common form in the aqueous extract, is a strong antioxidant (Wu et al., 2000) and free radical scavenger (Yokozawa et al., 1995). Recent pharmacological studies have indicated that MLB protects against renal dysfunction (Yokozawa et al., 1989, 1991, 1992, 1997), myocardial damage (Fung et al., 1993), and experimental hepatitis (Hase et al., 1997). RA and LA are also naturally occurring polyphenols with antioxidative, antibacterial and antiviral activities (Parnham et al., 1985). In addition, LA and MLB were reported to be potent and nontoxic inhibitors of human immunodeficiency virus type 1 integrase (Abd-Elazem et al., 2002).

Previous studies have indicated MLB (LSB) has extremely low oral bioavailability
(Zhang et al., 2004a) and that after intravenous administration in dog, it is eliminated quickly from serum (Li et al., 2004). Also, MLB is rapidly excreted into bile mostly as methylated metabolites (Zhang et al., 2004b). RA and its related metabolites have also been found in plasma and urine after oral administration (Baba et al., 2004). The strong antioxidative and free radical scavenging activity of these depside salts may help repair damage to vascular endothelial cells, thus reducing pathological changes in target organs and restoring vascular and tissue functions (Chen et al., 1999). The pharmacokinetics and distribution of these compounds in the target organs and their metabolic fate in serum, however, is unclear. Therefore, in the present study, we investigated the pharmacokinetics and distribution of LSB, RA, and LA as well as their metabolism and excretion following intravenous administration of *S. miltiorrhiza* depside salts to rats.
Materials and Methods

Chemicals. LSB, RA, LA (Fig. 1) and the corresponding internal standard silibinin (all were >99% pure) were provided by the Department of Phytochemistry, Shanghai Institute of Materia Medica (Shanghai, China). Depsides from S. miltiorrhiza were prepared from Radix Salviae Miltiorrhizae, which contains the salts of LSB (≥85.0%), RA (≥10.1%) and LA (≥1.9%). Acetonitrile (HPLC grade) was purchased from Fisher (Fair Lawn, NJ, USA), and all other reagents of HPLC or analytical grade were purchased from Sigma-Aldrich (St. Louis, MO, USA). Deionized distilled water was used for the preparation of all solution.

Animal Studies. Male Sprague-Dawley rats (8 weeks old, weighing 240-260 g) were purchased from the Animal Center of School of Agriculture and Biology, Shanghai Jiaotong University (Shanghai, China). The animals were acclimated to standard housing and environmental conditions (22 to 24 ºC; 50% relative humidity; and 12-h light/dark cycle) for 1 week. They were maintained in accordance with the Guidelines for Care and Use of Laboratory Animals at Shanghai Institute of Materia Medica, Chinese Academy of Sciences. At the end of the experiment, carbon dioxide was used for euthanasia of the animals.

Serum Kinetics. Six male rats received a single intravenous administration of 60 mg/kg S. miltiorrhiza depside salts dissolved in saline (1 ml/kg). Blank blood samples (0.3ml) were collected from the veins of each rat. After intravenous administration in rats, blood samples were withdrawn from the vein at 0.03, 0.08, 0.17, 0.33, 0.5, 0.75, 1, 1.5, 2, 3, 4, and 6 h. Serum was isolated from the blood samples by cooling them for 2
h at 4 °C, following by centrifugation at 3000×g for 10 min. All serum samples were frozen and stored at -20 °C until analysis.

LSB, RA, and LA were isolated from serum by liquid-liquid extraction according to our previously described procedure (Li et al., 2004). The serum samples were analyzed by liquid chromatography/tandem mass spectrometry (LC-MS/MS).

Data were analyzed using the Drug and Statistics version 2.0 program (Anhui Provincial Center for Drug Clinical Evaluation, China) with two-compartment model and a weighing function of 1/C² for data fitting and parameter estimation. All data were expressed as mean±SD.

**Tissue distribution Studies.** Sixty Sprague-Dawley male rats were given a single intravenous administration of 60 mg/kg *S. miltiorrhiza* depside salts through the femoral vein. Tissues (heart, liver, spleen, lung, kidney, and brain) were promptly removed at 0.08, 0.17, 0.33, 0.5, 0.75, 1, 1.5, 2, 3, and 4 h after dosing and washed with saline. Each tissue sample was diluted with three volumes (v/w) of saline containing 200 ng/ml silibinin as an internal standard and homogenized, and the homogenate was collected and stored at -20 °C until analysis. LSB, RA, and LA were isolated from the tissue extract as described for serum.

**Metabolism and Elimination Studies.** Six male rats were placed in separate metabolic cages and received a single intravenous administration of 60 mg/kg *S. miltiorrhiza* depside salts dissolved in saline (1 ml/kg). Urine and feces were collected the day before dosing and at 0-24 h after dosing for background measurements. The volume of urine samples was measured prior to storage at -20 °C. Feces were
homogenized in 100 volumes (v/w) of distilled water using a Polytron, and aliquots were stored at -20 °C until analysis.

In an additional six rats, bile was collected after dosing with 60 mg/kg *S. miltiorrhiza* depside salts under anesthesia induced by pentobarbital sodium. Bile fistulas in rats were cannulated with PE-10 polyethylene tubing. The bile was collected into successive vials on ice at 0-2 h and 2-6 h after dosing and stored at -20 °C until analysis.

To study the appearance of metabolites in the circulation, blood samples (~0.3ml) from six rats that received intravenous administration of *S. miltiorrhiza* depside salts were collected at 0.03, 0.08, 0.17, 0.33, 0.5, 0.75, 1, 1.5, 2, 3, 5, and 8 h. The serum was then obtained from the blood samples as described above (see “Serum Kinetics”).

Isolation and Quantitation of Biliary Metabolites. Bile was collected by biliary fistulization from 12 rats 10 h after intravenous administration of 60 mg/kg *S. miltiorrhiza* depside salts. The bile samples were combined, and the bile metabolites were isolated as described previously (Zhang et al., 2004b). HPLC analyses indicated that the purity of the three main metabolites, M1, M2, M3, was 98%, 95%, and 90%, after isolation. Metabolites in the bile, urine, feces, and serum were extracted and characterized using SCIEX API-3000 mass spectrometer using the isocratic system as described below.

LC-MS/MS Analysis. Serum samples and tissue samples were analyzed by LC-MS/MS as previously described (Li et al., 2004). The system was composed of an HPLC (Shimadzu, Japan) with a 5-µm CAPCELL PAK C18 column (50mm×2 mm
internal diameter; Shiseido, Japan) coupled to a Perkin-Elmer SCIEX API-3000 triple-quadruple mass spectrometer (Sciex, Concord, ON, Canada) equipped with an electrospray ionization source. The mobile phase for the HPLC column was 60 % water (containing 0.5% formic acid) /40 % acetonitrile. For the determination of LSB, RA, and LA, the negative ionization mode was selected, along with the following conditions: nebulizer gas, 12 L/min; curtain gas, 10 L/min; collision gas, 12 L/min; ionspray voltage, -4500 V; temperature of heated gas, 450°C. The mass spectrometer was operated in multiple reactions monitoring mode, with monitoring of the precursor-to-product ion transitions of m/z 717→519 for LSB, m/z 359→160 for RA, m/z 537→493 for LA, m/z 731→533 for M1, m/z 745→547 for M2, m/z 759→547 for M3, and m/z 481→301 for silibinin. Blank serum, tissue homogenate, bile, urine, and feces homogenate were used for the preparation of matrix-matched calibration curves so as to avoid different matrix effect. These samples were isolated by the same liquid-liquid extraction according to our previously described procedure (Li et al., 2004). Linear ranges of calibration curves were 16-4096 ng/mL for LSB with the lower limit of quantitation of 16 ng/mL, and 8-2048 ng/mL for all the other analytes with the lower limit of quantitation of 8 ng/mL.
Results

**Serum Pharmacokinetics.** Figure 2 shows the concentrations-time curves for LSB, RA, and LA after intravenous administration of 60 mg/kg *S. miltiorrhiza* depside salts. The concentration-time curves were adequately described by a two-compartment model, and the resulting Pharmacokinetic parameters are summarized in Table 1. The AUC$_{0-6h}$ of LSB, RA, and LA was 51.6, 6.6, and 25.2 mg·h/l, respectively, and the serum terminal elimination half-lives of LSB, RA, and LA were 1.04, 0.75, and 2.0 h, respectively (Table 1). A similar result of V and CL was found for LSB and RA, which was much larger than that of LA.

**Tissue Pharmacokinetics and Distribution.** The tissue concentrations of LSB, RA, and LA determined at 10 time points following intravenous administration of 60 mg/kg *S. miltiorrhiza* depside salts are shown in Fig.3, 4, and 5, respectively. The high concentration LSB was found in kidney, lung, and liver, and very little was found in the brain. A high concentration of RA was found in the kidney. The high concentration of LA was found in kidney, lung, and heart. LSB and LA were almost completely cleared from these tissues 4 h after administration, whereas RA was cleared within 2 h.

The pharmacokinetics and statistical matrix parameters for LSB, RA, and LA in individual tissues are summarized in Table 2. The mean C$_{max}$ values of LSB, RA, and LA for all the tissues analyzed were obtained at 0.08 h after intravenous administration. The elimination half-lives for LSB, RA, and LA in all these tissues were less than 2 h. The AUC values for the individual tissues following intravenous administration decreased as follow: for LSB, kidney > lung > liver > heart > spleen > brain; for RA,
kidney > lung > heart > liver > spleen > brain; and for LA, heart > lung > kidney > liver > spleen > brain. Fig. 6 described the ratio of the six total organ concentration compared with the serum concentration at each time points for LSB, RA, and LA. The highest ratio was found within 0.08 h followed by a rapid decrease within 0.5 h after the dose, which demonstrated they were quickly distributed into these organs and then released into the serum again rapidly.

**Metabolism and Excretion Study.** Following intravenous administration of *S. miltiorrhiza* depside salts, total mean recovery of LSB and its metabolites in the bile was about 86% (Table 3). Most of LSB was excreted within 6 h after administration, with the bile being the major route of excretion. In the bile, the mean recovery of unchanged LSB, RA, and LA was 13.8%, 0.09% and 7.0%, respectively. In the feces over a 24-h period, the mean recovery of LSB, RA, and LA was 3.9%, 0.1%, and 6.9%, respectively. On the other hand, the mean recovery in the urine over a 24-h period of LSB, RA, and LA was only 0.5%, 2.7%, and 1.6%, respectively.

Metabolite M1 accounted for 39.9% of the dose in bile and 0.15% of that in urine (Table 4). The product ion scans of *m/z* 731 ([M-H]⁻) generated major product ions at *m/z* 533 (Fig. 7). In the serum, this metabolite had a C\text{max} of 2.6 mg/l at 10 min after administration (Fig. 8). The AUC for this metabolite was 2.24 mg·h/l.

Metabolite M2 accounted for 26.3% of the dose in bile and 0.10% of that in urine (Table 4). The product ion scans of *m/z* 745 ([M-H]⁻) generated major product ions at *m/z* 547 (Fig. 7). In the serum, this metabolite had a C\text{max} of 0.25 mg/l at 10 min after administration (Fig. 8). The AUC for this metabolite was 0.28 mg·h/l.
Metabolite M3 accounted for 6.4% of the dose in bile and 0.06% of that in urine (Table 4). The product ion scans of $m/z$ 759 ([M-H]$^-$) generated major product ions at $m/z$ 547 (Fig. 7). Little M3 was found in the serum.
Discussion

In the present study, we found that the pharmacokinetics and distribution of LSB, RA, and LA in Sprague-Dawley rats after intravenous administration fit best to a two-compartment model. The half-lives of LSB, RA, and LA in serum were less than 2 h, indicating that all three compounds are rapidly eliminated.

LSB, RA, and LA were distributed widely amongst the tissues analyzed, with maximal levels reached within 5 min after dosing. The releasing ratio from organs into serum was extremely rapid with the decreased order of RA > LSB > LA within 0.5 h, then a relative releasing balance was maintained for LSB, RA, and LA. The levels in kidney, lung, and liver were similar to those in serum at the same time points. LSB, RA, and LA were almost completely cleared from these tissues after 4 h, with half-lives of less than 2 h (Table 2). The highest AUC value for LSB in these tissues was found in kidney, although it was lower than that in the serum. The AUC level of RA in kidney was much higher than those in other tissues, RA was also found to be eliminated mainly from kidney into urine, which was different with its analogues LSB and LA (Baba et al., 2005). Also, a low level of AUC value was found in brain for LSB, RA, and LA, suggesting that these three phenolic compounds do not efficiently cross the blood-brain barrier.

*S. miltiorrhiza* was used in clinics for the treatment of coronary heart disease and hepatitis, and the study also found the high AUC level of LSB in heart and liver after intravenous administration, the pharmacodynamic effect might be related to the high distribution level. Previous study also demonstrated a total bile recovery of LSB was
5.6% after oral administration (Zhang et al., 2004b). The preventive effect of LSB on experimental hepatitis induced by carbon tetrachloride or a combination of D-galactosamine and lipopolysaccharide (Hase et al., 1997) suggests that oral administration of *S. miltiorrhiza* depside salts may be useful for the treatment of hepatitis, but further studies are necessary to investigate their pharmacological properties.

LSB was reported to have extremely low oral bioavailability in Sprague-Dawley rats (Zhang et al., 2004a). After oral administration of 100mg/kg MLB to Sprague-Dawley rats, the peak concentration of LSB was beyond 50ng/ml in serum, which was much less than that after intravenous administration. The main metabolites (M1, M2, and M3) were also not observed in the serum after oral administration. On the other hand, only low levels of M1 and M2 were found in the serum after intravenous administration. These data indicate that the majority of the metabolites M1, M2, and M3 did not return to the blood circulation and were excreted directly into the bile. The mean recovery of LSB, M1, M2, and M3 in feces over a 24-h period was 3.9%, 10.9%, 5.0%, and 0.97%, respectively, whereas in the bile the mean recoveries were 13.8%, 39.9%, 26.3%, and 6.4%. These results demonstrate that the major LSB, M1, M2, and M3 were changed in the alimentary tract, and biotransformation occurred for these phenolic constituents in intestine.

In conclusion, the active components of *S. miltiorrhiza* depside salts are readily distributed to most tissues but can not efficiently cross the blood-brain barrier. The elimination of the active components in blood is rapid and the major route of
elimination of these compounds is excretion in the bile.
References


Baba S, Osakabe N, Natsume M, Terao J (2004) Orally administered rosmarinic acid is present as the conjugated and/or methylated forms in plasma, and is degraded and metabolized to conjugated forms of caffeic acid, ferulic acid and m-coumaric acid. *Life Sci* **75**:165-178.


Kasimu R, Tanaka K, Tezuka Y, Gong ZN, Li JX, Basnet P, Namba T, Kadota S


Footnotes

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Legends for figures

FIG. 1. The chemical structures of LSB, RA, and LA.

FIG. 2. The mean serum concentrations of LSB, RA, and LA in Sprague-Dawley rats following intravenous administration of 60 mg/kg S. miltiorrhiza depside salts.

FIG. 3. Biodistribution of LSB in Sprague-Dawley rats following intravenous administration of 60 mg/kg S. miltiorrhiza depside salts. The levels of LSB in ng/g are expressed as mean±SD.

FIG. 4. Biodistribution of RA in Sprague-Dawley rats following intravenous administration of 60 mg/kg S. miltiorrhiza depside salts. The levels of RA in ng/g are expressed as mean±SD.

FIG. 5. Biodistribution of LA in Sprague-Dawley rats following intravenous administration of 60 mg/kg S. miltiorrhiza depside salts. The levels of LA in ng/g are expressed as mean±SD.

FIG. 6. The ratio of total six organ concentration compared with the serum concentration at each time points for LSB, RA, and LA.

FIG. 7. The product ion scans of the metabolites M1, M2, and M3.

FIG. 8. The mean serum concentrations of M1 and M2 in rats following intravenous administration of 60 mg/kg S. miltiorrhiza depside salts.
TABLE 1

Serum pharmacokinetic parameters of LSB, RA, and LA in Sprague-Dawley rats following intravenous administration of 60 mg/kg *S. miltiorrhiza* depside salts.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>LSB (mg·h/l)</th>
<th>RA (mg·h/l)</th>
<th>LA (mg·h/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC(0→t)</td>
<td>51.6±12.4</td>
<td>6.6±1.8</td>
<td>25.2±2.3</td>
</tr>
<tr>
<td>AUC(0→∞)</td>
<td>52.3±12.6</td>
<td>6.9±1.7</td>
<td>26.6±3.1</td>
</tr>
<tr>
<td>MRT(0→∞)</td>
<td>0.55±0.09</td>
<td>0.32±0.07</td>
<td>1.75±0.16</td>
</tr>
<tr>
<td>V (l/kg)</td>
<td>1.89±0.68</td>
<td>1.13±0.51</td>
<td>0.12±0.02</td>
</tr>
<tr>
<td>T1/2α</td>
<td>0.13±0.07</td>
<td>0.12±0.04</td>
<td>0.13±0.06</td>
</tr>
<tr>
<td>T1/2β</td>
<td>1.04±0.09</td>
<td>0.75±0.14</td>
<td>2.00±0.60</td>
</tr>
<tr>
<td>CL (l/h/kg)</td>
<td>1.09±0.26</td>
<td>1.02±0.32</td>
<td>0.04±0.01</td>
</tr>
</tbody>
</table>
TABLE 2

Tissue pharmacokinetic parameters of LSB, RA, and LA in Sprague-Dawley rats following intravenous administration of 60 mg/kg *S. miltiorrhiza* depside salts.

<table>
<thead>
<tr>
<th></th>
<th>LSB</th>
<th></th>
<th></th>
<th>RA</th>
<th></th>
<th></th>
<th>LA</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AUC(0-tn)</td>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>T&lt;sub&gt;1/2&lt;/sub&gt;</td>
<td>AUC(0-tn)</td>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>T&lt;sub&gt;1/2&lt;/sub&gt;</td>
<td>AUC(0-tn)</td>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
</tr>
<tr>
<td>Heart</td>
<td>8.26</td>
<td>22.75±15.58</td>
<td>1.02</td>
<td>0.34</td>
<td>1.08±0.95</td>
<td>1.39</td>
<td>4.02</td>
<td>5.22±2.32</td>
</tr>
<tr>
<td>Liver</td>
<td>22.26</td>
<td>85.28±38.15</td>
<td>0.79</td>
<td>0.04</td>
<td>0.29±0.16</td>
<td>-</td>
<td>0.43</td>
<td>1.20±0.47</td>
</tr>
<tr>
<td>Spleen</td>
<td>3.24</td>
<td>11.29±5.84</td>
<td>0.49</td>
<td>0.01</td>
<td>0.07±0.06</td>
<td>-</td>
<td>0.29</td>
<td>0.46±0.21</td>
</tr>
<tr>
<td>Lung</td>
<td>27.14</td>
<td>102.60±43.33</td>
<td>0.57</td>
<td>1.68</td>
<td>3.00±0.82</td>
<td>0.19</td>
<td>2.81</td>
<td>4.87±0.55</td>
</tr>
<tr>
<td>Kidney</td>
<td>41</td>
<td>136.65±64.94</td>
<td>0.7</td>
<td>10.84</td>
<td>43.65±18.11</td>
<td>0.36</td>
<td>2.67</td>
<td>5.05±0.92</td>
</tr>
<tr>
<td>Brain</td>
<td>1.04</td>
<td>2.15±0.67</td>
<td>0.76</td>
<td>0.01</td>
<td>0.06±0.03</td>
<td>-</td>
<td>0.27</td>
<td>0.27±0.10</td>
</tr>
</tbody>
</table>
TABLE 3

Total recovery of LSB, M1, M2, and M3 excreted in bile after intravenous administration of 60 mg/kg *S. miltiorrhiza* depside salts in Sprague-Dawley rats.

<table>
<thead>
<tr>
<th>Time after administration(h)</th>
<th>Ratio to LSB Administration (%)</th>
<th></th>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>LSB</td>
<td>M1</td>
<td>M2</td>
<td>M3</td>
<td>total</td>
</tr>
<tr>
<td>0 to 2</td>
<td>13.7±4.1</td>
<td>38.6±2.7</td>
<td>24.3±5.9</td>
<td>5.5±2.3</td>
<td>82.1±6.6</td>
</tr>
<tr>
<td>2 to 6</td>
<td>0.10±0.03</td>
<td>1.28±0.19</td>
<td>2.08±0.52</td>
<td>0.96±0.59</td>
<td>4.43±1.07</td>
</tr>
<tr>
<td>0 to 6</td>
<td>13.8±4.5</td>
<td>39.9±3.0</td>
<td>26.3±6.9</td>
<td>6.4±3.2</td>
<td>86.5±7.9</td>
</tr>
</tbody>
</table>
TABLE 4

Percentage of LSB, M1, M2, M3, RA, and LA in bile and urine of Sprague-Dawley rats following intravenous administration of 60 mg/kg *S. miltiorrhiza* depside salts.

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>Matrix</th>
<th>1/M</th>
<th>2/M</th>
<th>3/M</th>
<th>4/M</th>
<th>5/M</th>
<th>6/M</th>
<th>Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSB</td>
<td>Bile</td>
<td>18.5</td>
<td>6.8</td>
<td>12.3</td>
<td>11.8</td>
<td>18.2</td>
<td>15.4</td>
<td>13.8±4.5</td>
</tr>
<tr>
<td></td>
<td>Urine</td>
<td>0.3</td>
<td>0.3</td>
<td>0.9</td>
<td>0.2</td>
<td>0.4</td>
<td>0.8</td>
<td>0.5±0.3</td>
</tr>
<tr>
<td>M1</td>
<td>Bile</td>
<td>43.6</td>
<td>39.9</td>
<td>36.1</td>
<td>36.5</td>
<td>41.3</td>
<td>41.8</td>
<td>39.9±3.0</td>
</tr>
<tr>
<td></td>
<td>Urine</td>
<td>0.15</td>
<td>0.06</td>
<td>0.19</td>
<td>0.08</td>
<td>0.20</td>
<td>0.22</td>
<td>0.15±0.07</td>
</tr>
<tr>
<td>M2</td>
<td>Bile</td>
<td>24.2</td>
<td>38.5</td>
<td>21.0</td>
<td>28.7</td>
<td>19.0</td>
<td>26.6</td>
<td>26.3±6.9</td>
</tr>
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<tr>
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<td>Bile</td>
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FIG. 6

![Graph showing total tissue concentration/serum concentration over time. The graph includes three lines representing LSB, RA, and LA. The x-axis represents time in hours (0.00 to 4.00) and the y-axis represents total tissue concentration/serum concentration (0.00 to 3.00).]