Full Length Research Paper

Determination of Arctiin in rat plasma by HPLC method and its application to pharmacokinetic studies

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The present study was to investigate the pharmacokinetics of arctiin in male and female rats after oral administration of Fructus arctii. Arctiin (30-70 mg/kg) was orally administered to rats. Blood samples were collected by orbital vein at time intervals after drug administration and the plasma concentrations of the studied components were determined by HPLC after the plasma protein was precipitated directly with acetonitrile. Arctiin was successfully separated using a C18 column with UV detection at 280 nm and a mobile phase of acetonitrile–water (31:69, v/v) pumped at 1.0 ml/min. The method has a lower limit of quantitation (LOQ) of 0.50 g/ml for arctiin with the limit of detection (LOD) 0.25 g/ml, based on a signal-to-noise ratio of 3. The assay was linear over a range 0.50 to 256.50 g/ml with coefficient of determination greater than 0.99 for analytes. The extraction recoveries was >87%. The method had shown tremendous reproducibility with intra- and inter-day precision <3.2% (RSD), and had proven to be highly reliable for the analysis of plasma samples. The main pharmacokinetic parameters of Arctiin in rats after administration dose range of 30 to 70 mg/kg was processed by Das 2.0, while calculating the pharmacokinetic parameters of one compartment model. From the experimental results, the AUC (area under the concentration-time curve) was not proportional to the administered dose. In the range of the doses examined, the absorption pharmacokinetics of arctiin in rats was based on nonlinear kinetics. The male and female rats on the absorption and elimination of arctiin are different. The female rat’s $t_{1/2}$ (half life), $k_e$ (elimination rate constant), $K_a$ (absorption rate constant), $C_{max}$ (peak concentration), $V_d$ (apparent volume), Cl (total body clearance) and AUC of arctiin was significantly higher than the male’s ($P < 0.05$).

Key words: Arctiin, Fructus arctii, pharmacokinetics, plasma.

INTRODUCTION

The lignans are a group of compounds formed by condensation of pairs of phenylpropane structures. Derived mainly from whole grain bread, various beans, seeds, fruits, berries and vegetables; they have several biological functions and cellular activities such as protein synthesis, steroid biosynthesis and metabolism, growth factor action, cell proliferation, cell transformation, cell differentiation and angiogenesis (Adlercreutz et al., 1997; Botta et al., 2001) Arctiin is one of the most important bioactive lignans extracted from seeds of Arctium lappa L. On the other hand, Arctiin have the biological activities on the treatment of the acute progress nephritis, chronic glomerulonephritis and nephritis (Takela et al., 1990). Arctiin also have such function as the enhancement of immunological activitives, anti-inflammatory (Yan and Li, 1993), PAF antagonist (Lwakami et al., 1996), Ca$^{2+}$ antagonist and antihypertension (Fujimoto et al., 1992; Kazuo et al., 1986).

Pharmacokinetic study of active ingredients in natural product and Chinese herbs is important to illustrate their action mechanism for the development of traditional Chinese medicine (Ma et al., 2007). To date, there are some reports on the pharmacokinetic of retrojusticidin B from Phyllanthus niruri L., (Wei et al., 2010) and five lignan constituents (schisandrin, schisandrol B, schisantherin A, schisanhenol, and deoxyshisandrin) of Schisandra chinensis (Murugaiyah and Chan, 2007). However, the pharmacokinetics of arctiin had not been...

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studied further. Several methods had been reported for the determination of arctiin, including HPLC, UPLC and HPLC/MS (Park et al., 2009; Bing-Sheng et al., 2003; Young et al., 2003; Yuan et al., 2005). The HPLC method with high specificity and sensitivity had been employed to determine arctiin in various extraction of arctiin. However, method for determination of arctiin in plasma had not been reported. It is necessary to develop an analytical method for its determination in biological samples, e.g. plasma. In this paper, a simple, rapid, sensitive and accurate method was applied to determine the plasma levels of arctiin and had been successfully applied to pharmacokinetic investigation after the oral administration of arctiin.

In addition, it was reported that plant lignans and isoflavonoid glycosides were converted by intestinal bacteria to hormone-like compounds with weak estrogenic and antioxidative activities (Herman et al., 1995) and it had been suggested that their general pattern of conjugates in urine was similar to that of endogenous estrogens (Adlercreutz et al., 1995). In order to verify whether sex difference could affect the absorption of the arctiin, the pharmacokinetic of arctiin in male and female rats respectively was investigated.

MATERIALS AND METHODS

Chemicals and reagent

Arctiin was extracted and purified from the crude fruit of A. lappa L. (purchased from a local Chinese pharmacy store) in our own laboratory. The authenticity of Arctiin was confirmed by using UV, IR, NMR and HPLC with a photodiode array detector and comparing the data and melting point with those reported in literature (Han et al., 1998). The purity of arctiin was higher than 99.8% tested by HPLC. Its chemical structure is shown in Figure 1. Baicalin was purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol and acetonitrile (all of HPLC grade) were provided by Fisher Scientific International, Inc. (Fair Lawn, NJ, USA). Purified water was prepared in a water purification system (Mili-Q Biocel., USA). All other reagents were analytical grade and purchased from Tianjin Kernel Chemical Reagents Co. Ltd. (Tianjin, China).

Chromatographic conditions

The RP-HPLC analysis was carried out on an Agilent high performance liquid chromatography system (series 1100, Agilent Technology, Palo Alto, CA, USA) equipped with a G1310A quaternary pump, a G1322A on-line degasser, a G1316A column-heating assembly and a G1314A VWD detector and 7725I manual sample injector. The chromatography data were recorded and processed with Agilent chemstation software. The separation was performed on an Agilent C18 HPLC column (250×4.6 mm, particle size 5 μm, Agilent Technologies, Beijing, China). The mobile phase was composed of acetonitrile and water in volumetric ratio of 31/69. The injection volume was 20 μl. The flow rate was set to 1.0 ml/min throughout the elution, and the temperature was set to 30ºC and wavelength to 280 nm.

Preparation of stock solutions

Accurately weighed 10.26 mg of standard sample of arctiin was transferred to a 10 ml volumetric flask which was then filled to mark with methanol. Final concentration of the resulted stock solution was 1.026 mg/ml. Standard curve solutions were freshly prepared based on diluting the above stock solution with methanol at appropriate ratios to yield concentrations of 2.00, 4.00, 16.03, 64.13, 128.25, 256.5, 513.0, 1026 μg/ml of arctiin. The stock solution and working standard solutions consisted of arctiin were stored at -4 and 4ºC, respectively. Internal standard stock solution of baicalin (0.213 mg/ml) was also prepared in methanol and diluted with methanol for a working solution of 2.13 μg/ml.

Extraction procedure

An aliquot of 1.0 ml acetonitrile was added to 200 μl plasma sample to precipitate proteins with 50 μl internal standard in a centrifuge tube and the mixture was vortexed for 3 min. After centrifuged at 4000 g for 5 min, the supernatant was transferred into another tube and evaporated to dryness at 35ºC. The residue was dissolved by 50 μl methanol.
HPLC validation procedures

Calibration curves and linearity

The standard calibration curves in plasma were prepared daily by spiking the blank rat plasma (200 μl) with 50 μl of the standard solutions and 50 μl internal standard prepared above and making them to the following concentrations of arctiin: 0.50, 2.00, 4.01, 8.02, 16.03, 64.13, 128.30, 256.50 μg/ml. The spiked samples were then treated following the sample extraction procedure.

Precision, accuracy and recovery

The intra-day accuracy and precision of five replicates quality control samples were determined within one day at concentrations of 1.00, 128.50 and 205.20 μg/ml for arctiin. The inter-day accuracy and precision of the quality control samples were determined on three different days within one week. The relative standard deviation (% RSD) of each concentration was calculated to determine the precision of the method. The recovery included extraction recovery and assay recovery. The extraction recovery was determined for three different concentrations of arctiin at 1.00, 128.50 and 205.20 μg/ml by comparing the peak area ratio of arctiin to puerarin in plasma with that in methanol at same concentration. The assay recovery was determined in the same method, but calculated by comparing the concentrations of the analyte from the calibration curves with that in the prepared sample. The mean assay recovery value (%, mean ± S.D.) represented the accuracy of the method.

Stability

The quality control plasma samples in triplicates at low, medium and high concentration were used, which were prepared by spiking the blank rat plasma (200 μl) with 50 μl of the appropriate arctiin solution and mixing them to yield the following concentrations 1.00, 128.50 and 205.20 μg/ml. Short-term temperature stability was assessed at room temperature (25°C) for 12 h. Long-term stability evaluations involved an analysis of quality control samples that were stored at -20°C for 30 days. Freeze-thaw stability was evaluated after three cycles of thawing at room temperature followed by re-freezing to -20°C. Post-preparation stability was also determined using the extracted samples that were kept at room temperature for 24 h prior to injection and analysis by HPLC.

Applicability of the method in pharmacokinetic studies

Preparation of arctiin solution for oral administration

The dosing arctiin was dissolved in water containing 10 mg/ml.

Animals

Adult Sprague-Dawley (SD) rats (purchased from Sippr-BK Experimental Animal Ltd Co, Shanghai, Grade II, Certificate No 00800161386, n=36) weighing 200±20 g were used. All these animals were housed in an air-conditioned room (temperature, 25°C; relative humidity, 55%), and allowed free access to food and water. The rats were fasted for 12 h before dosing.

Pharmacokinetics

The male rat and female rat were divided into three groups (six rats per group), respectively. These groups were assigned to receive arctiin solution by oral administration at a single dose of 30, 50, 70 mg/kg of arctiin, respectively. The dosage of oral administration was calculated as the content of arctiin (5%) in Fructus Arctii, mentioned in Chinese Pharmacopoeia. Serial blood samples (0.5 ml) were obtained via the rats orbital vein at 20, 40, 50, 60, 80, 100, 120, 180, 240 min after administration and collected into heparinized centrifuge tubes. The blood samples were immediately centrifuged at 4000 g for 5 min at room temperature. The plasma sample was extracted and analyzed according to the extraction procedure aforementioned. The data analysis of plasma concentration of arctiin versus time and calculation of pharmacokinetic parameters in rats were executed by the statistics software DAS 2.0. The pharmacokinetic parameters were estimated using compartmental and statistical moment (that is compartmental) analysis methods.

RESULTS AND DISCUSSION

Specificity

The purpose of this study was to develop a simple and rapid HPLC method for determination of arctiin in plasma. Various proportional solvent system was chosen, e.g. methanol (acetonitrile)–water, methanol (acetonitrile)–phosphoric acid, (acetic acid)–water, using isocratic elution or gradient elution. After trial and error, the isocratic elution with acetonitrile–water (31/69, v/v) as the mobile phase was finally used for the determination of arctiin.

Typical chromatograms obtained from blank plasma, spiked rat plasma and plasma samples were shown in Figure 2. The peaks of the analyte in the plasma were identified by comparing their retention time with that of the standard sample. In this study, the retention times of arctiin varied from 11.183 to 11.887 min. The specificity of the assay was investigated by injecting blank plasma. No peaks were observed that would interfere with the analysis in HPLC chromatograph.

In this study, several organic solvents were tested for precipitating protein and extracting the analyte from rat plasma. They included methanol, acetonitrile and methanol-ethyl acetate (1:1, v/v) and the volume of them were one to five times that of plasma sample. The result showed that the effect of precipitating protein and extraction recoveries were best when acetonitrile was chosen. The extraction recoveries of the analyte at low, middle and high were 89.65±0.20%, 90.8±1.80 and 89.65±0.20%, respectively. In addition, 10% salicylsulfonic acid was used to precipitate protein in the experiment. It has a conspicuous effect on precipitating protein, but defects of which showed a bad peak shape.

Linearity

The peak areas of the analyte were plotted against standard concentrations to establish calibration curves for arctiin. The curves were best fitted using a least square linear regression model y = mx+b, weighted by 1/x², in which y is the peak area ratio, m is slope of the
Figure 2. Chromatograms of rat plasma samples. (A) Blank plasma; (B) Blank plasma spiked with arctiin and IS (arctiin, t = 11.87 min; I.S., t = 23.04 min); (C) Plasma sample obtained 40 min after oral administration of arctiin at a dose of 50 mg/kg to rat; (D) Plasma sample obtained 60 min after oral administration of arctiin at a dose of 50 mg/kg to rat; (E) Plasma sample obtained 240 min after oral administration of arctiin at a dose of 50 mg/kg to rat.
Figure 2. Continued.
calibration curve, \( b \) is intercept of the calibration curve and \( x \) is the analyte concentration. The slopes of the calibration graphs were 0.137±0.19 (n = 6) with RSD of 2.68%, and the intercepts were -0.040±0.18 (n = 6) with RSD of 1.80% throughout the study. The correlation coefficients (r > 0.99) showed good linearities. This result showed the usefulness of the present HPLC method in the assays of arctiin from low to high plasma levels.

The limit of quantitation (LOQ) was achieved as the lowest point on the standard curve, 0.50 \( \mu g/ml \) for arctiin with RSD of 3.2% (n = 5). The limit of detection (LOD) was defined as the lowest concentration of the drug resulting in a signal-to-noise ratio of 3:1. The limit of detection (LOD) of the analyte in plasma was determined to be 0.25 \( \mu g/ml \) for arctiin.

Precision, accuracy and recovery

Quality control samples (n = 5) representing low, medium and high concentration contained 1.00, 128.50 and 205.20 \( \mu g/ml \) for arctiin. The maximum intra- and inter-day precision was 3.25% for arctiin. The assay showed that the intra- and inter-day precisions for arctiin with a RSD of less than 15% were obtained at the lowest concentrations. Data on accuracy about the recovery of arctiin was 97.3±3.08% (n = 5) at the lowest concentration of 0.32 \( \mu g/ml \) in spiked rat plasma samples (Table 1). The mean extraction recovery of the analyte (n = 5) from spiked rat plasma (Table 1) was satisfactory at low, medium and high concentrations, which varied from 87.47±3.97 to 90.8±1.80%. High recovery of arctiin from rat plasma suggested that there was negligible loss during drug extraction.

Stability

Acceptable analyte stability was demonstrated for all phases of storage and processing was shown in Table 2. The accuracy of low (1.00 \( \mu g/ml \)), medium (128.50 \( \mu g/ml \)) and high (205.20 \( \mu g/ml \)) concentrations of arctiin in rat plasma for 12 h room temperature stability (99 and 105%), three cycle freeze-thaw stability (96 and 104%), 30 days at -20\(^\circ\)C, stability (95 and 105%) and 24 h post-preparative stability (95 and 105%) were all acceptable. The RSD (%) for each of the stability experiments varied between 0.2 and 2.6%.

Application of the HPLC method for pharmacokinetic studies

The DAS program as validated methods had been successfully applied for pharmacokinetic studies of arctiin in rat plasma after oral administration of an arctiin solution. The mean plasma concentration versus time profiles of arctiin was shown in Figure 3. The pharmacokinetic parameters were listed in Table 3. With minimum akaike information criterion (AIC) values, a one-compartment open pharmacokinetic model was proposed. Arctiin appeared to be distributed most rapidly into a highly perfused central compartment. The phenomenon with a rapid distribution and a relatively slow distribution followed by a slower elimination phase was observed from the compartment model parameters \( t_{1/2} \) (half life).

The values of \( t_{1/2} \), \( ke \) (elimination rate constant), \( V_1 \), CL (total body clearance), \( ka \) (absorption rate constant) and \( C_{max} \) (peak concentration of arctiin) in female plasma was about 58.85 to 89.97 min, 0.02 to 0.07 min\(^{-1}\), 11.19 to 16.08 L/kg, 0.13 to 0.41 L/min/kg and 0.04 to 0.13 min\(^{-1}\), 0.85 to 4.79 mg/l at three different doses, respectively; and the values of \( t_{1/2} \), \( ke \), \( V_1 \), CL, \( ka \) and \( C_{max} \) of arctiin in male plasma was about 59 to 70 min, 0.02 to 0.04 min\(^{-1}\), 5.8 to 24.7 L/kg, 0.07 to 0.26 L/min/kg and 0.59 to 0.104 min\(^{-1}\), 0.78 to 1.88 mg/L at three different doses, respectively. The plasma concentrations and AUC (area under the concen-tration-time curve) of arctiin in female rat and in male plasma were not proportional to the administrated doses. Nonlinear pharmacokinetics of arctiin was shown using least squares regression analysis in rats after oral administration of arctiin at doses of 30 to 70 mg/kg. The plasma concentration-time curves are illustrated in Figure 3.

Statistical analysis

All the means were presented with their standard deviation (mean ± S.D.). An unpaired Student’s t-test was
Table 2. Stability of arctiin in a rat plasma sample and plasma extract (mean ± S.D. n=3).

<table>
<thead>
<tr>
<th>Storage conditions</th>
<th>Conc. added (µg/ml)</th>
<th>Conc. assayed (µg/ml)</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma sample</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25°C×12 h</td>
<td>1.0</td>
<td>0.98 ± 0.02</td>
<td>98.5 ± 1.83</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>128.5</td>
<td>125.54 ± 1.63</td>
<td>97.6 ± 1.27</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>205.2</td>
<td>210.42 ± 1.65</td>
<td>102.5 ± 0.80</td>
<td>0.8</td>
</tr>
<tr>
<td>−20°C×30 days</td>
<td>1.0</td>
<td>0.99 ± 0.04</td>
<td>98.8 ± 3.90</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>128.5</td>
<td>130.45 ± 1.74</td>
<td>101.5 ± 1.35</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>205.2</td>
<td>209.13 ± 0.66</td>
<td>101.9 ± 0.32</td>
<td>0.4</td>
</tr>
<tr>
<td>Three freeze–thaw</td>
<td>1.0</td>
<td>0.98 ± 0.04</td>
<td>97.8 ± 3.80</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>128.5</td>
<td>129.52 ± 0.38</td>
<td>100.7 ± 0.30</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>205.2</td>
<td>209.47 ± 0.49</td>
<td>102.0 ± 0.24</td>
<td>0.4</td>
</tr>
<tr>
<td>Plasma extract</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25°C×24 h</td>
<td>1.0</td>
<td>0.98 ± 0.03</td>
<td>98.2 ± 3.02</td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td>128.5</td>
<td>128.27 ± 1.74</td>
<td>99.8 ± 1.36</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>205.2</td>
<td>208.94 ± 1.41</td>
<td>101.8 ± 0.69</td>
<td>0.7</td>
</tr>
</tbody>
</table>

240 min; C_{max}: Peak concentration; t_{1/2}: half-life; ke: elimination rate constant ; V_z: apparent volume of distribution; Cl_z: total body clearance.

used to determine any significance difference between the males and females. The differences were considered to be significant at p < 0.05. The result was shown in Table 3. The AUC was not proportional to the administered dose. The sum of these results indicated that, in the range of the doses examined, the pharmacokinetics of arctiin in rat was based on nonlinear kinetics.

In our study, the male and female rats on the absorption and elimination of arctiin are different. The AUC of female administrated by arctiin was significantly higher (p < 0.05 at 30 and 50 mg/kg, p < 0.01 at 70 mg/kg) than the male. The absorption rate constant (k_a) of female with arctiin were significantly increased (P < 0.01 at 30, 50 and 70 mg/kg) compared to that of male. Peak concentration (C_{max}) of female with arctiin were significantly increased (P < 0.05 at 30 and 70 mg/kg, p < 0.01 at 50 mg/kg) compared to those of male. Apparent volume of distribution (V) of female with arctiin was significantly increased (P < 0.01 at 30 and 70 mg/kg) compared to that of male. Total body clearance (CL) of female with arctiin were significantly increased (P < 0.01 at 30, 50 and 70 mg/kg) compared to that of male. The half life (t_{1/2}) of female with arctiin were significantly increased (P < 0.01 at 70 mg/kg) compared to that of male. The elimination rate constant (ke) of female with arctiin were significantly increased (P < 0.01 at 70 mg/kg) compared to that of male.

Because of arctiin estrogen-like effect, the pharmacokinetic parameter of female rat was different with the male rat. The absorption of arctiin in female was rapid than in male and the elimination rate of arctiin in female was slower than in male, especially at the dose of 70 mg/kg.

The difference of the absorption and elimination of arctiin in the male and female rats is mainly in the different hepatic metabolism of male and female rats (Gandhi et al., 2004; Meibohm et al., 2002). In female rats, absorption, protein binding, volume of distribution, and metabolism of drugs may differ due to hormonal influences on physiological functions (Thürmann and Hompesch, 1998).

Conclusion

In conclusion, a simple HPLC assay method had been used for determination of arctiin in rat plasma with an internal standard. Treatment with acetonitrile for arctinin results in chromatograms free of interference. The procedures for the determination of arctiin by HPLC method have been validated, and the results demonstrate that the standard curve is linear over the concentration of 0.50 to 256.50 µg/ml. 200 µl plasma was required for analysis of the compound. The method is suitable for pharmacokinetic study of arctiin in rats after oral administration.

The results of the present study demonstrated that arctiin was absorbed and distributed quickly but eliminated slowly after oral administration at the doses of 30, 50 and 70 mg/kg in rats. From the experimental results, the AUC was not proportional to the administered dose. In the range of the doses examined, the absorption pharmacokinetics of arctiin in rats was based on
nonlinear kinetics. The male and female rats on the absorption and elimination of arctiin are different. The female rat's absorption $t_{1/2}$ (half life), $k_e$, $K_a$, $C_{max}$, $V_d$, $Cl$, and AUC of arctiin was significantly higher than the that of male ($P < 0.05$). Further study should be ongoing in our laboratory to further characterize the absorption profiles of arctiin.

Figure 3. Plasma concentration-time curves after oral administration of arctiin to rat at doses of 30, 50 and 70 mg/kg.

ACKNOWLEDGMENTS

Thanks for the funding of the National Eleventh-five-year Plan in R&D of Important New Medicine (2009ZX09103-423) and Supporting Plan Project for Excellent Researcher of Liaoning Provincial Education Department (2008RC34).
Table 3. Pharmacokinetic parameters for arctiin in SD rats (mean ± S.D., n = 6) after a single oral administration of arctiin.

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>Male 30mg/kg</th>
<th>Male 50mg/kg</th>
<th>Male 70mg/kg</th>
<th>Female 30mg/kg</th>
<th>Female 50mg/kg</th>
<th>Female 70mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>$t_{1/2}$ (min)</td>
<td>59.21 ± 6.218</td>
<td>63.70 ± 7.372</td>
<td>62.79 ± 4.321</td>
<td>58.85 ± 5.010</td>
<td>69.99 ± 8.733</td>
<td>89.97 ± 11.959**</td>
</tr>
<tr>
<td>$K_e$ (1/min)</td>
<td>0.02 ± 0.003</td>
<td>0.054 ± 0.009</td>
<td>0.04 ± 0.006</td>
<td>0.02 ± 0.003</td>
<td>0.06 ± 0.010</td>
<td>0.07 ± 0.011**</td>
</tr>
<tr>
<td>$V_1/F$ (L/kg)</td>
<td>5.83 ± 0.953</td>
<td>15.70 ± 3.260</td>
<td>24.69 ± 3.14</td>
<td>16.08 ± 2.283**</td>
<td>14.32 ± 2.260</td>
<td>11.19 ± 1.830**</td>
</tr>
<tr>
<td>$CL/F$ (L/min/kg)</td>
<td>0.07 ± 0.008</td>
<td>0.153 ± 0.024</td>
<td>0.26 ± 0.034</td>
<td>0.13 ± 0.026**</td>
<td>0.29 ± 0.079**</td>
<td>0.41 ± 0.062**</td>
</tr>
<tr>
<td>$AUC(0-t)$ (mg/L * min)</td>
<td>98.77 ± 9.78</td>
<td>111.17 ± 13.074</td>
<td>130.77 ± 6.728</td>
<td>112.25 ± 10.820*</td>
<td>134.29 ± 21.313*</td>
<td>168.08 ± 23.249**</td>
</tr>
<tr>
<td>$AUC(0-\infty)$ (mg/L * min)</td>
<td>131.17 ± 7.23</td>
<td>146.16 ± 15.050</td>
<td>177.67 ± 19.005</td>
<td>145.09 ± 10.751*</td>
<td>175.01 ± 26.106*</td>
<td>205.67 ± 19.964*</td>
</tr>
<tr>
<td>$K_a$ (1/min)</td>
<td>0.16 ± 0.01</td>
<td>0.17 ± 0.01</td>
<td>0.20 ± 0.01</td>
<td>0.18 ± 0.01**</td>
<td>0.20 ± 0.01**</td>
<td>0.22 ± 0.01**</td>
</tr>
<tr>
<td>$C_{max}$ (mg/L)</td>
<td>0.78 ± 0.03</td>
<td>1.10 ± 0.01</td>
<td>1.34 ± 0.01</td>
<td>0.85 ± 0.07*</td>
<td>1.10 ± 0.01**</td>
<td>1.24 ± 0.01**</td>
</tr>
</tbody>
</table>

REFERENCES


