Effects of Acute Hepatic and Renal Failure on Pharmacokinetics of Flunixin Meglumine in Rats

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Abstract: The aim of this study was to investigate the effects of hepatic and renal failure on the pharmacokinetics of flunixin in carbon tetrachloride (CCl\(_4\))- and glycerol-treated rats. After intravenous administration of flunixin (2 mg/kg), the plasma concentration of flunixin was measured by high-performance liquid chromatography. Both acute hepatic and renal failure resulted in significantly increased area under the curve (AUC), prolonged elimination half-life (\(t_{1/2,\beta}\)), and reduced total body clearance (\(C_l_{tot}\)) compared with respective controls (\(P<0.05\)). In conclusion, hepatic failure as well as renal failure modified the pharmacokinetics of flunixin.

Key words: acute renal and hepatic failure, flunixin, pharmacokinetics

Flunixin is a nonsteroidal anti-inflammatory drug (NSAID) used only in veterinary medicine, and has potent anti-inflammatory, analgesic and anti-pyretic activities [31]. Flunixin acts through the inhibition of cyclooxygenase, which synthesizes prostaglandins from arachidonic acid [12]. Flunixin has been used for the control of postoperative pain and the treatment of respiratory diseases, mastitis, osteoarthritis, and endotoxemia in a variety of animals [2, 6, 11]. Pharmacokinetic profiles of flunixin are characterized by good bioavailability, short half-life and a high protein binding property. Taylor et al. [28] and Lascelles et al. [14] reported that flunixin shows good absorption from the GI tract and has a bioavailability >100%. The plasma half-life of flunixin is about 1–3 h in mice, dogs, horses, and cattle [18–20, 23]. The protein binding of flunixin is 92.2% in dogs, 84.8% in goats, and 86.9% in horses [7]. Although flunixin is well known to be relatively safe among NSAIDs, its overdose and repeated administration may cause several adverse effects such as renal necrosis, myonecrosis, and gastric ulceration [15, 22, 26].

The liver and kidneys are the main routes of drug elimination, the removal of a drug from the body by metabolism and/or excretion. Hepatic and renal failure not only delay drug metabolism, but also decrease drug elimination [27]. The alteration of drug metabolism and elimination could induce adverse effects of flunixin via drug accumulation. Although flunixin is frequently used in critically ill cases as an anti-inflammatory therapy, there is no available information on the effects of renal or hepatic failure on the pharmacokinetics of flunixin. In the present study, we investigated whether CCl\(_4\)-induced hepatic or glycerol-induced renal failure affects the pharmacokinetics of flunixin.
All animal experiments were performed in accordance with the Guidelines for Animal Experiments of Chungnam National University (Daejeon, South Korea) and approved by the Animal Ethics Committee, Chungnam National University. Male Sprague-Dawley rats (250–270 g, 9-week-old) were obtained from Orient Bio Inc. (Sungnam, South Korea) and housed in an air-conditioned room under conditions of controlled temperature (23 ± 3°C), relative humidity (50 ± 10%), and light-dark cycle (12 h). Animals were provided with feed pellet and filtered water ad libitum.

After 18 h starvation, acute hepatic failure was induced by intraperitoneal administration of CCl₄ dissolved in olive oil (25% v/v, 2 ml/kg) 24 h before flunixin injection. For the induction of acute renal failure, rats were intramuscularly injected with glycerol dissolved in saline (50% v/v, 10 ml/kg) after 24 h water deprivation, and used in experiments 48 h after glycerol administration. Rats treated with olive oil (2 ml/kg) or saline (10 ml/kg) served as respective controls of hepatic and renal failure. To determine the induction of liver and kidney dysfunction, the biochemical parameters of treated animals measured before the pharmacokinetic study were serum creatinine, blood urea nitrogen (BUN), alanine aminotransferase (ALT) and aspartate aminotransferase (AST).

Flunixin meglumine (Fluximine®) was intravenously administered at a dose of 2 mg/kg via the tail vein. Blood samples were collected at 5, 15, 30, and 45 min, and 1, 1.5, 2, 4, 6 and 8 h post-treatment and the total volume of blood samples from each rat was less than 2 ml. The samples were collected in heparinized tubes and centrifuged at 3,000 × g for 10 min. The plasma samples were stored at −70°C until analysis.

For the determination of plasma flunixin concentration, the analytical method reported by Cheng et al. [3] and Baert and De Backer [1] was used with some modifications. Briefly, samples were analyzed by an Acme 9000 high performance liquid chromatography system (HPLC, Young-Lin, Seoul, South Korea) equipped with a UV spectrophotometric detector (UV725S) and data processor (Autochrom-3000). An Eclips plus C₁₈ column (3.5 µm, 4.6 × 100 mm, Agilent, Santa Clara, CA, USA) was used for separation. Isocratic elution was performed at a flow rate of 1.0 ml/min with acetonitrile: methanol: 0.04% acetic acid in water (40:40:20). One hundred microliter aliquots of plasma samples were acidified with 10 µl of 1 M hydrochloric acid and added to 1 ml diethyl ether. The samples were vortexed for 5 min and then centrifuged at 2,000 × g for 5 min. After centrifugation, the supernatant was evaporated under nitrogen at 40°C and the residue was re-dissolved in 100 µl of the mobile phase. Then, a 20 µl aliquot was injected into the HPLC system and flunixin was measured at 330 nm. Intra-day and inter-day coefficients of variation for flunixin were between 5.4 and 6.3%, respectively.

The appropriate pharmacokinetic model was determined by the Akaike information criterion (AIC) [30] and pharmacokinetic parameters were calculated using a two-compartmental analysis (WinNonlin 5.2.1, Pharsight Corp., St. Louis, MO, USA). The plasma concentration-time curves of flunixin after intravenous administrations were fitted to the following exponential equations:

\[ C = A_1 e^{-\alpha t} + A_2 e^{-\beta t} \]

where C is plasma concentration of flunixin; A₁ and A₂ are mathematical coefficients; \( \alpha \) is the rate constant for distribution phase; \( \beta \) is the rate constant for the terminal elimination phase; and t is time. The area under the plasma concentration-time curves (AUC) were calculated by the method of trapezoids. The elimination rate constant \( (K_{10}) \) of flunixin was calculated with the following equation:

\[ K_{10} = \frac{1}{(A_1/\alpha + A_2/\beta)} \]

From the data, the distribution half-life \( (t_{1/2d}) \), elimination half-life \( (t_{1/2e}) \), mean residence time (MRT), the apparent volume of distribution at steady state \( (V_{ss}) \), and total body clearance of drug from the plasma \( (Cl_{tot}) \) were estimated.

Data are expressed as the mean ± standard deviation. The distribution and elimination half-lives were calculated as harmonic means and the statistical significance of differences was evaluated with the Mann-Whitney U test. The other pharmacokinetic and biochemical parameters were analyzed with the unpaired t-test. All of the statistical analyses were performed using SPSS software (SPSS Inc., Chicago, IL, USA) with a chosen probability level of P<0.05.
After the treatment of rats with CCl₄ or glycerol, the induction of acute hepatic or renal failure was evaluated through biochemical examination. CCl₄-treated rats showed higher levels of AST (3264.3 ± 224.6 vs. 66.2 ± 9.2 IU/l) and ALT (214.3 ± 45.2 vs. 21.4 ± 5.2 IU/dl) than those of olive oil-treated rats (P<0.05), whereas the levels of creatinine and BUN between control and CCl₄-treated rats did not show significant differences. The levels of BUN (83.2 ± 4.9 vs. 19.4 ± 1.6 mg/dl) and creatinine (1.55 ± 0.14 vs. 0.32 ± 0.04 mg/dl) were significantly increased in the glycerol-treated rats (P<0.05). There were no differences of AST and ALT between saline treatment and glycerol treatment. In pharmacokinetic studies, CCl₄ and glycerol treatments are widely used for the induction of acute hepatic and renal failure in rats. CCl₄ treatment leads to lipid peroxidation of the hepatocyte membrane and centrilobular necrosis of the liver via the generation of free radical species [13]. The injection of hypertonic glycerol causes nephrotoxicity through renal vasoconstriction, tubular obstruction and direct heme-protein induced cytotoxicity. In the present study, CCl₄ treatment induced apparent liver dysfunction without renal damage, and glycerol treatment induced marked renal dysfunction with little liver injury. These results are mostly consistent with the previous studies [5, 10]. Therefore, our results confirm that CCl₄ and glycerol treatment induce selective hepatic and renal dysfunction.

The plasma flunixin concentration-time profile was first reported in rats with or without acute hepatic and renal failure. The plasma concentrations profiles of flunixin after single intravenous administration at a dose of 2 mg/kg are shown in Fig. 1. The disposition curves were best fitted by a bi-exponential equation. In CCl₄- and glycerol-treated rats, plasma concentrations of flunixin during all experimental periods after intravenous administration were higher than those of the respective controls. The pharmacokinetic parameters of flunixin after a single intravenous injection are summarized in Table 1.

After i.v. administration of flunixin, the mean elimination half-life (t½) of the drug was 1.60 and 1.78 h in the olive oil- or saline-treated rats, respectively. In the respective control animals, the apparent steady-state volume of distribution (Vss) was 0.44 ± 0.08 l/kg after olive oil-treatment and 0.46 ± 0.06 l/kg after saline treatment. The mean total body clearance (Cltot) was 0.31 ± 0.02 and 0.29 ± 0.06 l/kg/h in olive oil- and saline-treated rats, respectively. The CCl₄ treatment increased the AUC up to approximately 2-fold, compared with respective control (14.81 ± 3.45 vs. 6.57 ± 0.82 µg·h/ml; P<0.05). In CCl₄-treated rats, the half-life was significantly prolonged to 1.6-fold (2.56 ± 0.36 vs. 1.60 ± 0.15 h; P<0.05) and Cltot was reduced by about 50% (0.14 ± 0.04 vs. 0.31 ± 0.02 l/kg/h; P<0.05). Similar to CCl₄ treatment, glycerol treatment led to a markedly increased

![Fig. 1. Plasma flunixin concentration-time profiles after a single intravenous administration (2 mg/kg) in rats (n=5) treated with olive oil, carbon tetrachloride (CCl₄), saline or glycerol. Open circles (○), olive oil-treated animals; solid circles (●), CCl₄-treated animals; open squares (□), saline-treated animals; solid squares (◇), glycerol-treated animals. *P<0.05, olive oil treatment versus CCl₄ treatment or saline treatment versus CCl₄ treatment.](image-url)
AUC (13.38 ± 2.41 vs. 6.95 ± 0.69 µg·h/ml; P<0.05), prolonged t\textsubscript{1/2β} (2.16 ± 0.49 vs. 1.78 ± 0.23 h; P<0.05) and reduced Cl\textsubscript{tot} (0.15 ± 0.04 vs. 0.29 ± 0.06 l/kg/h; P<0.05) compared with the respective control.

The disposition and elimination of flunixin are related with at least two active transporters, including organic anion transporter polypeptide (OATP)-2 in the liver and renal active transporter in the kidney. After a single intramuscular administration of \textsuperscript{14}C-flunixin (10 mg/kg) in rats, fecal and urinary excretions accounted for 61 and 29% of the dose, respectively [4]. Miyazaki et al. [16] reported that in rabbits hepatic OATP-2 and tubular secretion were involved in the distribution and total body clearance of flunixin as well as the renal excretion. The profiles of disposition and elimination of flunixin in cats are similar to those reported in rabbits [9]. In horses, the renal clearance of flunixin is much higher than the glomerular filtration rate, implying an active transporter pathway in the kidney [25]. Hepatic failure as well as renal failure results in different expressions of active transporters in the liver. Acute hepatic failure induced by CCl\textsubscript{4} led to the down-regulation of mRNA expression of OATP-2 in relation to disease severity [8, 21]. The expression of OATP-2 in the liver was decreased by chronic renal failure, which may indicate a decrease in drug elimination [17]. In the present study, both hepatic and renal failure changed the profile of flunixin pharmacokinetics as seen in the increased AUC, prolonged half-life, and decreased total body clearance in Fig. 1 and Table 1. These findings suggest that both hepatic and renal failure can affect the pharmacokinetic parameters of flunixin in relation to active transporters.

Although flunixin is a relatively safe NSAID, several researchers have reported its adverse effects. Flunixin in combination with endotoxin resulted in gastric ulceration at a dose of 1.1 mg/kg in dogs [26]. Macallister et al. [15] reported that administration of flunixin (1.1 mg/kg) for 16 days caused renal crest necrosis in horses. In cats, ALT after the administration of flunixin (1 mg/kg) for 7 days increased from 11.4 to 21.3 IU/l [14, 29]. In goats, the intravenous administration of flunixin (2.2 mg/kg) for 12 days twice a day resulted in the increases of AST, ALP, ALT, and creatinine, and the hydropic degeneration of hepatocytes [24]. These results suggest that repeated administration of flunixin can lead to potential hepatic and renal toxicity. In the present study, the excretion of flunixin was decreased in both hepatic and renal dysfunction. Flunixin could cause adverse effects via the reduction of drug elimination in patients with hepatic and/or renal diseases.

In conclusion, acute hepatic and renal failure in rats not only increased the plasma concentration of flunixin but also prolonged its half-life. Further studies should investigate the effects of the hepatic and renal failure on the pharmacokinetics of flunixin in various other species.

### Table 1. Pharmacokinetic parameters of flunixin after a single intravenous administration (2 mg/kg) in rats treated with olive oil, carbon tetrachloride (CCl\textsubscript{4}), saline or glycerol

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Acute hepatic failure</th>
<th>Acute renal failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>K\textsubscript{10} (h\textsuperscript{-1})</td>
<td>1.27 ± 0.34</td>
<td>1.69 ± 0.35</td>
</tr>
<tr>
<td>t\textsubscript{1/2α} (h)</td>
<td>0.21 ± 0.04</td>
<td>0.22 ± 0.07</td>
</tr>
<tr>
<td>t\textsubscript{1/2β} (h)</td>
<td>1.60 ± 0.15</td>
<td>2.56 ± 0.36*</td>
</tr>
<tr>
<td>AUC\textsubscript{0→∞} (µg·h/ml)</td>
<td>6.57 ± 0.82</td>
<td>14.81 ± 3.45*</td>
</tr>
<tr>
<td>Cl\textsubscript{int} (l/h/kg)</td>
<td>0.31 ± 0.02</td>
<td>0.14 ± 0.04*</td>
</tr>
<tr>
<td>V\textsubscript{ss} (l/kg)</td>
<td>0.44 ± 0.08</td>
<td>0.35 ± 0.08</td>
</tr>
<tr>
<td>AUMC (µg·h\textsuperscript{2}/ml)</td>
<td>9.71 ± 2.46</td>
<td>39.36 ± 4.92*</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>1.46 ± 0.31</td>
<td>2.62 ± 0.69</td>
</tr>
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</table>

The values are expressed as mean ± SD (n=5). K\textsubscript{10}, elimination rate constant; t\textsubscript{1/2α}, the distribution half-life; t\textsubscript{1/2β}, the elimination half-life; AUC, the area under the plasma concentration-time curve from zero to infinity; Cl\textsubscript{int}, the total body clearance of drug from the plasma; V\textsubscript{ss}, the apparent volume of distribution at steady state; AUMC, area under the first moment curve; MRT, mean residence time. *P<0.05, olive oil treatment versus CCl\textsubscript{4} treatment or saline treatment versus glycerol treatment.

In the present study, both hepatic and renal failure changed the profile of flunixin pharmacokinetics as seen in the increased AUC, prolonged half-life, and decreased total body clearance in Fig. 1 and Table 1. These findings suggest that both hepatic and renal failure can affect the pharmacokinetic parameters of flunixin in relation to active transporters.
References