Changes in Digoxin Pharmacokinetics Treated with Lipopolysaccharide in Wistar Rats

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Lipopolysaccharide (LPS) is a highly bioactive substance that can cause local as well as systemic damage to various organs of both humans and animals, even at very low doses. However, there are a few reports on drug pharmacokinetics during endotoxemia. In this study, we analyzed the pharmacokinetics of digoxin (a therapeutic agent for cardiac insufficiency) as a probe drug for a two-compartment model in a rat model of endotoxemia induced by LPS for 5 d. Digoxin was given to Wistar rats intravenously (i.v.), orally (p.o.), and intra-intestinally using an in situ closed-loop method (loop). The AUC∞ was significantly increased in the LPS (+) group throughout the experiment (p<0.05). There was significant decrease in V2 (volume of distribution of tissue compartment) on Day 1—3 (p<0.05). On Day 1—2 after LPS administration, the AUC∞ was significantly increased in the LPS (+) group (p<0.05). The AUC/LP was significantly increased throughout the experiment (p<0.05). The elimination rate constant was unchanged. Thus LPS administration affected the absorption but not the excretion of digoxin. The findings of this study suggest that digoxin absorption increased and the volume of distribution of tissue compartment decreased after LPS administration (5 mg/kg, i.p.). It appears that digoxin pharmacokinetics recover over 3 d after LPS administration.

Key words lipopolysaccharide; digoxin; absorption; distribution

It has been proposed that endotoxia is a systemic inflammatory response syndrome (SIRS) that is induced by infection.1) The pathophysiology of sepsis is characterized by hypercytokinemia.2—4) Many reports have described sepsis due to Gram-negative bacterial infections.5—11)

Composed of polysaccharides, fatty acids, and phosphoric acids, LPS (lipopolysaccharide) is the principal structural component of the outer membrane of Gram-negative bacteria. LPS is a highly bioactive substance and can damage multiple organs at very low doses. LPS facilitates the production and release of such cytokines as tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β). LPS induces various local and systemic pathological reactions, and blood-clotting abnormalities such as disseminated intravascular coagulation (DIC).12) In addition, changes in drug pharmacokinetics during sepsis have been reported.13—15) However, there are few reports about the process of recovery of drug pharmacokinetics during sepsis.16)

In this study, we analyzed the pharmacokinetics of digoxin (a therapeutic agent for cardiac insufficiency) in a rat model of endotoxemia induced by LPS for 5 d. Digoxin was used as a probe drug for a two-compartment model.

MATERIALS AND METHODS

Materials LPS from Escherichia coli (O55: B5, Lot No. 064K4010) and digoxin were purchased from Sigma-Aldrich Co. (St. Louis, MO, U.S.A.). Other reagents were commercially available, extra-pure grade chemicals.

Subjects Male Wistar ST rats (8—9 weeks of age, weighing 260—300 g) were purchased from Japan SLC (Hamamatsu, Japan). Animals were reared under normal environmental conditions (24±1°C, humidity 55±10%, and illumination 6:00 to 18:00) and allowed to drink tap water and eat solid feed (NMF, Oriental Yeast Co., Ltd., Tokyo, Japan) ad libitum. Animals were acclimated for at least 1 week before use in experiments. Experiments were conducted in accordance with the animal experiment guidelines of Osaka University of Pharmaceutical Sciences.

Preparation and Administration of LPS LPS (2 mg/ml) was dissolved in sterile distilled water and administered intraperitoneally (i.p.) once at 2.5 ml/kg to each rat in the LPS (+) group. Physiological saline (2.5 ml/kg, i.p.) was administered to control rats (control group).

Measurement of Body Temperature and Blood Pressure Body temperature was measured by inserting a thermometer directly into the rectum just before and 3, 6, 9, and 12 h after LPS administration. At the same times, blood pressure was measured using a tail cuff method.

Measurement of Plasma Cytokines Blood samples (0.5 ml) were collected from the cervical vein under anesthesia with diethyl ether just before and 3, 6, 9, and 12 h after LPS administration. Blood samples were transferred to a tube containing 5 units of heparin and centrifuged (600×g, 15 min). Resultant plasma was obtained and stored at 4°C until analysis. TNF-α and IL-1β were measured in each plasma sample using ELISA kits (Quantikine® TNF-α, IL-1β, R&D Systems, Minneapolis, MN, U.S.A.).

Digoxin Pharmacokinetics after Intravenous Administration Digoxin solution (0.1 mg/ml) for administration was prepared in physiological saline and administered at a dose of 0.1 mg/kg. Rats were fasted overnight before oral ad-
ministration of digoxin but had free access to water. Digoxin was administered intravenously after physiological saline (2.5 ml/kg, i.p.) administration in the control group and was administered intravenously on Day 1—3, and Day 5 after LPS (5 mg/kg, i.p.) administration in the LPS (+) group. Blood samples (0.5 ml) were collected from the cervical vein under anesthesia with diethyl ether just before and 2 min, 5 min, 10 min, 30 min, 1 h, 2 h, 4 h, and 6 h after digoxin administration. Blood samples were transferred to a tube containing 5 units of heparin and centrifuged (600 × g, 15 min). Resultant plasma was obtained and stored at 4 °C until analysis.

**Digoxin Pharmacokinetics after Oral Administration**

Digoxin solution (0.1 mg/ml) for administration was prepared in physiological saline and administered at a dose of 0.1 mg/kg. In the control group, digoxin was administered orally after physiological saline (2.5 ml/kg, i.p.) administration. In the LPS (+) group, digoxin was given orally on Day 1—3, and Day 5 after LPS (5 mg/kg, i.p.) administration. Blood samples (0.5 ml) were collected from the cervical vein under anesthesia with diethyl ether just before and 1, 2, 4, 6, 8, 12, and 24 h after digoxin administration. Plasma was obtained by the method described in the section on intravenous administration.

**Absorption from the Intestinal Loop**

A closed-loop for drug administration was inserted through the proximal end and ligated with thread over the tissue to produce a closed intestinal loop. Digoxin solution (50 μg/ml) for administration was prepared in physiological saline, and 1 ml of the solution was administered. After the administration of digoxin, the needle was removed and the ligation was secured. Blood samples (0.2 ml) were collected from the portal vein at 30 min, 45 min, 1 h, 2 h, and 4 h after digoxin administration. Plasma was obtained by the method described in the section on intravenous administration.

**Measurement of Digoxin**

Deproteinizing agent (200 μl, Abbott Laboratories, Abbott Park, IL, U.S.A.) was added to 200 μl of each plasma sample, mixed with a vortex mixer for 30 s, and centrifuged (600 × g, 5 min). Digoxin was assayed using a fluorescence polarization immunoassay (FPIA: TDx View, CA, U.S.A.). With the values of body temperature and blood pressure remaining almost constant (39.1 ± 0.6 °C and 80.6 ± 16.9 mmHg, respectively) throughout the period of experimentation in the control group. In the LPS (+) group, body temperature and blood pressure decreased markedly (37.1 ± 0.6 °C and 80.6 ± 16.9 mmHg, respectively) at 3 h after LPS administration (p<0.05) and tended to return to baseline thereafter. There were no significant between-group differences in temperature (Fig. 1a) or blood pressure (Fig. 1b) at 6, 9, and 12 h after LPS administration.

**Pharmacokinetic Analysis**

The plasma concentration (Cp) versus time (t) profiles of digoxin were analyzed by a two-compartment model with first-order absorption, where the plasma concentrations after intravenous administration (without absorption) and after oral administration (with absorption) are described by Eqs. 1 and 2, respectively, as follows:

\[ Cp = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t} \]  
\[ Cp = L \cdot e^{-\lambda t} + M \cdot e^{-\beta t} - N \cdot e^{-\kappa t} \]  

where

\[ L = \frac{A \cdot F \cdot K_a}{K_s - \alpha} \]  
\[ M = \frac{B \cdot F \cdot K_a}{K_s - \beta} \]  
\[ N = L + M \]

and where A, B, α, and β are constants and K_s and F are the absorption rate constant and bioavailability, respectively. The values of A, B, α, and β were estimated by fitting Eq. 1 to the concentration versus time profiles after intravenous administration using a computer program for nonlinear regressions, WinNonlin Professional Version 5.2 (Pharsight Co., Mountain View, CA, U.S.A.). With the values of A, B, α, and β fixed, the values of K_s were estimated by fitting Eq. 2 to the concentration versus time profiles after oral administration. The model-based values of the area under the concentration curve (AUC), the total body clearance (CL), the volume of distribution of central compartment (V_1), and the volume of distribution of tissue compartment (V_2) were calculated as follows.

\[ AUC_{iv} = \frac{A}{\alpha + \beta} \]  
\[ AUC_{po} = \frac{L}{\alpha + \beta} - \frac{N}{K_s} \]  
\[ CL = \frac{Dose}{AUC_{iv}} \]  
\[ V_1 = \frac{Dose}{A + B} \]  
\[ V_2 = \frac{AB(\alpha - \beta)\gamma}{(\beta + B\alpha)\gamma} \times V_1 \]

where AUC_{iv} and AUC_{po} are those for intravenous and oral administration, respectively.

**Statistical Analysis**

Results are shown as mean±S.D. AUC_{int} was calculated using a trapezoidal method. Student’s t-test or Dunnett’s test was used for statistical analyses. p<0.05 was statistically significant.

**RESULTS**

**Changes in Body Temperature and Blood Pressure**

Body temperature and blood pressure remained almost constant (39.1±0.5 °C, 90.4±3.56 mmHg) throughout the period of experimentation in the control group. In the LPS (+) group, body temperature and blood pressure decreased markedly (37.1±0.6 °C and 80.6±16.9 mmHg, respectively) at 3 h after LPS administration (p<0.05) and tended to return to baseline thereafter. There were no significant between-group differences in temperature (Fig. 1a) or blood pressure (Fig. 1b) at 6, 9, and 12 h after LPS administration.

**Changes in Plasma TNF-α and IL-1β Concentrations**

Plasma TNF-α and IL-1β increased significantly in the LPS (+) group (p<0.05), peaking at 3 and 6 h, respectively, after
LPS administration. There were no significant between-group differences in plasma TNF-α level at 9 and 12 h after LPS treatment (Fig. 2a) and no between-group differences in plasma IL-1β at 12 h after LPS treatment (Fig. 2b).

Disposition after Intravenous Administration The concentration profiles were well described by the two-compartment model (Fig. 3), and pharmacokinetic parameters are summarized in Table 1. Compared with the control group, $AUC_{i.v.}$ was significantly increased in the LPS (+) group ($p<0.05$ on Day 1—3; $p<0.01$ on Day 5). There were no significant between-group differences in $V_1$ but significant differences in $V_2$ on Day 1—3 ($p<0.05$).

Digoxin Pharmacokinetics after Oral Administration The concentration profiles were well described by the two-compartment model with first-order absorption (Fig. 4), and pharmacokinetic parameters are summarized in Table 1. Compared with the control group, there were no between-group differences in $K_a$ though $AUC_{p.o.}$ increased significantly in the LPS (+) group ($p<0.05$ on Day 1—2).

Absorption from the Intestinal Loop Compared with the control group, $AUC_{loop}$ was significantly increased ($p<0.05$ on Day 2—3, and Day 5; $p<0.01$ on Day 1). $C_{max}$ was significantly increased in the LPS (+) group on Day 1—2 ($p<0.01$) (Fig. 5, Table 2).

DISCUSSION

This study used LPS from *E. coli* as an inducer of endotoxemia. Though LPS (5 mg/kg, i.p.) was able to induce endotoxemia in all rats, all rats survived in this study. This dose (the equivalent of $10^9$ bacterial cells) is thought to be sufficient to cause severe SIRS in humans.

In this study, body temperature and blood pressure decreased until 3 h after LPS administration and gradually returned to pre-administration levels by 12 h (Fig. 1). These results were similar to those previously reported.\(^{18}\) It has been...
reported that LPS stimulates monocytes/macrophages, activates NF-kB in these cells, and subsequently stimulates the production of pro-inflammatory cytokines such as TNF-α and IL-1β.\textsuperscript{19–21} In the present study, serum TNF-α and IL-1β increased transiently at 3 and 6 h, respectively, after LPS administration, and then decreased to pre-administration levels by 12 h after LPS administration (Fig. 2). In the control group, serum IL-1β was slightly increased at 9 h. The increase of IL-1β may have been due to repeated blood collection with a needle. This difference between TNF-α and IL-1β has been previously reported\textsuperscript{22} and may indicate that LPS induced TNF-α before IL-1β induction via a TNF-α-mediated pathway. Since these findings were similar to those in previous reports of endotoxemia,\textsuperscript{2,18} a model of reversible and transient endotoxemia was successfully established in Wistar rats using LPS (5 mg/kg, i.p.).

The pharmacokinetics of digoxin in pathophysiological conditions were examined in this model. Digoxin is a cardiac glycoside widely used as a therapeutic agent for tachycardiac conditions were examined in this model. Digoxin is a cardiac glycoside widely used as a therapeutic agent for tachycardiac conditions in pathophysiological conditions (such as atrial fibrillation) and congestive heart failure.\textsuperscript{23} In this study, digoxin was used as a probe drug for a two-compartment model, and administered

Table 1. Pharmacokinetic Parameters of Digoxin in Rats after Intravenous and Oral Administration

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>LPS (5 mg/kg, i.p.)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 2</td>
</tr>
<tr>
<td>A (ng/ml)</td>
<td>164.3±24.5</td>
<td>120.9±4.0</td>
</tr>
<tr>
<td>B (ng/ml)</td>
<td>9.3 ±3.6</td>
<td>19.5±3.6*</td>
</tr>
<tr>
<td>α (h(^{-1}))</td>
<td>25.3±8.5</td>
<td>25.7±2.6</td>
</tr>
<tr>
<td>β (h(^{-1}))</td>
<td>0.307±0.156</td>
<td>0.305±0.026</td>
</tr>
<tr>
<td>K(_{a}) (h(^{-1}))</td>
<td>1.77±1.45</td>
<td>0.91±0.28</td>
</tr>
<tr>
<td>AUC(_{0-24}) (ng·h/ml)</td>
<td>39.5±8.9</td>
<td>69.1±14.1**</td>
</tr>
<tr>
<td>CL (l/h/kg)</td>
<td>2.63±0.66</td>
<td>1.49±0.33**</td>
</tr>
<tr>
<td>AUC(_{0-24}) (ng·h/ml)</td>
<td>42.7±7.0</td>
<td>75.4±20.9**</td>
</tr>
<tr>
<td>V(_{1}) (l/kg)</td>
<td>0.58±0.100</td>
<td>0.71±0.035</td>
</tr>
<tr>
<td>V(_{2}) (l/kg)</td>
<td>7.63±3.59</td>
<td>3.81±0.63*</td>
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Table 2. Pharmacokinetic Parameters of Digoxin after Administration into the Jejunal Loop

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>LPS (5 mg/kg, i.p.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 2</td>
</tr>
<tr>
<td>AUC(_{0-24}) (ng·h/ml)</td>
<td>15.0±5.33</td>
<td>45.1±11.0**</td>
</tr>
<tr>
<td>C(_{\text{max}}) (ng/ml)</td>
<td>14.2±4.96</td>
<td>42.3±16.2**</td>
</tr>
</tbody>
</table>

Fig. 4. Plasma Concentrations of Digoxin in Lipopolysaccharide (LPS)-Treated Rats

Digoxin was given orally on Day 1–3, and Day 5 after LPS (5 mg/kg, i.p.) administration. Results are shown as means±S.D. (n=4–6); control group; ○, Day 1 after LPS administration; ▲, Day 2 after LPS administration; ●, Day 3 after LPS administration; ■, Day 5 after LPS administration. Lines represent computer-fitted profiles.

Fig. 5. Plasma Concentrations of Digoxin in the Portal Vein after Administration into the Intestinal Loop in Lipopolysaccharide (LPS)-Treated Rats

Rats were administered with digoxin (50 μg [=39 μg/ml]) into the jejunal loop on 1–5 d after LPS administration. Control is no LPS treated group. LPS, lipopolysaccharide; AUC, area under the plasma concentration–time curve; CL, total body clearance; V\(_{1}\), volume of distribution of central compartment; V\(_{2}\), volume of distribution of tissue compartment; LPS, lipopolysaccharide; i.p., intraperitoneally. Data are shown as means±S.D. (n=3–6); *p<0.05 and **p<0.01, compared with Control.
digoxin intravenously. The $AUC_{1\rightarrow\infty}$ increased significantly, and $CL$ decreased significantly throughout the experiment after LPS administration (Fig. 3, Table 1). Although there were no significant between-group differences in $V_2$, there were significant decreases in $V_2$ on Day 1—3 after LPS administration. However, $\beta$ (elimination rate constant) remained relatively unchanged throughout the experiment, even after LPS treatment. The pharmacokinetics of digoxin is well described by a two-compartment model, and digoxin has a large $V_2$ (high tissue distribution). It appears that increase of $AUC_{1\rightarrow\infty}$ is due to decrease of $V_2$.

After oral administration of digoxin, the $AUC_{p.o.}$ of digoxin in the LPS (+) group increased significantly on Day 1—2 after LPS administration (Fig. 4, Table 1). However, $\beta$ remained relatively unchanged throughout the experiment, even after LPS treatment. These findings suggested that the amount of digoxin absorption increases and the volume of distribution decreases.

Then we examined gastrointestinal permeability using an in situ closed-loop method. Plasma digoxin level increased significantly and $AUC_{\text{loop}}$ increased throughout the experiment after LPS administration, suggesting that increase in digoxin absorption by the small intestine plays an important role in the above increases (Fig. 5, Table 2). In rats, cytochrome P450 (CYP) 3A and P-glycoprotein (P-gp) in intestine are involved in digoxin absorption and metabolism. Decreases in intestinal CYP3A2 and P-gp may accompany these changes. In the pathologic state induced by LPS (5 mg/kg, i.p.), the excretion phase was not affected, but the absorption phase was greatly affected.

In conclusion, the present study showed that digoxin absorption was increased and the volume of distribution of the tissue compartment decreased after LPS administration (5 mg/kg, i.p.). It appears that digoxin pharmacokinetics recover over 3 d after LPS administration.

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REFERENCES