Pharmacokinetics of Theophylline: Effects of Hepatic Fibrosis in Rats Induced by Bile Duct Ligation

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This experiment was performed to evaluate the usefulness of an experimental fibrosis model by bile duct ligation as a pharmacokinetic model of a disease state. First, experimental liver fibrosis was produced by bile duct ligation. At 4 weeks postoperation, a fibrotic condition was characterized by measurement of the aminoterminal procollagen type III peptide (PIIINP) level in serum, total collagen content in liver and light microscopic histology.

Four weeks after bile duct ligation there was an increase in total collagen content of the liver to 430% of the initial values, accompanied by an increase of serum-PIIINP (385%). Secondly, we examined the pharmacokinetics of theophylline in the fibrotic rat induced by bile duct ligation. An i.v. dose of 8 mg of theophylline per kg of body weight was administered, and the levels of theophylline in serum were assayed by high performance liquid chromatography. The area under the serum concentration–time curve of theophylline was increased significantly in fibrotic rats compared with that of the control, and the total clearance of drug in fibrotic rats was low, averaging 22.6 mg/kg/h vs. 36.1 and 60.9 ml/kg/h in the control and the normal rat, respectively. However, the value of distribution during the β-phase was not significantly affected by experimental liver fibrosis.

Key words liver fibrosis; bile duct ligation; aminoterminal procollagen type III peptide; pharmacokinetics; theophylline; rat

Liver cirrhosis is the one of most common causes of death and results from various liver injuries such as viral infection, ethanol abuse, cholestatic disease and other conditions. Because of the high morbidity and mortality related to cirrhosis/fibrosis, a reliable experimental animal model is difficult to find for understanding and overcoming this disease. Multiple doses of carbon tetrachloride have been chosen in the past for the convenience and ease of producing cirrhosis/fibrosis, though several problems have repeatedly arisen: i) variable responsiveness in individual animals, ii) high mortality iii) unclear validity for fibrosis/cirrhosis, iv) a longer period, 10–12 weeks, required to produce cirrhosis/fibrosis.2) Therefore bile duct ligation was used to produce liver cirrhosis/fibrosis because of its morphological compatibility to human and its high yield of cirrhosis/fibrosis.3) The acceptance of liver cirrhosis/fibrosis induced by bile duct ligation was validated by measuring the aminoterminal procollagen peptide type III (PIIINP), which is reported to be a reliable ongoing marker and superior to the conventional serum parameters such as serum glutamic-oxaloacetic transaminase (sGOT), serum glutamic-pyruvic transaminase (sGPT) and alkaline phosphatase (ALP), etc.4,5)

Hepatic disease is well known to change the pharmacokinetics of a number of drugs. Various effects from hepatic disease make prediction of the pharmacokinetics of drugs difficult. We examined theophylline, which is eliminated extensively by liver and has a narrow therapeutic range. The aim of this study is to evaluate the usefulness of an experimental fibrosis model by bile duct ligation as a pharmacokinetic model in a disease state.

MATERIALS AND METHODS

Animals Female Sprague-Dawley rats, weighing 230–280 g, were used throughout this study. Animals were housed in a 12 h light–dark, constant temperature environment for 1 week prior to study with free access to drinking water and commercial pellet diet (Jeil Combination Feed Co., Seoul, Korea). All rats were fasted for 24 h prior to and during the experiment, although water was available ad libitum.

Chemicals NaCl and goat anti-rabbit IgG antiserum were obtained from Amersham Co., and theophylline was obtained from Wako Chemical Co. (Japan). β-Hydroxyethyltheophylline was used as an internal standard and was obtained from Sigma Chemical Co. Other chemicals were generally available or purchased from Merck Co.

Experimental Protocols Two series of experiments were carried out. (i) In the first, experimental liver fibrosis was produced in rats under anesthesia (ketamine: 20 mg/kg, Rompun®: 10 mg/kg) by ligation of the common bile duct, as was done previously.3) The animals were sacrificed in groups of six each week for 8 weeks and characterized by measurement of clinical biochemical parameters (GOT, GPT, ALP, cholesterol and bilirubin) in serum with a commercial kit (Gilford Co., U.S.A.); PIIINP levels in serum and total collagen content in liver. Serum PIIINP levels were measured by a radioimmunoassay method6) (with a gamma counter, autogamma 5500, Packard, Downers Grove, IL, U.S.A.). To determine the total collagen content, total hydroxyproline content in the liver was determined according to the methods described by Jamall et al.7) The liver was fixed in formalin and embedded in paraffin sections of 2 μm thickness, which were then stained with hematoxylin and eosin and examined by microscopy. (ii) In the second experiment, to access the usefulness of an experimental fibrosis model by the bile duct ligation as a pharmacokinetic model of a disease state, a pharmacokinetic experiment using theophylline (as a model drug) in experimental fibrotic rats was performed. Rats were divided into 3 groups: 1) normal, 2) sham-operation group as the control, 3) experimental liver fibrotic group induced by bile duct ligation.

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ligation. The i.v. dose was administered in the upper part of the tail vein in lightly anesthetized rats as aminophylline. Blood samples (about 70 μl) were obtained from the tail vein at 10, 30, 45, 60, 90, 120, 240, 480 and 720 min after administration, and serum was obtained by centrifugation at 3000 rpm for 10 min, the serum was then frozen until assayed.

Theophylline Assay Serum theophylline was quantitated by high-pressure liquid chromatography. Briefly, serum was mixed with an equal volume of acetonitrile containing the internal standard, β-hydroxyethyltheophylline, to precipitate serum proteins. After centrifugation, a 20 μl of sample was injected. The HPLC conditions are as follows: UV absorbance detector, 254 nm; column, μ-Bondapak C18; mobile phase, acetonitrile: 10 mM acetate buffer (pH 4.0); flow rate, 2.0 ml/min; sensitivity, 0.005 a.u.f.s.

Pharmacokinetic Analysis The pharmacokinetics of theophylline could be described by a two-compartment open model. The serum data was fitted to the equation of the model with the aid of the nonlinear regression program MULT. The area under the serum concentration–time curve (AUC) was calculated by the trapezoidal rule. The area from the last data point (Ct) to infinite time was estimated by the quotient of Ct and the beta disposition constant (β).

Statistical Analysis Data are expressed in the mean ± standard error of the mean. For the detection of significant differences, the Student’s t-test was used. A p value of less than 0.05 was considered significant.

RESULTS

During the postoperative period, both the sham-operated and bile duct ligated groups showed a slight increase in body weight, of 5.8% and 9.0%, respectively, and there was no significant difference in body weight between the two groups. A marked increase in liver weights was observed in the bile duct ligated group (7.03 g/100 g body weight) compared with the sham-operated group (3.45 g/100 g body weight). There was a significant difference between two groups in serum bilirubin, the activities of sGOT, sGPT, and ALP, the PIINP in serum, as well as in the hydroxyproline content of the liver (Table I, Fig. 1). The elevation of these biochemical parameters are the major characteristics of biliary liver fibrosis. Proliferation of the biliary duct was prominent, and degeneration and necrosis of hepatocytes were observed by a light microscope (Fig. 2).

The level of PIINP in serum shows two maxima, at 2 and 4 weeks, respectively, and tended to decline after 4 weeks. This phenomenon was also seen in the report published by Boeker et al. A good correlation between PIINP level in serum and the degree of liver fibrosis has been substantiated by increased hydroxyproline content in the liver and by histological data. Accordingly, it might be proposed that liver fibrosis was sufficiently induced at 4 weeks after bile duct ligation, though the reason why the value of PIINP at 2 weeks shows a sharp increase cannot be explained at this moment.

Fig. 1. The Serum Level of PIINP (A) and Hydroxyproline Content in Liver (B) after Bile Duct Ligation

Mean values ± S.E., for n see Table 1.

<table>
<thead>
<tr>
<th>Weeks</th>
<th>n</th>
<th>sGOT (U/l)</th>
<th>sGPT (U/l)</th>
<th>ALP (U/dl)</th>
<th>t-Bili (mg/dl)</th>
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<tr>
<td>0</td>
<td>6</td>
<td>58 ± 3.7</td>
<td>70 ± 2.4</td>
<td>17 ± 1.6</td>
<td>0.28 ± 0.03</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>179 ± 10.8**</td>
<td>117 ± 8.8**</td>
<td>46 ± 3.2**</td>
<td>9.6 ± 0.87**</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>294 ± 24.9**</td>
<td>121 ± 9.9**</td>
<td>30 ± 4.1**</td>
<td>7.5 ± 0.57**</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>129 ± 17.9**</td>
<td>72 ± 8.9**</td>
<td>24 ± 3.6</td>
<td>4.44 ± 0.66**</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>84 ± 16</td>
<td>44 ± 6.5*</td>
<td>20 ± 3.5</td>
<td>1.67 ± 0.76*</td>
</tr>
</tbody>
</table>

Each data represent the mean ± S.E. The significance of differences as compared with the control group. * p < 0.05, ** p < 0.01. n: number of rats. sGOT: serum glutamic-oxaloacetic transaminase. sGPT: serum glutamic-pyruvic transaminase. ALP: alkaline phosphatase. t-Bili: total bilirubin.

<table>
<thead>
<tr>
<th>Groups parameters</th>
<th>Normal group</th>
<th>Sham operation group</th>
<th>Bile duct ligation group</th>
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<tr>
<td>VDP (ml/kg)</td>
<td>522.6 ± 60.6</td>
<td>506.6 ± 106.5</td>
<td>434.3 ± 82.2</td>
</tr>
<tr>
<td>AUC (μg·h/ml)</td>
<td>157.4 ± 36.9</td>
<td>223.8 ± 13.5</td>
<td>367.7 ± 38.5*</td>
</tr>
<tr>
<td>CL (ml/h/kg)</td>
<td>60.9 ± 15.0</td>
<td>36.1 ± 2.0</td>
<td>22.6 ± 2.7</td>
</tr>
<tr>
<td>t1/2 (h)</td>
<td>6.56 ± 1.11</td>
<td>8.30 ± 1.79</td>
<td>17.55 ± 5.53*</td>
</tr>
</tbody>
</table>

Theophylline was administered intravenously at a dose of 8 mg/kg. Each value represents the mean ± S.E. from 4 rats. a) p < 0.05, between normal, sham operation groups and bile duct ligation group. b) p < 0.05, between normal group and bile duct ligation group.

The retention times of theophylline and the internal standard were 8.8 and 10.8 min, respectively. No interfering peak appeared on the chromatogram of serum from the control rats. Figure 3 shows the curves of theophylline serum levels in normal, sham-operated, and bile duct
ligated groups, when aminophylline was administered at a dose of 10 mg/kg. There was no difference between the normal and sham-operated groups. However, the theophylline serum levels in the bile duct ligated group were markedly higher than in the normal and sham operated control groups.

The pharmacokinetic parameters are summarized in Table II. The duration of the distribution phase was reduced in the experimental fibrotic rats compared with sham-operated rats. The $AUC$ value was significantly greater in the experimental fibrotic rats compared with the normal and sham-operated rats. A significant difference was found in the clearance ($Cl$) value among the normal and sham operated groups and the bile duct ligated group. The apparent volume of distribution at the $\beta$-phase ($V_{\beta}$) was decreased from 522.6 ml/kg in the normal group, 506.6 ml/kg in control to 434.3 ml/kg in experimental fibrotic rats, but the difference was not statistically significant.

**DISCUSSION**

Bile duct ligation was reported previously by Cameron et al. A new insight into this model has been generated
recently, and bile duct ligation is used frequently to study liver fibrosis. PIIINP has been regarded as a marker of fibrogenesis, since extracellular removal of the N- and C-terminal propeptides by a specific enzyme permits fibrosis of the collagen to grow via lateral alignment. In this experiment, the PIIINP level was elevated significantly in serum, the weight of rats was increased and hepatic function was lowered after the operation. According to light microscopic study, bile duct hyperplasia, newly formed ducts and forming bands around the lobules constituted the prominent characteristic of liver fibrosis.

The biotransformation of theophylline is largely attained in the liver. A significant decrease in plasma theophylline clearance might be due to hepatic cellular dysfunction, since the body clearance of drugs with a low hepatic extraction ratio is not a function of hepatic blood flow but of hepatic metabolizing enzyme activity.12,13

A serum theophylline half-life is increased three-fold in fibrotic rats because of a change in clearance. The volume of distribution at the β-phase (V_{β}) was not significantly affected by experimental fibrosis, despite lower protein binding of theophylline to plasma protein (preliminary data in our laboratory) and an increased bilirubin level which might reduce the binding of theophylline to plasma protein. Further studies should be carried out to elucidate the exact mechanism of this phenomenon. No significant change of V_{β} on theophylline is in accordance with previous clinical results.14

From the results of the present study, it was considered that the experimental fibrosis model by bile duct ligation is useful as a pharmacokinetic model in a disease state.

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REFERENCES AND NOTES

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