EFFECT OF HYPOALBUMINEMIA ON THE DISPOSITION OF THEOPHYLLINE
Comparative Study with Sprague-Dawley Rats and a Mutant Sprague-Dawley Hyperlipidemic Strain with Hypoalbuminemia

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ABSTRACT:
We demonstrated the effect of hypoalbuminemia on theophylline disposition in rats. The pharmacokinetic parameters in Sprague-Dawley rats (SDRs) were compared with those in spontaneously hyperlipidemic rats (HLRs), which had approximately one half the serum albumin concentration of the SDRs, after a single 10 mg/kg injection (iv) of theophylline. Theophylline clearance (CL) in the HLRs was increased 1.6-fold, and the AUC was decreased by 36%. Although the elimination t1/2 was not significantly different between the two types of rats, the distribution volume (Vd) was increased significantly in the HLRs, compared with the SDRs. The free theophylline concentration in the SDRs was one half of the total concentration. In contrast, the free theophylline concentration in the HLRs was approximately equal to the total concentration. The enzymatic activities and apoprotein expression levels of CYP1A were decreased significantly in the HLRs, compared with the SDRs. The total theophylline CL was increased in HLRs with hypoalbuminemia, even though they exhibited lower enzymatic activity and CYP1A expression than did the SDRs. Because the unbound fraction and Vd of theophylline in HLRs were much larger than those in SDRs, we conclude that hypoalbuminemia may contribute to an increase in the Vd and a decrease in the CL for theophylline.

Theophylline is a bronchodilator that is frequently used in the treatment of asthma and chronic obstructive pulmonary disease (Murciano et al., 1984, 1989). Because the therapeutic range of theophylline is reportedly narrow (10–20 μg/ml), therapeutic drug monitoring is essential. The theophylline dosage required to achieve therapeutic concentrations varies among subjects, largely because of differences in metabolism (Boobis et al., 1991). Another pharmacokinetic determinant is protein binding (Shaw et al., 1982; Du Souch et al., 1993). Theophylline binds mainly to albumin, with protein binding of approximately 50% (Buss et al., 1983). Hypoalbuminemia has been found to affect the protein binding of theophylline in some situations. Decreased theophylline binding and physiological hypoalbuminemia have been observed in the third trimester of pregnancy (Dean et al., 1980; Connelly et al., 1990). Patients with poor glycemic control of insulin-dependent diabetes mellitus, decreases in serum albumin levels are accompanied by increases in the free fraction of theophylline (Karrapati et al., 1995). This increased free fraction of theophylline with hypoalbuminemia implies an effect on pharmacokinetic parameters such as Vd and/or theophylline CL (Karrapati et al., 1995).

We established a mutant strain of HLRs, which were bred from SDRs (Watanabe et al., 1996; Nakura et al., 1997). Reduction of P450 cholesterol 7α-hydroxylase (CYP7A1) activity in HLRs may contribute to a decrease in cholesterol elimination, resulting in increased serum cholesterol levels (Brass et al., 1996). Because HLRs show extremely low serum albumin concentrations, compared with SDRs, we examined the effect of hypoalbuminemia on theophylline disposition using HLRs. This study provides instructive information concerning the pharmacokinetics for the theophylline treatment of patients with hypoalbuminemia.

Materials and Methods

Chemicals. Theophylline, 7-hydroxycoumarin, 3,3′-diaminobenzidine, and benzo[a]pyrene were obtained from Wako Pure Chemicals (Tokyo, Japan). Glucose-6-phosphate, glucose-6-phosphate dehydrogenase, and NADP+ were purchased from Oriental Yeast (Tokyo, Japan), and 7-ethoxycoumarin was purchased from Aldrich Chemical Co. (Milwaukee, WI).

Determination of Theophylline Pharmacokinetics. HLRs, exhibiting 3-fold higher serum cholesterol levels than do original SDRs, have been raised and bred in our laboratory for more than 60 generations. Eight-week-old male SDRs and HLRs weighing 219 to 242 g were raised in our breeding colony after brother-sister mating. These rats were maintained in air-conditioned quarters with 12-hr light/dark cycles and were given laboratory chow (CE-2; Clea Japan, Tokyo, Japan) and water ad libitum. Theophylline (10 mg/kg) dissolved in saline was injected into the tail veins of SDRs (N = 5) and HLRs (N = 5); blood samples (300 μl) were collected at 5 and 30 min and 1, 2, 3, 6, and 10 hr after injection. The serum theophylline concentrations were measured by an immunofluorescence method using a TDX® system (Dynabot, Tokyo, Japan). Pharmacokinetic parameters were calculated using noncompartmental methods. The slope of the terminal elimination phase (β) was obtained by least-squares linear regression analysis. The elimination t1/2 was...
calculated as ln 2/β. The AUC was calculated with the trapezoidal rule. The values were extrapolated to infinity by dividing the last measured plasma concentration by β. CL and Vd were calculated as $CL = \frac{dose}{AUC}$ and $V_d = CL/\beta$, respectively. For a second set of HLRs ($N = 5$) and SDRs ($N = 5$), which were used to measure the free fraction of theophylline, the same dose was administered iv and blood samples were collected at 1, 2, 3, and 6 hr after the injection. The unbound theophylline concentrations were measured after ultrafiltration using Ultrafree-MC filters (Millipore Corp., Bedford, MA). The pH values of these serum specimens varied from 7.8 to 8.0. Serum albumin concentrations were measured using a kit (Albumin B-Test Wako; Wako Pure Chemicals).

Determination of Enzymatic Activities in Liver Microsomes. Eight-week-old male SDRs and HLRs weighing 230–254 g were killed by decapitation in the morning; the livers were removed and homogenized with 3 volumes of ice-cold 1.15% potassium chloride. Liver microsomes were prepared by sequential centrifugation at 9000 $g$ for 20 min and then at 105,000 $g$ for 60 min. The amounts of microsomal protein were determined by the method of Lowry et al. (1951), using bovine serum albumin as the standard. A typical incubation mixture for the assay of ECOD and AHH activities consisted of 100 mM sodium/potassium phosphate buffer (pH 7.4), 0.05 mM EDTA, 0.5 mg of microsomal protein, an NADPH-generating system (5 mM magnesium chloride, 0.5 mM NADP$^+$, 5 mM glucose-6-phosphate, and 1 unit of glucose-6-phosphate dehydrogenase), and a substrate (0.5 mM ethoxycoumarin or 3.2 mM benzo(a)pyrene), in a final volume of 1 ml. The reaction was started by the addition of the NADPH-generating system. After a 2-min preincubation, the reaction mixture was incubated at 37°C for 10 min and 5 min for ECOD and AHH, respectively. One milliliter of 5% trichloroacetic acid (for ECOD) or 1 ml of acetone (for AHH) was added to the mixture to stop the reaction. The activity of ECOD was estimated by determination of 7-hydroxycoumarin (Nebert, 1978). The activities of both enzymes were measured spectrophotometrically (excitation/emission wavelengths of 380/460 nm and 396/518 nm for ECOD and AHH, respectively).

Immunoblot Analysis of CYP1A1/1A2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed according to the methods of Laemmli (1970) and Guengerich et al. (1982), with a 3% stacking gel and a 10% separating gel (gel size, 13 × 10 cm), and electrophoresis was conducted at 30 mA for 2 hr. Western blot analysis was performed with a polyclonal antibody specific to rat CYP1A1/1A2 (Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan). The bands were detected by the developed peroxidase reaction antibody specific to rat CYP1A1/1A2 (Daiichi Pure Chemicals Co., Ltd., at 30 mA for 2 hr. Western blot analysis was performed with a polyclonal antibody specific to rat CYP1A1/1A2 (Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan). The bands were detected by the developed peroxidase reaction.

Statistical Analysis. Data are presented as mean ± SE. Data were compared using an unpaired t test. A $p$ value of <0.05 was considered significant.

Results

The HLRs had significantly lower albumin concentrations than did the SDRs (2.0 ± 0.05 and 4.1 ± 0.03 mg/dl, respectively). No significant differences were seen between the HLRs and SDRs in body or liver weight (table 1).

Serum concentration-time curves for theophylline in HLRs and SDRs are shown in fig. 1, and the pharmacokinetic parameters are summarized in table 2. The AUC from 0 to 10 hr was significantly lower for the HLRs than for the SDRs. Conversely, the CL was significantly greater in the HLRs than in the SDRs. The theophylline $V_d$ was significantly increased in the HLRs, compared with the SDRs. No significant difference was noted between the HLRs and the SDRs in the theophylline $t_{1/2}$.

The theophylline free concentration in the SDRs was approximately 30% of the total concentration at each sampling point. In contrast, the unbound theophylline concentration in HLRs was approximately equal to the total concentration. The bound fraction of theophylline was lower in the HLRs than in the SDRs (fig. 2).

Table 3 shows the P450 contents and the ECOD and AHH activities. The P450 content in HLR liver microsomes was four fifths of that in SDR liver microsomes. ECOD and AHH activities in HLR liver microsomes were 78 and 59%, respectively, of the activities in SDR liver microsomes. The CYP1A1/1A2 apoprotein levels were determined by immunoblot analysis, and the CYP1A1/1A2 apoprotein levels in HLRs were 57% of those found in SDRs (fig. 3).

Discussion

In the present study, we demonstrated the effect of hypoalbuminemia on theophylline pharmacokinetics, with HLRs serving as the animal model because their serum albumin and theophylline protein binding levels were significantly lower than those found in SDRs. Theophylline is metabolized by 8-hydroxylation to 1,3-dimethyluric acid, which accounts for about one half of the CL of the drug in humans (Ogilvie, 1978), and by N-demethylation to 3-methylxanthine and 1-methylxanthine. Although the former reaction is catalyzed by several P450 subfamilies (Zhang and Kaminsky, 1995; Sarkar et al., 1991), CYP1A2 is reported to play a major role at lower substrate concentrations (Zhang and Kaminsky, 1995). CYP1A2 is also responsible for the latter reaction (Sarkar et al., 1991; Sarkar and Jackson, 1994).

The increase in theophylline $CL$ cannot be explained by the observed lower levels of CYP1A1/1A2 apoprotein and activities in HLRs, compared with those in SDRs. It could be related to the increase in the theophylline $V_d$. The lack of a significant difference in

![FIG. 1. Concentration-time profiles for theophylline in SDRs and HLRs. Each point with error bar represents the mean ± SE from five SDRs (○) or HLRs (○).]

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Body weight, relative liver weight, and concentration of serum albumin for SDRs and HLRs</th>
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<tbody>
<tr>
<td><strong>Body weight (g)</strong></td>
<td><strong>Relative liver weight</strong></td>
</tr>
<tr>
<td>HLRs</td>
<td>SDRs</td>
</tr>
<tr>
<td>221 ± 6</td>
<td>236 ± 5</td>
</tr>
</tbody>
</table>

All values represent mean ± SE ($N = 5$). *$p < 0.001$, significantly different from SDRs.

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Pharmacokinetic parameters for theophylline in SDRs and HLRs</th>
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<tr>
<td><strong>AUC (mg h/ml)</strong></td>
<td><strong>$K_{el}$ (hr$^{-1}$)</strong></td>
</tr>
<tr>
<td>HLRs</td>
<td>SDRs</td>
</tr>
<tr>
<td>45.0 ± 1.2*</td>
<td>70.3 ± 5.6</td>
</tr>
</tbody>
</table>

All values represent mean ± SE ($N = 5$). *$p < 0.01$, significantly different from SDRs.

TABLE 3

Comparison of P450 contents and CYP1A activities in HLRs and SDRs

<table>
<thead>
<tr>
<th></th>
<th>P450 Contents</th>
<th>ECOD Activity</th>
<th>AHH Activity</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>nmol/g protein</td>
<td>nmol/min/mg protein</td>
<td>nmol/min/mg protein</td>
</tr>
<tr>
<td>HLRs</td>
<td>0.93 ± 0.02***</td>
<td>0.68 ± 0.02***</td>
<td>33.5 ± 0.4***</td>
</tr>
<tr>
<td>SDRs</td>
<td>1.20 ± 0.01</td>
<td>0.85 ± 0.02</td>
<td>56.4 ± 0.5</td>
</tr>
</tbody>
</table>

All values represent mean ± SE (N = 5).

* $p < 0.001$, significantly different from SDRs.

The amounts of microsomal protein applied in each lane were 50 μg.

The theophylline $t_{1/2}$ between the HLRs and the SDRs might support this assumption that the increase in CL resulted from an increase in the theophylline $V_d$. An increase in the unbound fraction might contribute to an increase in the theophylline $V_d$. A linear relationship between the propranolol $V_d$ and the plasma unbound fraction was reported by Branch et al. (1976), which agrees with the results of this study. In our study, the theophylline $V_d$ was increased and the serum concentration was reduced in rats with hyperalbuminemia, in comparison with the theophylline $V_d$ and serum concentration in rats with normal albumin levels.

Karrapati et al. (1995) reported a positive correlation between hemoglobin Alc levels and plasma theophylline CL in patients with insulin-dependent diabetes mellitus, and they suggested that patients with poor glycemic control have higher theophylline CL values. Those authors also observed lower serum albumin concentrations and higher theophylline free fractions in those patients. In patients with poor glycemic control, an increase in the $V_d$ caused by hyperalbuminemia might contribute to an increase in theophylline CL.

The HLRs exhibited higher serum cholesterol and triglyceride concentrations, compared with the SDRs. Because hyperlipidemia reportedly does not affect theophylline pharmacokinetics in rabbits (Wojcicki et al., 1996), the difference in theophylline pharmacokinetics between SDRs and HLRs might not be related to the higher serum cholesterol or triglyceride concentrations in HLRs.

In summary, the rats with hyperalbuminemia had significantly greater $V_d$ values, which resulted in an increase in the CL, although the rats had lower CYP1A1/1A2 activity and expression than did the rats with normoalbuminemia. Hyperalbuminemia is associated with a number of diseases and disorders (Steinfeld, 1964; Toporovski et al., 1982). Patients with asthma or chronic obstructive pulmonary disease may develop hyperalbuminemia. It is hoped that our current results will inform further investigations concerning theophylline pharmacokinetics in patients with hyperalbuminemia. Further study is required to demonstrate the effects of an increase in the unbound fraction on the theophylline pharmacodynamics with hyperalbuminemia.