Effect of Simultaneous Administration of Drugs on Absorption and Excretion. XIX. 1) Binding of Acetoheaxamide and Its Major Metabolite, (−)-Hydroxyhexamide, to Human Serum Albumin

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The binding of acetoheaxamide and its major metabolite, (−)-hydroxyhexamide, to human serum albumin (HSA) was examined by using the equilibrium dialysis method. (−)-Hydroxyhexamide was isolated from the urine of rabbits after oral administration of acetoheaxamide. The Scatchard plots suggested that acetoheaxamide interacts with two kinds of binding sites on HSA, whereas (−)-hydroxyhexamide interacts with only one kind of binding sites. In addition, the binding parameters obtained led us to conclude that serum protein binding of (−)-hydroxyhexamide is considerably lower than that of acetoheaxamide.

Keywords—acetoheaxamide; (−)-hydroxyhexamide; human serum albumin; serum protein binding; equilibrium dialysis method; Scatchard plot; pharmacologically active metabolite; reductive metabolism

It is generally accepted that serum protein binding is an important determinant affecting the pharmacokinetics and pharmacodynamics of a drug. Thus, serum protein binding of many drugs has been already reported. 2) For example, Chignell 3) demonstrated that nonsteroidal anti-inflammatory drugs such as phenylbutazone and flufenamic acid are extensively bound to human serum albumin (HSA). However, serum protein binding of metabolites has not yet been fully examined. This is of interest, because a metabolite often retains the same function as the parent drug.

Acetoheaxamide is widely used as an orally antidiabetic drug. In humans, acetoheaxamide has been reported to be mainly biotransformed to (−)-hydroxyhexamide, which is a pharmacologically active metabolite. 4) The purpose of this study was to isolate (−)-hydroxyhexamide from the urine of rabbits after oral administration of acetoheaxamide, and to compare the binding of acetoheaxamide to HSA with that of (−)-hydroxyhexamide.

Experimental

Materials—Acetoheaxamide was kindly supplied by Shionogi Pharmaceutical Co., Ltd. Human serum albumin (HSA, Fraction V) was purchased from Miles Lab., Inc. The molecular weight of HSA was regarded as 66000. 5) (±)-Hydroxyhexamide, mp 143—147°C, was synthesized from acetoheaxamide by the method of Girgis-Takla et al. 6)

Animal Experiments—Male albino rabbits weighing 2.5—3.2 kg were fasted for 38—42 h prior to the experiments, but drinking water was allowed ad libitum. Acetoheaxamide (100 mesh powder) was suspended in about 80 ml of water, and administered orally to rabbits. The dose of acetoheaxamide was 100 mg/kg. The urine was collected up to 24 h after oral administration of acetoheaxamide.

Equilibrium Dialysis Method—The binding of acetoheaxamide and (−)-hydroxyhexamide to HSA was examined by using the equilibrium dialysis method. 7) The apparatus for equilibrium dialysis was purchased from Sanko Plastic Co. HSA, acetoheaxamide and (−)-hydroxyhexamide were dissolved in 1/15 M phosphate buffer (pH 7.4). Each cell of the apparatus was divided into two spaces with a cellulose membrane (Visking Co.). HSA solution (2.7 ml, 5 × 10−5 M) was placed in one space, and drug solution (2.7 ml, 4—200 × 10−9 M) was placed in the other. The apparatus was then
shaken for 6 h at 37 °C.

Measurement of Acetoxyamide and (−)-Hydroxyamide Concentrations—Each sample solution was diluted with 1/15 M phosphate buffer (pH 7.4), and the acetoxyamide and (−)-hydroxyamide concentrations were measured by ultraviolet (UV) spectrophotometry.

General Procedures for Identification of (−)-Hydroxyamide—Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-4S digital polarimeter. Infrared (IR) spectra were measured in KBr disks with a JASCO IRA-1 spectrometer. Thin-layer chromatography (TLC) was performed on Silica gel 60 HF254 plates (Merck).

Data Analysis—Data analysis was carried out in the Computer Center of Kumamoto University according to the method of Goto et al. 89

Results and Discussion

Isolation and Identification of (−)-Hydroxyamide

The reductive metabolism of ketones is well known to be stereoselective.9 The best animal model for studies of the reductive metabolism of ketones is the rabbit.10 Thus, we attempted to isolate (−)-hydroxyamide from the urine of rabbits after oral administration of acetoxyamide. A major metabolite of acetoxyamide was isolated by the method shown in Chart 1 (mp 142—145 °C). The metabolite was found to have optical activity, [α]D20 −17.0° (c = 5.0, CHCl3). The specific optical rotation was almost equal to that of (−)-hydroxyamide isolated from human urine.4 Its identity was confirmed by IR absorption spectrometry and TLC. (±)-Hydroxyamide was used as the authentic sample. The IR spectrum of the metabolite showed the hydroxy band at 3520 cm−1 and completely coincided with that of the authentic sample. In addition, TLC of the metabolite gave a single spot, and

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<table>
<thead>
<tr>
<th>urine</th>
<th>extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) adjust to pH 5 with HCl</td>
<td></td>
</tr>
<tr>
<td>2) saturate with NaCl</td>
<td></td>
</tr>
<tr>
<td>3) extract with benzene–ethyl acetate (1:1)</td>
<td></td>
</tr>
</tbody>
</table>

extract layer

<table>
<thead>
<tr>
<th>residue</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) dry over anhyd. Na2SO4</td>
</tr>
<tr>
<td>2) evaporate to dryness at 40°C</td>
</tr>
</tbody>
</table>

residue

<table>
<thead>
<tr>
<th>filtrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>concentrate to a small volume in vacuo at 40°C</td>
</tr>
</tbody>
</table>

metabolite (432 mg)
```

Chart 1. Isolation of a Major Metabolite from the Urine of Rabbits after Oral Administration of Acetoxyamide

The total dose of acetoxyamide was about 7 g.
Table I. Thin-Layer Chromatography of a Major Metabolite of Acetohexamide

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Solvent system I</th>
<th>Solvent system II</th>
</tr>
</thead>
<tbody>
<tr>
<td>(±)-Hydroxyhexamide (authentic)</td>
<td>0.21</td>
<td>0.26</td>
</tr>
<tr>
<td>Acetohexamide</td>
<td>0.50</td>
<td>0.66</td>
</tr>
</tbody>
</table>

* a) Solvent system I, chloroform-formic acid (97:3, v/v); II, benzene-acetone-acetic acid (90:10:1, v/v).

its *Rf* value was in fair agreement with that of the authentic sample, as shown in Table I. From these findings, the metabolite was identified as (−)-hydroxyhexamide.

**Serum Protein Binding of Acetohexamide and (−)-Hydroxyhexamide**

The Scatchard plots for the binding of acetohexamide and (−)-hydroxyhexamide to HSA are shown in Figs. 1 and 2, respectively. The Scatchard plots for the binding of acetohexamide to HSA gave a curve which corresponds to the summation of two straight lines. This suggests that acetohexamide interacts with two kinds of binding sites on HSA. Consequently, the data were fitted to the following equation:

\[
r = \frac{n_1 K_1 C_t}{1 + K_1 C_t} + \frac{n_2 K_2 C_t}{1 + K_2 C_t}
\]

where *r* is the number of bound drug molecule per protein molecule, *n_1* is the maximum number of primary binding sites, *n_2* is the maximum number of secondary binding sites, *K_1* is the binding constant at the primary binding sites, *K_2* is the binding constant at the secondary binding sites, and *C_t* is the concentration of unbound drug.

In contrast, the Scatchard plot for the binding of (−)-hydroxyhexamide to HSA gave a straight line. This suggests that (−)-hydroxyhexamide interacts with only one kind of binding sites on HSA. Consequently, the data were fitted to the following equation:

\[
r = \frac{n_1 K_1 C_t}{1 + K_1 C_t}
\]

![Fig. 1. Scatchard Plot for the Binding of Acetohexamide to Human Serum Albumin](image1)

![Fig. 2. Scatchard Plot for the Binding of (−)-Hydroxyhexamide to Human Serum Albumin](image2)
TABLE II. Binding Parameters for the Interaction of Acetohexamide or (-)-Hydroxyhexamide with Human Serum Albumin

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Acetohexamide</th>
<th>(-)-Hydroxyhexamide</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_1 \text{ (m}^{-1}\text{)}$</td>
<td>$4.66 \times 10^4$</td>
<td>$1.50 \times 10^3$</td>
</tr>
<tr>
<td>$n_1$</td>
<td>1.55</td>
<td>6.60</td>
</tr>
<tr>
<td>$n_1K_1 \text{ (m}^{-1}\text{)}$</td>
<td>$7.22 \times 10^4$</td>
<td>$9.90 \times 10^3$</td>
</tr>
<tr>
<td>$K_2 \text{ (m}^{-1}\text{)}$</td>
<td>$2.20 \times 10^3$</td>
<td>—</td>
</tr>
<tr>
<td>$n_2$</td>
<td>4.27</td>
<td>—</td>
</tr>
<tr>
<td>$n_2K_2\text{ (m}^{-1}\text{)}$</td>
<td>$9.39 \times 10^3$</td>
<td>—</td>
</tr>
</tbody>
</table>

The binding parameters obtained are summarized in Table II. It is evident from these binding parameters that the serum protein binding of (-)-hydroxyhexamide is considerably lower than that of acetohexamide.

Recently, McMahon et al.\(^{13}\) reported that (-)-hydroxyhexamide is 2.4 times as potent as acetohexamide. In addition, Smith et al.\(^{11}\) reported that the biological half-life of (-)-hydroxyhexamide is longer than that of acetohexamide. These results suggest that the pharmacologically active metabolite plays an important role in the overall hypoglycemic activity after oral administration of acetohexamide. However, further studies are necessary to evaluate accurately the contribution of (-)-hydroxyhexamide to the overall hypoglycemic activity after oral administration of acetohexamide.

In this paper, we present evidence that the serum protein binding of (-)-hydroxyhexamide is considerably lower than that of acetohexamide. This is significant in relation to the pharmacokinetics and pharmacodynamics of (-)-hydroxyhexamide.

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**References**