[Chem. Pharm. Bull. 33(3)1281—1284(1985)]

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

YORISHIGE IMAMURA,* YUICHIRO KOJIMA, and HISASHI ICHIBAGASE

Faculty of Pharmaceutical Sciences, Kumamoto University, 5-1, Oe-honmachi, Kumamoto 862, Japan

(Received June 30, 1984)

The binding of acetohexamide 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 acetohexamide. The Scatchard plots suggested that acetohexamide 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 acetohexamide.

Keywords——acetohexamide; (−)-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.

Acetohexamide is widely used as an orally antidiabetic drug. In humans, acetohexamide 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 acetohexamide, and to compare the binding of acetohexamide to HSA with that of (−)-hydroxyhexamide.

Experimental

Materials——Acetohexamide 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 acetohexamide 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*. Acetohexamide (100 mesh powder) was suspended in about 80 ml of water, and administered orally to rabbits. The dose of acetohexamide was 100 mg/kg. The urine was collected up to 24 h after oral administration of acetohexamide.

Equilibrium Dialysis Method——The binding of acetohexamide 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, acetohexamide 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^{-5} M) was placed in the other. The apparatus was then

shaken for 6 h at 37 °C.

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

General Procedures for Identification of (−)-Hydroxyhexamide——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 HF_{254} plates (Merck).

Data Analysis——Data analysis was carried out in the Computer Center of Kumamoto University according to the method of Goto *et al.*[8]

Results and Discussion

Isolation and Identification of (−)-Hydroxyhexamide

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 (−)-hydroxyhexamide from the urine of rabbits after oral administration of acetohexamide. A major metabolite of acetohexamide was isolated by the method shown in Chart 1 (mp 142—145 °C). The metabolite was found to have optical activity, $[\alpha]_D^{20}$: −17.0° ($c=5.0$, $CHCl_3$). The specific optical rotation was almost equal to that of (−)-hydroxyhexamide isolated from human urine.[4] Its identity was confirmed by IR absorption spectrometry and TLC. (±)-Hydroxyhexamide 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

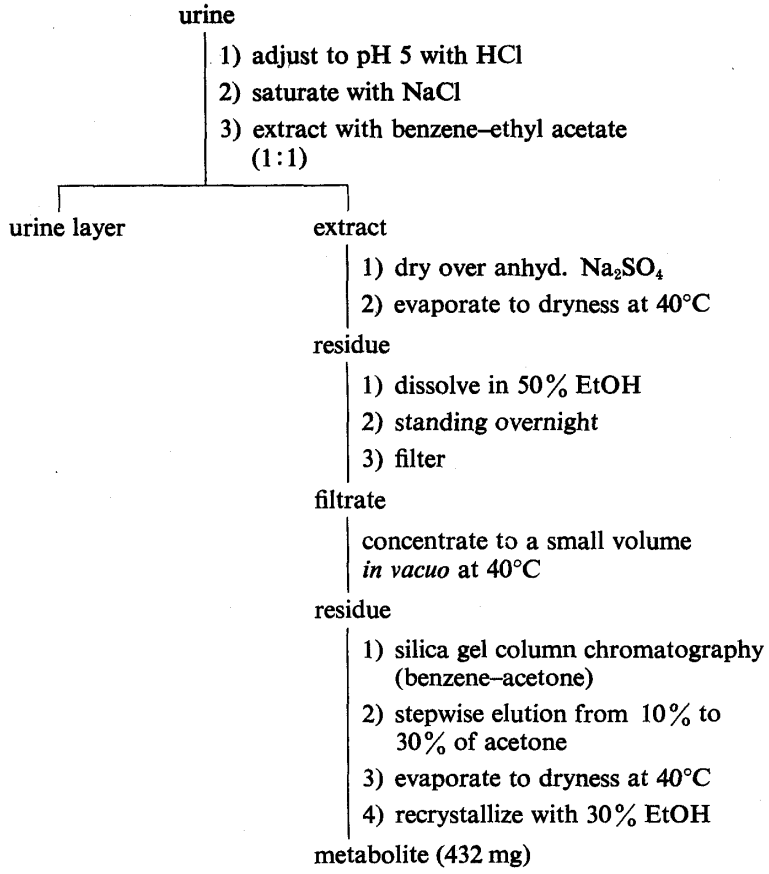

Chart 1. Isolation of a Major Metabolite from the Urine of Rabbits after Oral Administration of Acetohexamide

The total dose of acetohexamide was about 7 g.

TABLE I. Thin-Layer Chromatography of a Major Metabolite of Acetohexamide

	Rf Solvent system[a]	
	I	II
Metabolite	0.21	0.26
(±)-Hydroxyhexamide (authentic)	0.22	0.27
Acetohexamide	0.50	0.66

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;

$$r=\frac{n_1K_1C_f}{1+K_1C_f}+\frac{n_2K_2C_f}{1+K_2C_f} \quad (1)$$

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_f 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;

$$r=\frac{n_1K_1C_f}{1+K_1C_f} \quad (2)$$

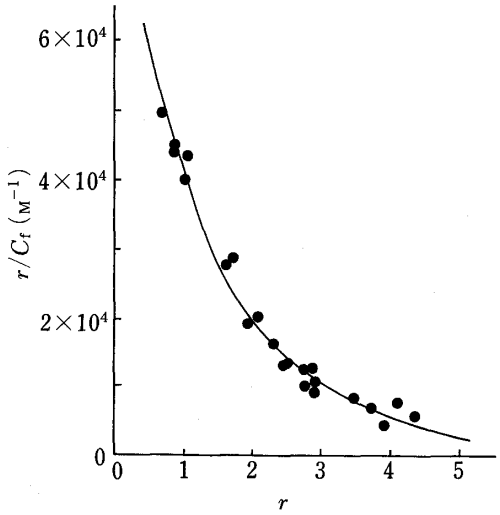

Fig. 1. Scatchard Plot for the Binding of Acetohexamide to Human Serum Albumin

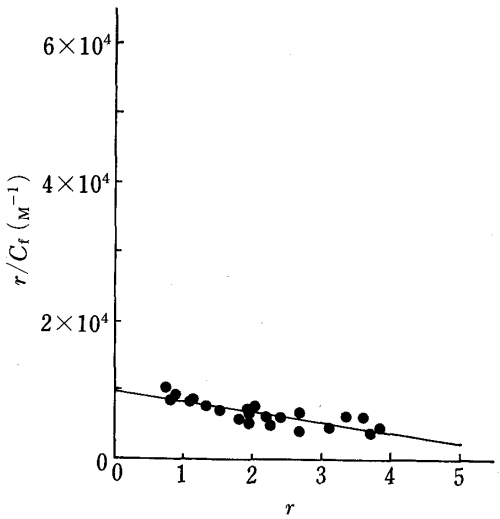

Fig. 2. Scatchard Plot for the Binding of (−)-Hydroxyhexamide to Human Serum Albumin

TABLE II. Binding Parameters for the Interaction of Acetohexamide or (−)-Hydroxyhexamide with Human Serum Albumin

Parameter	Acetohexamide	(−)-Hydroxyhexamide
K_1 (M^{-1})	4.66×10^4	1.50×10^3
n_1	1.55	6.60
n_1K_1 (M^{-1})	7.22×10^4	9.90×10^3
K_2 (M^{-1})	2.20×10^3	—
n_2	4.27	—
n_2K_2 (M^{-1})	9.39×10^3	—

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.*[4] 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.

Acknowledgement The authors are very grateful to Dr. Y. Kaneo, Faculty of Pharmacy and Pharmaceutical Sciences, Fukuyama University, for data analysis.

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