Communications to the Editor

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METABOLIC REDUCTION OF ACETOHEXAMIDE BY RABBIT TISSUES

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The in vitro metabolic reduction of acetohexamide was studied using rabbit tissue preparations. Acetohexamide is mainly reduced in the cytosols of rabbit liver and kidney, and its reduction is catalyzed by ketone reductases.

KEYWORDS —— acetohexamide; active metabolite; rabbit tissue preparation; cytosolic fraction; in vitro metabolic reduction; ketone reductase

It is well known that many drugs having a ketone group are metabolized to free secondary alcohols and/or alcohol conjugates.[1] However, studies of the enzymology of the reductive metabolism of these drugs have been very limited. The purpose of the present study is to characterize the reductive metabolism of these drugs, using rabbit tissue preparations. As a model drug, acetohexamide, an oral antidiabetic drug, was chosen. (-)-Hydroxyhexamide, an active metabolite of acetohexamide, is of particular interest clinically.[2]

First, we examined the acetohexamide reducing activity of the cytosols of various rabbit tissues. As shown in Fig. 1, the cytosol of liver or kidney exhibited the highest activity of all the tissues studied. Next, the acetohexamide reducing activity was examined in the subcellular fractions of rabbit liver and kidney. As shown in Table I, the highest activity was observed in the cytosolic fraction. In the microsomal fraction, the activity was not detected at all. On the basis of these results, the cytosolic fractions of rabbit liver and kidney were used in the subsequent experiments.

Carbonyl reductases (aldehyde and ketone reductases) and alcohol dehydrogenase are typically distinguished by their preference for NADPH or NADH as a cofactor.[3,4] So we tested the in vitro metabolic reduction of acetohexamide in the presence of NADPH or NADH. As shown in Table II, NADPH is better than NADH as a cofactor for the acetohexamide reduction. This implies that alcohol dehydrogenase is not an acetohexamide reducing enzyme.

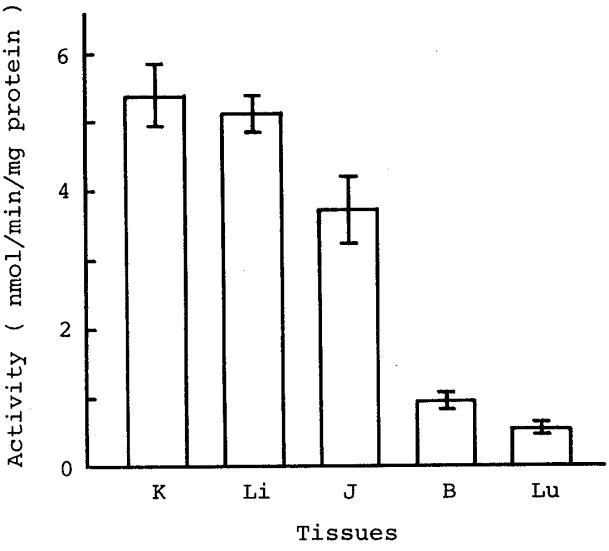

Fig.1. Tissue Distribution of Acetohexamide Reducing Activity

K, Kidney; Li, Liver; J, Jejunum; B, Brain; Lu, Lung.

Values represent means ± S.D. (n=3).

TABLE I. Subcellular Localization of Acetohexamide Reducing Activity

Tissue	Activity (nmol/min/mg protein)			
	Nuclei	Mitochondria	Microsomes	Cytosol
Liver	0.61 ± 0.34	0.69 ± 0.17	n.d.[a)]	4.53 ± 1.36
Kidney	0.30 ± 0.14	0.15 ± 0.03	n.d.	5.12 ± 2.24

Values represent means ± S.D. (n=3).
a) Not detected.

TABLE II. Cofactor Requirement of Acetohexamide Reducing Activity

Tissue	Activity (nmol/min/mg protein)		
	NADPH	NADH	None
Liver	2.07 ± 0.44	0.06 ± 0.04	n.d.[a)]
Kidney	2.57 ± 0.75	0.10 ± 0.02	n.d.

The concentration of NADPH or NADH was 0.075 mM.
Values represent means ± S.D. (n=3).
a) Not detected.

The effects of various inhibitors on the in vitro metabolic reduction of acetohexamide are summarized in Table III. Pyrazole was used as a classical inhibitor of alcohol dehydrogenase,[5] and barbiturates (barbitar and phenobarbitar) were used as potent inhibitors of aldehyde reductase.[4] In addition, quercitrin and quercetin were used as effective inhibitors to distinguish ketone reductases from alcohol dehydrogenase or aldehyde reductase.[4] As seen in Table III, quercitrin and quercetin markedly inhibited the acetohexamide reducing activity. On the other hand, neither pyrazole nor barbiturates had any effect on the acetohexamide reducing activity. This indicates that the reductive metabolism of acetohexamide is catalyzed by ketone reductases.

Ketone reductases appear to cause the stereoselective reduction of acetohexamide.[2] In fact, we isolated (-)-hydroxyhexamide from the urine of rabbits after oral administration of acetohexamide.[6] Further studies including the analysis of (-)-hydroxyhexamide and the purification of individual ketone reductase are in progress.

TABLE III. Effects of Various Inhibitors on Acetohexamide Reducing Activity

Inhibitor	Concn. (mM)	Relative activity (%)	
		Liver	Kidney
Pyrazole	10	100.0	97.4
Barbitar	1	97.0	101.7
Phenobarbital	1	89.0	97.5
Quercitrin	0.1	31.5	43.7
Quercetin	0.1	37.8	51.2

EXPERIMENTAL

Male albino rabbits (2.0 - 3.0 kg) were exsanguinated from the carotid artery. The tissues were carefully removed and homogenized with 0.01 M phosphate buffer, pH 7.4, containing 1.15% KCl. The homogenate was centrifuged at 10000 x g for 20 min, and the supernatant solution was centrifuged at 113000 x g for 60 min. The resultant supernatant fraction (cytosolic fraction) was used to assay for enzyme activity. When individual subcellular fractions were desired, each rabbit tissue was homogenized and prepared according to the method of Hogeboom.[7]

In assaying the acetohexamide reducing activity, the typical incubation mixture consisted of 1 mM acetohexamide, 0.25 mM of $NADP^+$, 6.25 mM of glucose 6-phosphate, 6.25 mM of $MgCl_2$, 0.25 units of glucose 6-phosphate dehydrogenase, the cytosolic fraction, and 0.1 M phosphate buffer (pH 7.4) in a final volume of 4 ml, unless otherwise noted. The reaction was started by the addition of cofactor, and continued at 37°C for 10 min under aerobic conditions. The reaction was stopped by the addition of 0.5 ml of 1 N HCl to the reaction mixture.

The reduction product (hydroxyhexamide) was determined by high-performance liquid chromatography (HPLC).[8] Each reaction mixture was extracted with benzene-ethyl acetate (1:1), containing the internal standard (tolazamide), after adjusting to pH 5 by adding 0.25 ml of 1 N NaOH. The organic phase was evaporated in vacuo and the residue was dissolved in the mobile phase of HPLC. HPLC was carried out using a Hitachi 655 HPLC apparatus equipped with a reversed phase LiChrosorb RP-18 (10 μm, Cica-MERCK) column.

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