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A new aspect of tolbutamide metabolism in the rabbit: the role of 1-butyl-3-(*p*-formylphenyl)sulphonylurea

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Abstract—We investigated the metabolism of tolbutamide by using synthetic 1-butyl-3-(*p*-formylphenyl)sulphonylurea (ATB), an intermediate in the metabolic pathway of tolbutamide. ATB (40 mg kg^{-1}) administered intravenously to rabbits was oxidized to 1-butyl-3-(*p*-carboxyphenyl)sulphonylurea (CTB) and also reduced to 1-butyl-3-(*p*-hydroxymethylphenyl)sulphonylurea (HMTB). Therefore, it is likely that in the metabolism of tolbutamide, the oxidation of HMTB to ATB involved the reverse reaction, suggesting the reduction of ATB to HMTB. The oxidation of ATB to CTB was inhibited by disulfiram pretreatment. ATB was detected in the blood following intravenous administration of HMTB in rabbits pretreated with disulfiram. These results, confirm that ATB is an intermediate in the oxidative metabolism of tolbutamide in the rabbit.

Tolbutamide is a sulphonylurea derivative used as an oral hypoglycaemic agent for the treatment of diabetes mellitus. The metabolism of tolbutamide has been extensively studied in man (Louis et al 1956; Wittenhagen et al 1959; Nelson & O'Reilly 1961; Thomas & Ikeda 1966) and laboratory animals (Wittenhagen et al 1959; Thomas & Ikeda 1966; Tagg et al 1967; Shibasaki et al 1973a). Although pronounced species differences have been recognized, similar metabolic patterns were found in man, rabbit and rat. McDaniel et al (1969) indicated that tolbutamide is metabolized to 1-butyl-3-(*p*-hydroxymethylphenyl)sulphonylurea (HMTB) and HMTB is further metabolized to 1-butyl-3-(*p*-carboxyphenyl)sulphonylurea (CTB), presumably via 1-butyl-3-(*p*-formylphenyl)sulphonylurea (ATB).

Materials and methods

Materials. HMTB, ATB (Makaya et al 1983) and CTB (Shibasaki et al 1973b) were prepared as previously reported. Disulfiram and 2,4-dinitrophenylhydrazine were of reagent grade and purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Tolbutamide and chlorpropamide were of pharmaceutical grade and were gifts from Hoechst Japan Ltd (Tokyo, Japan). All other chemicals were of reagent grade.

Synthesis of 2,4-dinitrophenylhydrazone of ATB. A solution of 2,4-dinitrophenylhydrazine (8 mg) in 1 mL acetic acid-ethanol (1:9, v/v) was added to a solution of ATB (10 mg) in ethanol (1 mL). The mixture was heated under reflux for 1 h. Distilled water was added until the mixture became turbid. The mixture was left overnight at room temperature (21°C). The precipitate was collected by filtration and crystallized from ethanol (mp > 220°C). Analysis: calculated for $C_{18}H_{21}N_6O_7S$: C, 46·55; H, 4·31; N, 18·10: found: C, 46·29; H, 4·37; N, 17·74. 2,4-Dinitrophenylhydrazine was used for the determination of aldehyde. Therefore, tolbutamide, HMTB and CTB do not give any reaction product with 2,4-dinitrophenylhydrazine.

In-vivo experiments. Male albino rabbits, 1·8–2·0 kg, were individually housed in cages in an air-conditioned room and maintained on a standard laboratory diet (ORC4, Oriental Yeast Co. Ltd, Tokyo, Japan). Rabbits were fasted for about 18 h before the experiments but had free access to water. ATB, tolbutamide and HMTB (100 mg) were dissolved in an equimolar NaOH solution (10 mL). Drug solution (40 mg kg^{-1}) was administered via an ear vein. Blood samples were taken with a heparinized syringe at appropriate time intervals from an ear vein. Urine collections were made through a catheter inserted into the bladder. Five millilitres of distilled water was used to rinse the bladder at every collection and the washings were combined for the analysis.

In-vivo metabolic inhibition experiments using disulfiram. Disulfiram (1 g kg^{-1}), an inhibitor of aldehyde dehydrogenase, suspended in 0·5% methylcellulose was administered orally by a stomach tube before ATB, tolbutamide or HMTB administration. To examine the time necessary for onset of the inhibitory effect of disulfiram, ATB was administered 3 and 12 h after disulfiram treatment. The effect was examined from the amount of CTB excreted in the urine. Three hours after disulfiram treatment, no difference was found in the amount of CTB in the urine compared with the control. Significant decrease of CTB in the urine was recognized in rabbits treated with disulfiram before 12 h. Therefore, the rabbits were treated with disulfiram 12 h before administration of drug.

HPLC. ATB, HMTB and CTB in blood and urine were determined by the method described in the previous report (Shibasaki et al 1985). Blood (0·5 mL) or appropriately diluted urine was mixed with 50 μL ethanol and centrifuged at 4000 rev min^{-1} for 5 min. The supernatant (0·2 mL) was mixed with 0·2 mL of acetonitrile containing 20 μg chlorpropamide as internal standard and centrifuged at 10000 rev min^{-1} for 5 min. A portion of the supernatant (10 μL) was chromatographed. The chromatographic apparatus (model 6000A pump, model U6K injector; Waters Associates, Inc., USA) was fitted with a variable wavelength UV absorbance detector (UVILDG-5III, Oyo-Bunko Kiki Co. Ltd, Tokyo, Japan). The stationary phase used was a Cosmosil $5C_{18}$ packed column (diam., 4·6 mm; length, 150 mm, Nacalai Tesque Inc.) and the analyte was detected at 250 nm. The column was used at room temperature. The mobile phase consisted of acetonitrile–H_2O–acetic acid (35:65:1, v/v/v) at flow rate of 1·0 mL min^{-1}. The retention times of HMTB, CTB, ATB and chlorpropamide were 5·3, 6·6, 10·6 and 16·1 min, respectively. Quantitation of HMTB and CTB was by peak height ratio and that of ATB by peak area ratio.

Identification of ATB in the blood after administration of HMTB. A rabbit pretreated with disulfiram was given an intravenous administration of HMTB (40 mg kg^{-1}) and then anaesthetized with intraperitoneal secobarbitone (50 mg kg^{-1}). Within 30 min of anaesthetization, about 50 mL blood was obtained with a heparinized syringe from the inferior vena cava. Five millilitres of the blood sample was mixed with 5 mL 0·1 M acetic acid buffer solution (pH 4·0). The mixture was extracted twice with 25 mL ether. The ether layer was collected and dried over Na_2SO_4. After the ether was evaporated to dryness, the residue was dissolved in 5 mL ethanol. Then, 0·2 mL of the ethanol solution was mixed

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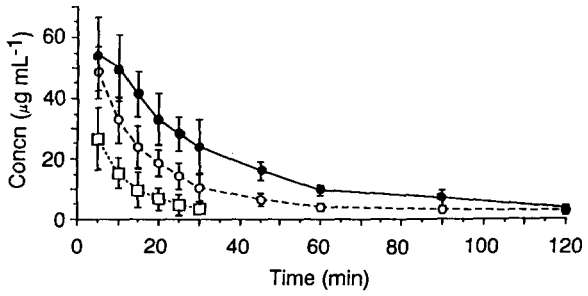

FIG. 1. Blood concentration of ATB (□), HMTB (○) and CTB (●) following intravenous administration of ATB (40 mg kg^{-1}) in rabbits. Blood concentration of HMTB and CTB: ATB equivalent. Results are expressed as the mean ± s.d.

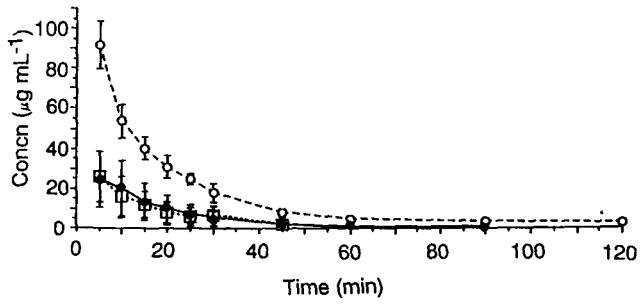

FIG. 2. Blood concentration of ATB (□), HMTB (○) and CTB (●) following intravenous administration of ATB (40 mg kg^{-1}) in rabbits pretreated with disulfiram. Blood concentration of HMTB and CTB: ATB equivalent. Results are expressed as the mean ± s.d.

with 0·2 mL of 1 M HCl containing 20 μg 2,4-dinitrophenylhydrazine. After centrifugation at 10000 rev min^{-1} for 5 min, the supernatant was subjected to HPLC using the same apparatus and the stationary phase as described above, with a mobile phase consisting of methanol–H_2O–acetic acid (70:30:1, v/v/v) at a flow rate of 0·8 mL min^{-1} and UV detection at 383 nm.

Results

ATB administration. Fig. 1 shows the blood concentration of ATB, HMTB and CTB following intravenous administration of ATB (40 mg kg^{-1}) to rabbits. The blood concentration of ATB decreased rapidly and disappeared within 40 min after dosing. HMTB and CTB were detected immediately after the dose of ATB. Urinary recovery of HMTB and CTB is shown in Table 1. HMTB excreted was about a half the amount of CTB. ATB was not found in the urine. From these results, it was demonstrated that ATB was not only oxidized to CTB but also reduced to HMTB.

The effect of an inhibitor of aldehyde dehydrogenase on the metabolism of ATB was examined in rabbits treated with disulfiram 12 h before ATB administration. Blood concentration of HMTB exceeded that of CTB as shown in Fig. 2, and the amount of HMTB in the urine exceeded that of CTB (Table 1).

Tolbutamide administration. The effect of disulfiram on the metabolism of tolbutamide was examined following intravenous administration (40 mg kg^{-1}). The results are presented in Table 1. The amount of urinary recovery of CTB decreased after disulfiram pretreatment. Thus, it might be considered that disulfiram also inhibited the oxidation of ATB resulting from tolbutamide. The poor urinary recoveries may be mainly due to very slow elimination of tolbutamide from the blood as reported previously (Shibasaki et al 1973a). In addition, ATB was not detected in the urine or blood even after disulfiram pretreatment. Simultaneous pretreatment with disulfiram and the inhibitor of aldehyde oxidase might be needed for the detection of ATB resulting from tolbutamide.

HMTB administration. Experiments following intravenous administration of HMTB (40 mg kg^{-1}) in rabbits with or without disulfiram pretreatment were carried out. HMTB and CTB were excreted in the urine, the former being predominant. The inhibitory effect of disulfiram was not recognized (Table 1). However, the blood sample obtained from the disulfiram-pretreated rabbits after the dose of HMTB gave a peak corresponding to ATB on HPLC while those from rabbits without pretreatment of disulfiram did not give this peak. The identification of ATB was further confirmed by HPLC after derivatization of ATB with 2,4-dinitrophenylhydrazine. As shown in Fig. 3, the formation of the expected 2,4-dinitrophenylhydrazone of ATB was demonstrated compared with the authentic compound.

Discussion

Following intravenous administration of ATB, intact ATB, HMTB and CTB were detected in the blood and the latter two metabolites were found in the urine, indicating the complete conversion of ATB to HMTB and CTB. In the blood and urine, the amount of CTB was larger than that of HMTB. By the pretreatment with disulfiram, the amounts of the two metabolites were reversed, suggesting that the metabolic inhibition by disulfiram was involved in the oxidative process of ATB to CTB.

The metabolic pattern (CTB ratio in Table 1) following

Table 1. Urinary excretion of HMTB and CTB following intravenous administration of ATB, tolbutamide and HMTB to rabbits.

Drug	Disulfiram pretreatment	Collection period (h)	% of dose HMTB	% of dose CTB	% of dose Total	CTB ratio (%)
ATB (5)	–	0–5	36·6 ± 3·4	63·8 ± 2·7	100·5 ± 4·2	63·6 ± 2·3
ATB (4)	+	0–5	69·4 ± 10·2	30·9 ± 8·6	100·3 ± 3·6	30·2 ± 7·7*
Tolbutamide (3)	–	0–5	21·4 ± 4·4	28·6 ± 6·0	49·9 ± 9·8	57·1 ± 3·0
Tolbutamide (3)	+	0–5	20·1 ± 2·3	9·2 ± 2·7	29·3 ± 0·9	31·1 ± 8·9**
HMTB (4)	–	0–4	61·9 ± 10·9	14·5 ± 5·8	76·4 ± 10·9	19·0 ± 6·7
HMTB (4)	+	0–4	79·4 ± 11·2	14·7 ± 1·8	94·0 ± 9·6	16·0 ± 3·7

Dose of ATB, tolbutamide and HMTB: 40 mg kg^{-1}. Each value represents the mean ± s.d. Numbers in parentheses represent number of experiments. Results were compared statistically using Student's *t*-test. *Significantly smaller than ATB alone ($P < 0·001$). **Significantly smaller than tolbutamide alone ($P < 0·01$).

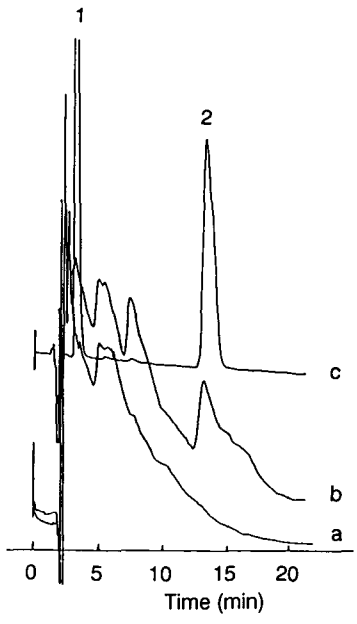

FIG. 3. HPLC chromatograms of blood samples following intravenous administration of HMTB (40 mg kg^{-1}) in rabbits pretreated with disulfiram. a, Blood sample; b, blood sample after treatment with 2,4-dinitrophenylhydrazine; c, authentic sample of ATB 2,4-dinitrophenylhydrazone + 2,4-dinitrophenylhydrazine. Peak 1, 2,4-dinitrophenylhydrazine; peak 2, 2,4-dinitrophenylhydrazone of ATB.

intravenous administration of tolbutamide agreed closely with that of intravenous administration of ATB. The metabolic pattern after intravenous administration of HMTB was different from that of intravenous administration of ATB and tolbutamide.

In general, formyl derivatives as intermediates in metabolic pathways are too reactive to be isolated. Several studies have investigated the identification of formyl derivatives in the biological fluid of man and experimental animals receiving the compounds containing methyl group. For example: from the urine of rats given toluene-4-sulphonamide, traces of the corresponding aldehyde were detected (Ball et al 1978); from the urine of human (Iguchi et al 1975a) and experimental animals

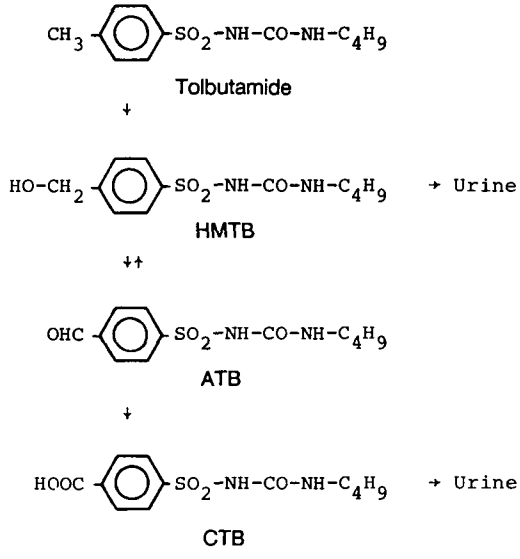

FIG. 4. Proposed metabolic pathway of tolbutamide.

(Iguchi et al 1975b) given aminopyrine, 4-formylaminoantipyrine was detected.

We could not detect ATB in the blood and urine following intravenous administration of tolbutamide. However, we succeeded in identifying ATB in the blood following intravenous administration of HMTB in rabbits pretreated with disulfiram. In the present study, we have obtained evidence that ATB is an intermediate in the metabolic pathway of tolbutamide, following the hypothesis of McDaniel et al (1969) that ATB might be an intermediate.

Kirkpatrick et al (1983) studied the metabolism of salicylidene benzylamine in rats and dogs. They reported that salicylidene benzylamine was first hydrolysed completely to salicylaldehyde and benzylamine, and no salicylaldehyde was detected but two biotransformation products, salicylic acid and salicyl alcohol resulting from oxidation and reduction of the aldehyde group, respectively, were the major metabolites. In the present study, it is considered that the ATB resulting from tolbutamide was also partly oxidized to CTB and partly reduced to HMTB. Finally, we propose the scheme shown in Fig. 4 as a new overall metabolic pathway of tolbutamide.

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