Short Communication

Reductive Metabolism In Vivo of Trans-4-Phenyl-3-buten-2-one in Rats and Dogs

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ABSTRACT:

The reductive metabolism in vivo of a flavoring additive, trans-4-phenyl-3-buten-2-one (PBO; trans-methyl styryl ketone) was investigated in rats and dogs. In both species, the double bond-reduced product, 4-phenyl-2-butanone (PBA), was detected by HPLC as the predominant species in blood after i.v. administration of PBO. PBA detected in rat blood was identified by comparison to the authentic sample. In contrast, the carbonyl-reduced product, trans-4-phenyl-3-buten-2-ol (PBOL) was also detected as a minor metabolite of PBO in both species. The area under the curve of PBOL in rat blood was only 3% of that of PBA. PBO was mutagenic in the Ames test using Salmonella typhimurium TA 100 when S-9 mix was added, but PBA and PBOL were not. It appears that PBO is mainly metabolized to PBA in vivo in rats and dogs as a detoxification pathway.

Experimental Procedures

Materials. PBO, PBA, and 4-phenyl-2-butanol were purchased from Nacalai Tesque Inc. (Kyoto, Japan). Aroclor 1254-induced rat liver S-9 and the cofactors were obtained from Oriental Yeast Co. (Tokyo, Japan). PBO was prepared by the method of Chaikin and Brown (1949). S. typhimurium TA 100 was obtained from the Institute for Fermentation (Osaka, Japan).

Animals and Drug Administration. Male Wistar (Slc:Wistar/ST) rats, 7 to 8 weeks old (weighing 210–240 g), were purchased from Japan SLC, Inc. (Shizuoka, Japan). Two female beagle dogs, 10 and 11 months old and weighing 10.7 and 12.5 kg (Shimizu Jikkenzairyo Inc., Kyoto, Japan), were used.

PBO or PBA was dissolved in dimethyl sulfoxide at the concentration of 50 mg/ml for dosing and was i.v. administered at 25 mg/kg to rats. PBO was also dissolved in dimethyl sulfoxide at the concentration at 250 mg/ml and was i.v. administered at 25 mg/kg to dogs. About 0.5 ml of blood was collected from these animals. A 0.2-ml aliquot of the blood was added to 0.4 ml of 0.01 M phosphate buffer and the hemolyzed blood was used for isolation and determination of metabolites of PBO.

Identification of Reductive Metabolites of PBO in Rats. The reductive metabolites (M-1 and M-2) of PBO were extracted with 20 ml of diethyl ether from 10 ml of the pooled hemolyzed blood of rats that had received PBO. The supernatant was evaporated to about 50 µl at 0°C and mixed with 100 µl of ethanol. The solution was subjected to HPLC and gas chromatography (GC)-Mass analysis.

Determination of PBA, PBOL, and 4-phenyl-2-butanol in Blood of Rats and Dogs. Reductive metabolites were determined in blood of rats and dogs given PBO or PBA. To determine the amounts of PBO, PBA, PBOL, and 4-phenyl-2-butanol in the blood, 0.6 ml of hemolyzed blood containing 10 µg of methyl p-aminobenzoate (an internal standard) was extracted with 5 ml of diethyl ether. The extract was evaporated to about 50 µl at 0°C and mixed with 100 µl of ethanol. An aliquot of the extract was subjected to HPLC. If the extracts were dried by vacuum centrifugation at room temperature, as in the earlier work (Sauer et al., 1997a,b), the amounts of these metabolites were markedly decreased.

HPLC. The system for separation and determination of PBO and its metabolites in rat and dog blood consisted of a Hitachi 655A HPLC system (Tokyo, Japan) equipped with an ODS column (Inertsil ODS-2, 150 × 4.6-mm i.d., GL Science, Tokyo, Japan). The mobile phase was acetonitrile/water (40:60, v/v) and the flow rate was 0.5 ml/min. The chromatogram was

Send reprint requests to: Dr. Shigeyuki Kitamura, Institute of Pharmaceutical Sciences, Hiroshima University, School of Medicine, 1–2–3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan. E-mail: kitamura@pharm.hiroshima-u.ac.jp
monitored with a UV detector set at 260 nm. The elution times of PBOL, 4-phenyl-2-butanol, PBO, and PBA were 14.5, 15.6, 19.2, and 23.0 min, respectively.

**GC-Mass.** GC-mass was performed using a Shimadzu GC-17A/QP-5000 (Kyoto, Japan) equipped with a DB-5 fused-silica capillary column (30 m × 0.25 mm i.d., J & W Scientific, Inc., Folsom, CA). The column temperature was held at 50°C for 1 min, then increased at the rate of 20°C/min to 200°C. The retention times of PBA, PBOL, and PBO were 5.9, 6.5, and 6.8 min, respectively.

**Mutation Assay.** The assay was carried out as described by Ames et al. (1975) with a slight modification. A test compound was preincubated for 30 min at 37°C with the tester strain in the presence of S-9 mix in 0.1 M phosphate buffer. Soft agar was added, and the mixture was poured into an agar plate. After incubation for 2 days at 37°C, the numbers of revertants were counted. The numbers on control plates and plates with 0.1 mg of AF-2 (positive control) were 164 and 748/plate, respectively.

**Results and Discussion**

**Reductive Metabolites of PBO in Rats and Dogs.** When PBO was administered i.v. to rats and dogs, two metabolites (M-1 and M-2) were detected in the HPLC chromatograms of the extract of the blood of rats and dogs. These peaks were not observed in the chromatograms of control blood. M-1 and M-2 gave retention times corresponding to those of PBA and PBOL, respectively (Fig. 1). M-1 and M-2 were isolated from the blood of rats as described in Experimental Procedures. The mass spectrum of M-1 showed the molecular ion at m/z 148 and fragment ions at m/z 133, 105, 91, and 77 (Fig. 2). The UV spectrum of the metabolite revealed an absorption maximum at 260 nm with a shoulder at 280 nm. The mass spectrum of M-2 gave the molecular ion at m/z 148 and fragment ions at m/z 115, 105, 91, and 77. The UV spectrum of the metabolite revealed an absorption maximum at 250 nm with shoulders at 283 and 296 nm. The mass and UV spectra and the HPLC behaviors of these metabolites were identical with those of authentic samples of PBA and PBOL (data not shown).

**Blood Concentration of PBA and PBOL in Rats and Dogs.** PBA was detected in blood after i.v. administration of PBO to male rats. The area under the curve (AUC0 –120 ) of PBA was 392 mg·min/ml. PBOL was detected in a small amount, in addition to the unchanged PBO. The AUC 0 –120 values of unchanged PBO and PBOL after dosing of PBO were 72.1 and 4.1 mg·min/ml, which correspond to 18.4 and 1.0% of that of PBA, respectively. The Cmax values for PBA and PBOL at 5 min were 9.46 and 0.12 mg/ml, respectively. Vd for PBO was 2449 ml/kg. In this case, 4-phenyl-2-butanol, the reduction product of both the carbonyl group and the double bond of PBO, was not detected in the blood (Fig. 3A). However, when PBA was administered i.v. to rats, 4-phenyl-2-butanol was detected in a small
The enzyme exhibited a significant double bond reductase activity toward the double bond was detected with trans-stilbene or styrene, which have no carbonyl group adjacent to the double bond. The double bond of PBO may be reduced by this α,β-ketoalkene double bond reductase in the animal body.

PBA was detected in much larger amounts than PBOL in the blood of rats and dogs given PBO i.v. Sauer et al. (1997a,b) reported that PBOL was detected, together with benzylic alcohol in the blood of rats and mice given PBO, and PBOL was further metabolized to the glycine conjugate via phenylacetic acid (Fig. 4). However, they did not detect the double bond-reduced metabolite, PBA, in rat blood. The discrepancy with the results of this study may be due to strain differences of the rats used in the experiments, but another possible explanation is the high volatility of PBA, which may be lost if the extract of the blood is evaporated to dryness, as in the earlier work.

**References**


