Metabolism of o,p′-DDD in Humans: A Novel Metabolic Pathway Forming Methylthio-containing Metabolites

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Urine samples were obtained from four patients with Cushing's syndrome who had been treated with o,p′-DDD[2,2-(2-chlorophenyl,4'-chlorophenyl)1,1-dichloroethane]. Methylthio-containing (MC) metabolites and other metabolites of o,p′-DDD were detected in the urine samples by gas chromatographic mass spectrometry. These MC metabolites showed smaller peaks in the gas chromatogram than other peaks derived from other metabolites of o,p′-DDD. Although the biological significance of the pathway forming these MC metabolites is not known at present, the mechanism of the metabolic pathway was discussed.

Key words: Methylthio-containing metabolite, o,p′-DDD, GC/MS.

The most common metabolic pathways in drug metabolism are the oxidative and conjugative reactions. The drugs often, but not always, proceed to undergo oxidation followed by conjugation with glutathione (GSH) in the liver and are secreted in the bile as mercapturic acid pathway (MAP) metabolites. The biliary MAP metabolites are modified in the intestine, reabsorbed and metabolized further before being excreted. An outline of drug metabolism in the experimental animals (1-3) are proposed as mentioned above. However, it is not clear that the drug metabolism which has been described about the experimental animals extends also to humans, since these metabolic reactions may be species specific. In order to determine whether these metabolic reactions occur in humans, the metabolic fate of o,p′-DDD which had been administered to the patients of Cushing’s syndrome was studied. This drug acts on cortisol synthesis and lowers cortisol production (4) and is used to treat Cushing’s syndrome (5).

The present paper reports that the gas chromatographic mass spectrometric (GC/MS) analysis of o,p′-DDD metabolites in the urine provides evidence for the existence of a novel metabolic pathway in humans.

MATERIAL AND METHODS

Chemicals: o,p′-DDA and o,p′-DDA-OH were gifts from Roussel Medica K.K. (Tokyo). All other chemicals and solvents were of analytical grade and were supplied by Nakarai Chemicals Ltd. (Kyoto).

Synthesis of methylthio o,p′-DDA: This com-
pound was synthesized by a modification of the method of Müller (6). Namely, glyoxylic acid (2g) was dissolved in sulfuric acid (2 ml) and added 4-chlorothioanisole (2 ml), chlorobenzene (2 ml) and ferric III chloride as a catalytic agent. This solution was stirred for 48 h in the room temperature. The product was extracted with ether, and dried.

Specimens and Pretreatment: 24 hr-urine samples were obtained from 4 patients of Cushing's syndrome (2 males, 2 females) who had been treated with 0.5-1.5 g of o,p'-DDD orally after meals for 1-2 yrs. Ten ml of urine sample was added to 1 N HCl (1 ml) and then extracted with ether. The extract was dried.

Methylation: o,p'-DDA, o,p'-DDA-OH, methylthio o,p'-DDA and the extracted urine samples were each methylated in methanol containing 5% hydrogen chloride (7). This procedure was carried out to selectively achieve methylation of carboxylic groups.

GC/MS conditions: A JEOL Model DX-300 GC/MS (JEOL Ltd, Tokyo) was connected on-line with a JEOL Model DA-5000 mass data analyzer (JEOL Ltd, Tokyo). A 30-m (film thickness of 0.25 \( \mu \)m) fused silica capillary column DB-1 (J. & W. Scientific, Inc.) was employed for separation. The carrier gas was helium, and the flow rate was 1.5 ml/min. The column temperature was programmed for 180°C to 250°C (4°C/min). Mass spectrometry was performed at an ion source temperature of 250°C, an ionization voltage of 70 eV, an ionization current of 30 \( \mu \)A and an acceleration energy of 3.0 KV.

RESULTS

The total ion and m/z 340 ion current chromatograms of methylated extracts from one patient's urine sample are shown in Fig. 1. Peaks in the chromatogram were analysed. Peaks (A and C) were identified to be o,p'-DDA methyl ester (ME) and o,p'-DDA-OH (ME) by GC/MS comparison with the chromatograms of synthetic chemicals. Peaks (B:B' and D:D') were surmised to be o,p'-DDE-OH and o,p'-DDD-OH, or their position-isomers by comparison of the mass spectra reported in the literature (8) since we had no synthetic chemicals. The mass spectra of o,p'-DDA (ME), o,p'-DDA-OH (ME), o,p'-DDE-OH, and o,p'-DDD-OH have been already reported in the literature (7, 8). The new metabolites (E, F and G

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![Fig. 1. Total ion chromatogram of methylated extract of a urine sample taken from an o,p'-DDD-treated patient. A: o,p'-DDA (ME), B: o,p'-DDE-OH, C: o,p'-DDA-OH (ME), D: o,p'-DDD-OH. B', C' and D' are isomers of B, C and D respectively. E: methylthio o,p'-DDA. F and G are isomers of E.](image)

![Fig. 2. Electron-impact mass spectra of urinary metabolites (as methyl ester) o,p'-DDD. E, F and G correspond to chromatographic peaks E, F and G in Fig. 1.](image)
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in Fig. 1) detected were proposed to be position-isomers of one another since their mass spectra (Fig. 2) were identical while their chromatographic retention times were different. Their mass spectra (Fig. 2) showed a molecular ion at m/z 340, accompanying a base fragment ion at m/z 281 (M⁺-59). These compounds were found to contain two chloride ions, judging from the ratio of relative peak intensities at m/z 340 and m/z 342. When methylthio o,p'-DDA prepared by synthesis and a new metabolite (E) were compared in GC/MS, their mass spectral and chromatographic properties were identical. Therefore the new metabolite (E) was identified to be methylthio o,p'-DDA. These metabolites of o,p'-DDD mentioned above were also identified in the other patients. Based on the comparison of the chromatographic peak areas, the amounts of new metabolites (E, F and G) are 3% less than that of other metabolites of o,p'-DDD. In view of the results so far analysed, metabolic pathways of o,p'-DDD in humans are proposed as shown in Fig. 3.

DISCUSSION

Miller and Miller et al. first reported that methylthio-containing (MC) metabolites of compounds such as aminobiphenyl (9), acetylaminofluorene (10) and dimethylaminoazobenzene (11) were isolated as alkaline degradation products from liver protein in rats treated with these compounds. Since then, the serial methylthio pathway has been studied for several compounds such as naphthalene (12), S-(1,2-dichlorovinyl)cysteine (13), penta chloronitrobenzene (14) and bromazepam (15). The presence of a MC metabolite of polychlorinated biphenyls (16) has also been reported in humans.

Formerly, Miller and Miller et al. proposed that the production of MC metabolites would involve the direct attachment of compounds to the sulfur atom of the methionyl residue of liver protein and splitting of the methylthio group from the electrophilic intermediate interacting between the aromatic amine and the methionyl residue in liver protein (9-11). However, the formation of thioether derivatives, including GSH, cysteinylglycine and cysteine conjugates, is now generally considered a pathway for the detoxification of reactive intermediates. For example, aromatic hydrocarbons are arene-epoxided by cytochrome P-450 and then conjugated with GSH by GSH-S-transferase and appear as cysteinylglycine and cysteine conjugate in urine. Thus, in vivo studies for thioether excretion may indicate that formation of a chemically reactive intermediate has occurred (17, 18). A thiol-containing metabolite is produced through scission of the S-C bond of cysteine conjugates by β-lyase, which is found in the liver cytosol (15) or in the intestinal flora (19) and S-methylated with S-adenosylmethionine (SAM) to form MC metabolites which are excreted in the urine. By means of administration of CD₃-methionine, it was confirmed that the methyl group in the MC metabolites was derived from methionine (20). Methylation of the thiol by SAM may be important in inhibiting covalent binding of reactive intermediates with biocomponents, similar to the GSH conjugate for the detoxification of epoxides.
It has been reported that o,p'-DDD undergo oxidation and/or reduction to be o,p'-DDD-OH, o,p'-DDE-OH, o,p'-DDA and o,p'-DDA-OH (8). In this study it was shown that o,p'-DDA was further metabolized to methylthio o,p'-DDA. The metabolic pathways for o,p'-DDD are outlined in Fig. 3.

As the amounts of MC metabolites are 3% less than that of other metabolites of o,p'-DDD in the urine, this methylthio pathway might be a minor route in the detoxification process. The biological significance of the methylthio pathway reported in this paper is not known at present, but the appearance of methylthio o,p'-DDA may indicate that o,p'-DDA was by-passed in the metabolism of o,p'-DDD.

REFERENCES