Methylsulfone Formation and PCB Metabolism

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Introduction

Polychlorinated biphenyls (PCBs) are ubiquitous environmental pollutants, and methylsulfones of PCBs were found along with PCBs [1)] in seal blubber the same year as 2-methylsulfones were reported as metabolites of 2-chloro-N-isopropyl acetanilide[2)]. The following year PCB methylsulfones were shown to accumulate as persistent residues in the tracheobronchial mucosa of lungs[3)]. Lund et al.[4)] subsequently characterized a lung cystolic protein that had a high affinity for binding PCB methylsulfones. Larsen et al.[5)] have described binding proteins for other methylsulfones in other tissues. Environmental residues of pentachlorothioanisole and bis-methylthiotetrachlorobenzene have been attributed to the use and metabolism of hexachlorobenzene and pentachloronitrobenzene[6)]. The physiological significance of these methylsulfone-containing residues has yet to be established. Speculation that they could be involved in decreased reproductive efficiency and respiratory problems needs further study.

Over fifty compounds have been found to be excreted in part by rats as methylthio-, methyl sulfoxyl- or methylsulfonyl-containing metabolites[7)]. Many of these compounds were also excreted as mercapturic acids. No connection was made between these two observations, because the prevailing pathway cited for their formation was the reaction of activated intermediates with methionine to form sulfonium ion adducts as was shown by DeBraun et al.[8)] in the formation of methylthio-containing 2-acetylaminoflourine adducts that were isolated from liver proteins after basic hydrolysis.

Another possible pathway involving glutathione (GSH) conjugation came from the proposal of Collucci and Buyske[9)] that the thiol formed in benzothiazole-2-sulfonamide metabolism resulted from glutathione (GSH) displacement of the sulfonamide and subsequent cleavage of the C-S bond to produce the thiol (benzothiazole-2-thiol). This particular thiol was excreted as the S-glucuronide, however, Weisiger et al.[10)] described the role of thiol-S-methyl transferase in detoxication of thiols. The enzyme was most active in caecal mucosa, colonic mucosa and liver. These discoveries indicated a method for introduction of methylthio groups into xenobiotics that is mediated by glutathione conjugation, thiol formation, and S-methylation.

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The objective of our studies was to determine and describe the most common pathway leading to introduction of methylthio groups into xenobiotics in vivo.

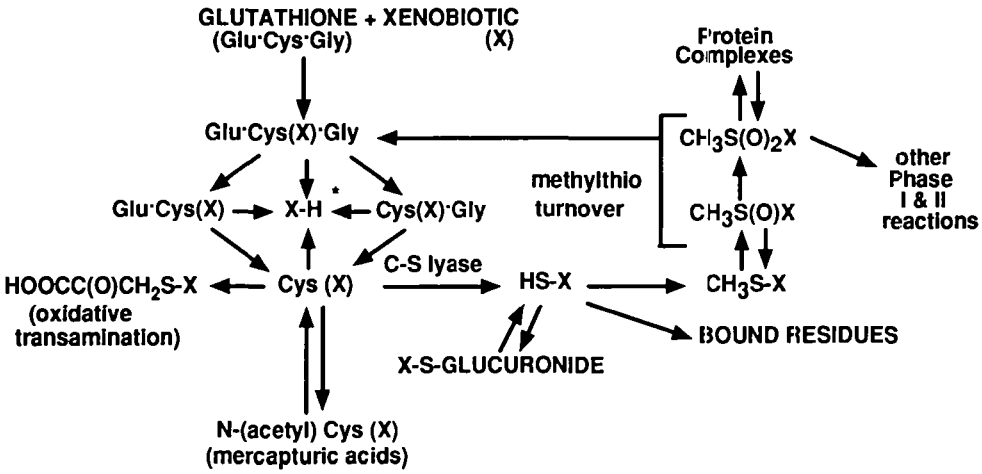
Materials and Methods

Control, bile-duct cannulated and germfree rats were dosed orally with appropriately radiolabelled xenobiotics and the excreted radiolabelled metabolites isolated, characterized and quantitated. These compounds included 2-chloro-N-isopropylacetanilide[2], naphthalene[11], pentachlorothioanisole[12], 2-acetamido-4-(chloromethyl)thiazole[13], 2,4',5-trichlorobiphenyl[14,15],bis-methylthiotetrachlorobenzene[16], 2,6-dichlorobenzonitrile[17], 1,2,4-trichlorobenzene[18]

Results

Methylthio groups were shown to be introduced into xenobiotics by catabolism of glutathione conjugates in the overall biochemical pathways shown in figure 1, however, inter-organ processing is also important[14,15,19].

Figure 1: Catabolism of glutathione conjugates [Glu·Cys (X)·Gly]



*** Formation of X-H is proposed to result from glutathione-mediated reductive displacement that forms the corresponding peptide disulfides and the defunctionalized xenobiotic.[20,21]**

The formation of the GSH conjugates of 2,4',5-trichlorobiphenyl is assumed to involve a P_{450} catalyzed oxidation to the 3,4-epoxide. Formation of the corresponding dihydrohydroxy-GSH adducts was evidenced by isolation of 3- and 4-methylsulfonyl-2,4',5-trichlorobiphenyl from the feces and lungs and the dihydrodiol from the bile.

Enzymes that catalyze the reactions shown in figure 1 are present in the tissues, but the methylthiolation process (formation of CH_3S-X) is usually enhanced by biliary excretion of mercapturic acid pathway metabolites and S-glucuronides which are subsequently hydrolyzed to the cysteine conjugate and the xenobiotic thiol, respectively, in the intestinal lumen.

The cysteine conjugate formed in the lumen can either be transported into the general circulation for further tissue metabolism or cleaved to the thiol of the xenobiotic moiety by microfloral cysteine conjugate ß-lyase (C-S lyase). The thiols formed from the cysteine conjugates or by hydrolysis of the S-glucuronides can then be methylated, after translocation into intestinal tissue, by intestinal mucosal S-methyltransferases[10]. The methylthio-containing metabolites can be translocated either to the intestinal lumen for faecal excretion or to the general circulation for further metabolism and excretion. This further tissue metabolism can involve S-oxidation to the methyl sulfoxides and/or methyl sulfones that can again be displaced by glutathione which results in methylthio-turnover. Methylsulfones can also form complexes with tissue proteins which result in persistent tissue residues, such as the PCB-methyl sulfones in lung tissue[4].

The role of enterohepatic circulation and microfloral metabolism in 2,4',5-trichlorophenyl methylsulfone formation was demonstrated in the levels of 3-&4-methylsulfonyl-2,4',5-trichlorobiphenyls as protein complexes in the lungs of control, bile-duct-cannulated, and germfree rats dosed with 2,4',5-trichlorobiphenyl (Table I).

Table I. Lung residues of 3-, and 4-methylsulfonyl-2,4',5-trichlorobiphenyl 48 h after dosing rats with ^{14}C-2,4',5-trichlorobiphenyl (16 mg/kg).

rats	3-&4-methylsulfonyl, 2,4'5-trichlorobiphenyl (ppm)
control	12.5 ± 1.8
cannulated bile ducts	1.3 ± 0.3
germfree	0.6 ± 0.3

When biliary excretion was prevented there was a 10-fold decrease in lung tissue residues of the methylsulfones; the residues were even lower in germfree lungs probably due to enterohepatic circulation of mercapturic acid pathway metabolites in the germfree rats which was prevented in the cannulated rats.

Methylthio turnover was apparently not of significance in PCB metabolism and therefore not responsible for the persistence of methylsulfone residues in rat lungs. This was concluded because when $^{14}CH_3$-labelled 4-methylthio-2,2',5,5'-tetrachlorobiphenyl was dosed orally to rats only 0.2 ± 0.0% of the dose was excreted as $^{14}CO_2$ and 0.5 ± 0.1% was excreted in the urine. CO_2 is a major metabolite from displacement of $CH_3S(O)$-groups, and urinary $CH_3SO_2^-$ a major metabolite from methylsulfones[22]. For comparison, 45% of doses of $^{14}CH_3$-labelled

pentachlorothioanisole (a metabolite from hexachlorobenzene, and pentachloronitrobenzene) were excreted as $^{14}CO_2$ (23%) and urinary $^{14}CH_3SO_2^-$ (23%).

Another possibility for the persistence of pulmonary PCB methylsulfones is muco-ciliary escalation of the protein complexes from the lungs back into the alimentary canal and reabsorption of the sulfones.

Any physiological significance, beneficial or adverse, associated with the formation of methylsulfones may result from their competing as ligands for various carrier proteins in vivo.

For a more comprehensive review of glutathione conjugate catabolism see reference 23.

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