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In vitro metabolism of tributyltin and triphenyltin by human cytochrome P-450 isoforms

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Abstract

The metabolic fate of tributyltin and triphenyltin may contribute to the toxicity of these chemicals. We used human hepatic cytochrome P-450 (CYP) systems to confirm the specific CYP(s) involved in the in vitro metabolism of tributyltin and triphenyltin. There were no significant sex differences in the metabolic pattern of tributyltin or triphenyltin, indicating that the CYP(s) responsible for the metabolism of these chemicals in humans is/are not sex-specific form(s). Six major drug-metabolizing isoforms of cDNA-expressed human CYPs and the CYP2C subfamily were tested to determine their metabolic capacities for tributyltin and triphenyltin. CYP2C9, 2C18, 2C19, and 3A4 significantly mediated both dealkylation and dearylation of these triorganotins. Furthermore, the metabolism of tributyltin and triphenyltin was significantly inhibited in vitro by pretreatment with selective inhibitors, azamulin for CYP3A4 and *N*-3-benzylnirvanol for CYP2C19. Since the CYP2C18 content of hepatic microsomes in humans is relatively low, CYP2C9, 2C19, and 3A4 might be the main isoforms of CYP that are responsible for tributyltin and triphenyltin metabolism in the human liver.

Keywords: Tributyltin; Triphenyltin; Metabolism; Hepatic microsome; Human cytochrome P-450; In vitro

1. Introduction

Organotin compounds have become important commercial organometals, used mainly as agricultural biocides, marine antifouling paints, and stabilizers to lessen the effects of heat and light in polyvinyl chloride plastics (Blunden and Evans, 1990). With increased usage of organotin compounds, considerable attention has focused on potential toxicity. Numerous studies have assessed exposure and toxicological effects in humans. Triorganotin groups are usually more toxic than di- or mono-compounds of the same chain length. Tributyltin and triphenyltin compounds have embryotoxic, myotoxic, genotoxic and immunotoxic effects in mammals (Boyer, 1989; Kang et al., 1997; Chao et al., 1999; Cima et al., 2003; Wilson et al., 2004) and have been associated with occupational poisoning, hepatic injury, acute nephropathy, and mucous membrane irritation (Colosio et al., 1991; Lin and Hsueh, 1993; Wax and Dockstader, 1995). Derivatives of these compounds are known to induce imposex (the superimposition of male sex organs, penis and vas deferens, in females) in marine neogastropods and to cause developmental and reproductive toxicity in mammals by disrupting endocrine systems. These toxic effects have recently become a matter of substantial concern (Horiguchi et al., 1998; Matthiessen and Gibbs, 1998; Cooke, 2002; Grote et al., 2004).

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After ingestion, tributyltin and triphenyltin compounds undergo dealkylation and dearylation, respectively, by cytochrome P-450 (CYP) enzyme systems in mammals. These metabolic processes generally produce metabolites that are less toxic than the parent compounds (Blunden and Evans, 1990; Suzuki et al., 1992; Ohhira et al., 1999; Appel, 2004). The toxicity of tributyltin and triphenyltin compounds is thus considered to be related to their metabolic fate. We have previously demonstrated that acute triphenyltin toxicity induces hyperglycemia and hypertriglyceridemia in hamsters and that these effects are suppressed by pretreatment with phenobarbital, which increases levels of CYP and accelerates the metabolism of triphenyltin (Ohhira et al., 1999). A better understanding of the metabolism of these triorganotins in humans may help to reduce the risk of associated toxicity. We investigated the metabolism of tributyltin and triphenyltin by using human hepatic CYP systems to confirm the specific CYP that is responsible for the metabolism of these compounds in humans.

2. Materials and methods

2.1. Chemicals

Tributyltin chloride (95%) and triphenyltin chloride (98%) were obtained from Tokyo Chemical Industry (Tokyo, Japan). (±)-Dithiothreitol (DTT), glucose 6-phosphate dehydrogenase (G-6-PDH), D-glucose 6-phosphate disodium salt hydrate (G-6-P), and nicotinamide adenine dinucleotide phosphate oxidized form ($NADP^+$) were obtained from Wako Pure Chemical Industries (Osaka, Japan). Azamulin [14-*O*-(5-(2-amino-1,3,4-triazolyl)thioacetyl)-dihydromutilin] and (*S*)-(+)-*N*-3-benzylnirvanol, which were initially dissolved in acetonitrile and then diluted with pure water before use, were from Gentest Corp. (Woburn, MA). The final acetonitrile concentration of reaction mixtures was less than 1%. All other chemicals were of the highest grade commercially available.

2.2. Sources of human hepatic microsomes and complementary DNA-expressed human microsome P-450s

Hepatic microsomes were used to study the metabolism of tributyltin and triphenyltin in vitro. Mixed, male, and female human hepatic microsomes, derived as pooled human microsomes from 33, 17, and 10 donors, respectively, were purchased from Gentest Corp. The total CYP content of each microsome (picomole CYP per mg protein) (pM/mg) was spectrophotometrically determined by the supplier as follows: 330 (mixture), 310 (male), and 350 (female).

The in vitro metabolism of tributyltin and triphenyltin was also studied with the use of single enzyme systems. Microsomes prepared from baculovirus-infected insect cells (SUPERSOMES) were obtained from Gentest Corp. These microsomes also contained complementary DNA (cDNA)-expressed human P-450 reductase and human cytochrome b_5. The CYP content of each preparation (pM/mg) was spectrophotometrically determined by the supplier as follows: 149 (CYP1A2); 67 (CYP2A6); 159 (CYP2B6); 313 (CYP2C8); 476 (CYP2C9); 244 (CYP2C18); 233 (CYP2C19); 116 (CYP2D6); 444 (CYP2E1); and 128 (CYP3A4).

2.3. Assays of the metabolism of tributyltin and triphenyltin with human hepatic microsomes and cDNA-expressed human cytochrome P-450s

The metabolism of tributyltin and triphenyltin was assayed in vitro as described previously (Ohhira et al., 2004, 2006). Briefly, the incubations were carried out in 0.1 M potassium phosphate buffer, pH 7.4, containing 0.1 mM EDTA, 0.1 mM DTT, and an NADPH-generating system including 6 mM $MgCl_2$, 0.33 mM $NADP^+$, 8 mM G-6-P, and 0.1 U/ml of G-6-PDH. Hepatic microsomes (0.2 mg of protein) or microsomes containing cDNA-expressed CYP enzymes (50 pM) obtained from baculovirus-infected insect cells were added to the buffer system, and the final volume for each reaction was 1 ml. The reaction was initiated by adding tributyltin chloride or triphenyltin chloride (each tin compound was 0.5 μg as tin), each of which had been dissolved in acetone. The reaction mixture was then diluted to an appropriate concentration with distilled water. (The final acetone concentration in the incubation mixture was less than 0.05%.) This concentration of organotin was adapted from the optimal concentration used in an earlier study (Ohhira et al., 2003) examining the metabolism of tributyltin and triphenyltin by rat, hamster, and human hepatic microsomes. The reaction mixture was incubated for 180 min at 37 °C, except for dealkylation of tributyltin with cDNA-expressed CYP enzymes (reaction time, 15 min at 37 °C) (Ohhira et al., 2006). The reaction was terminated by adding 5 ml of 1.2 M hydrochloric acid and 10 ml of saturated sodium chloride. The reaction mixtures were extracted with organic solvents for tin analysis. Microsomes containing baculovirus vectors only (Gentest Corp.) served as controls for the assays of tributyltin and triphenyltin metabolism with cDNA-expressed human CYPs.

To study the inhibition of tributyltin and triphenyltin metabolism in vitro, mixed human hepatic microsomes were preincubated with azamulin or *N*-3-benzylnirvanol in the buffer system described above (final concentration, 5 μM) for 10 min at room temperature before assaying the metabolism of tributyltin and triphenyltin. Control incubation mixtures contained equivalent amounts of acetonitrile.

2.4. Measurement of tin metabolites

After studying the in vitro metabolism of tributyltin and triphenyltin, the parent triorganotins and their metabolites (di- and mono-organotins and inorganic tin) in the reaction mixtures were assayed by gas chromatography (GC) as described previously (Ohhira et al., 2003).