REDUCTIVE METABOLISM OF *p,p'*-DDT AND *o,p'*-DDT BY RAT LIVER CYTOCHROME P450

Shigeyuki Kitamura, Yuri Shimizu, Yuko Shiraga, Kazumi Sugihara, and Shigeru Ohta

Graduate School of Biomedical Sciences, Hiroshima University, Japan

Introduction

p,p'-DDT (1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane) is a broad-spectrum insecticide, which was used from the 1940s in large quantities, but was banned in many countries in the 1970s because of its persistence in the environment. It was replaced in part by less persistent alternatives. However, this pesticide is still used mainly in developing countries. *p,p'*-DDT and related compounds, many of which are carcinogenic and mutagenic, are also known to be environmental estrogens. *p,p'*-DDT is a phenobarbital-type inducer which induces the cytochrome P450 (CYP) 2B and 3A subfamily[1, 2]. *p,p'*-DDT and its metabolites accumulate in animal tissues and induce various enzymes [3]. The higher levels of *p,p*'-DDE (1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene) than *p,p*'-DDT in tissues reflect its stability. These compounds mimic hormones and bind to the estrogen receptor and androgen receptor [4]. *p,p'*-DDT and related compounds, *p,p'*-DDD (1,1-dichloro-2,2-bis(4-chlorophenyl)ethane), *o,p*'-DDT (1,1,1-trichloro-2,2-bis(2-chlorophenyl-4-chlorophenyl)ethane) and dicofol (1,1-bis(4-chlorophenyl)-2,2,2-trichloroethanol), are known to be xenobiotic estrogens, but *p,p'*-DDE is an antiandrogen [5]. In order to assess the possible risks associated with human exposure to the pesticide, it is essential to thoroughly elucidate its metabolism in mammalian species, birds, and marine and freshwater species.

p,p'-DDT is converted by reductive dechlorination to *p,p'*-DDD in insects, birds and animals. Several reports have indicated that *p,p'*-DDT is mainly metabolized to *p,p'*-DDE by dehydrochlorination in mammalian species, insects and microorganisms, and *p,p'*-DDD is an intermediate of the reaction, which may proceed via a-hydroxyl DDD [6]. *p,p'*-DDT and *p,p'*-DDE are further oxidized to 2,2-bis(4-chlorophenyl)acetic acid (*p,p*'-DDA), the major excreted metabolite in animals. The dechlorination is of considerable significance, because this reaction is the first and rate-limiting step in the metabolism of *p,p'*-DDT in mammals and microorganisms. A role of CYP in the microsomal reduction of *p,p*'-DDT was suggested [3, 7, 8]. However, the mechanism of microsomal reductive dechlorination of *p,p'*-DDT is not known in detail. Furthermore, the metabolism of *o,p*'-DDT, which contaminates technical-grade DDT to the extent of about 20%, has not been established. In the present study, the *in vitro* metabolism of *p,p'*-DDT and *o,p*'-DDT by rat liver microsomes was examined, focusing on reductive dechlorination to DDD isomers.

Methods and Materials

Animals

Male rats (Slc:SD, 180-210 g, Slc:Japan, Shizuoka, Japan) were used. In some experiments, phenobarbital was administered to rats intraperitoneally at the dose of 80 mg/kg for 3 days, 3-methylcholanthrene at 25 mg/kg for 3 days, dexamethasone at 100 mg/kg for 4 days, clofibrate at 250 mg/kg for 3 days, and acetone at 3 g/kg orally for one day.

Assay of Reductase Activity

The incubation mixture consisted of 0.1 mmol of *p,p'*-DDT or *o,p'*-DDT (50 ml of methanol

solution), 1 mmol of NADPH or NADH, and 0.2 ml of liver microsomes in a final volume of 1 ml of 0.1 M K,Na-phosphate buffer (pH 7.4). Incubation was performed using a Thunberg tube under anaerobic conditions for 30 min at 37 °C. The mixture was extracted once with 5 ml of *n*-hexane and the extract was evaporated to dryness *in vacuo*. The residue was dissolved in 0.1 ml of methanol and then subjected to high-performance liquid chromatography (HPLC).

Results and Discussion

Metabolism of p,p'-DDT by rat liver microsomes

p,p'-DDT was incubated with liver microsomes of untreated rats in the presence of NADPH for the detection of metabolites. Two peaks, *p,p'*-DDD and *p,p'*-DDE, were detected in an HPLC chromatogram of the extract of the incubation mixture.

In the case of boiled microsomes, these metabolites were not detected. The metabolites corresponding to *p,p'*-DDT and *p,p'*-DDE were isolated and identified unequivocally as *p,p'*-DDT and *p,p'*-DDE by mass spectrometry, TLC and HAPLY comparison with authentic samples. The further metabolized products, *p,p'*-DDMU and *p,p'*-DDA, were not detected by HAPLY or TLC as metabolites of *p,p'*-DDT with liver microsomes.

Dechlorinating activity of rat liver microsomes toward p,p'-DDT

The liver microsomes of untreated rats catalyzed the reductive dechlorination of *p,p'*-DDT to *p,p'*-DDD in the presence of NADPH or NADH under anaerobic conditions. The NADH- and NADPH-linked dechlorinating activities of the microsomes toward *p,p'*-DDT were inhibited by SKF 525-A, metyrapone and carbon monoxide. The NADPH-linked activity was also inhibited by ketoconazole. However, the activity was not affected by disulfiram, sulfaphenazole and quinidine. These results suggest that CYP 3A1 functions in the dechlorination of *p,p'*-DDT in untreated rats. These facts suggest that the reductive dechlorination of *p,p'*-DDT was catalyzed by the microsomal CYP system, but not DT-diaphorase, xanthine oxidase or aldehyde oxidase. The effect of the pretreatment of rats with various inducers on the reductive dechlorination of *p,p'*-DDT was examined. When the reductase activities in liver microsomes from untreated, phenobarbital-treated, 3-methylcholanthrene-treated, acetone-treated, clofibrate-treated and dexamethasone-treated rats were compared in the presence of NADPH, enhanced activity compared with that of untreated rats was observed in phenobarbital-treated and dexamethasone-treated rats (Fig. 1)

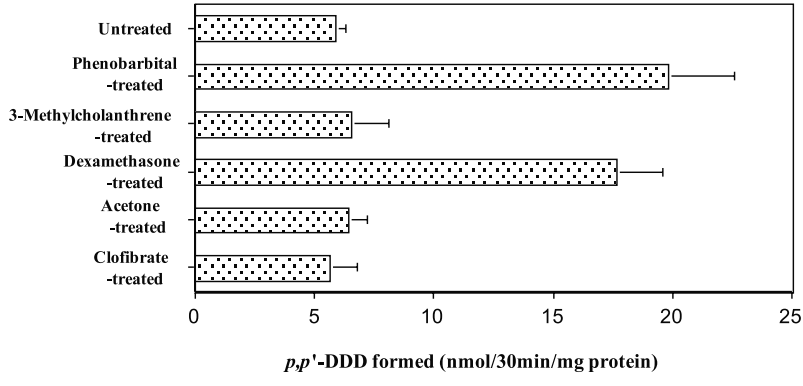

Figure 1. Dechlorination of p,p'-DDT by liver microsomes of rats pretreated with inducers of the cytochrome P450 system.

Identification of CYP Isoforms Involved in the Dechlorination

Identification of CYP isoforms involved in the dechlorination of *p,p'*-DDT in liver microsomes was attempted by using recombinant rat and human CYPs. Recombinant rat CYP 2B1 and 3A1 catalyzed this dechlorination in the presence of NADPH at rates of 1.16 and 1.03 nmol/min/nmol CYP, respectively. Recombinant human CYP 3A4 and 2B6 exhibited substantial activities for dechlorination of *p,p'*-DDT in the presence of NADPH. Human CYPs 1A1, 1A2, 2A6, 2C9, 2D6, 2E1 and 4A11 also showed some dechlorinase activity (Fig. 2). Control microsomes of human B lymphoblastoid cells exhibited no appreciable dechlorinase activity. These facts suggest that the dechlorination of *p,p'*-DDT is catalyzed predominantly by phenobarbital- and dexamethasone-inducible CYPs, but other CYPs also contribute to the dechlorination.

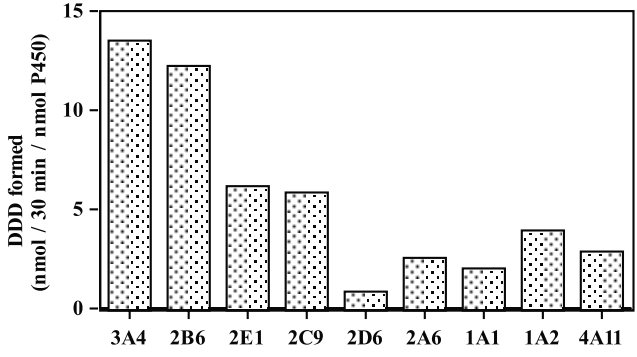

Figure 2. Dechlorination of p,p'-DDT by microsomes from human B lymphoblastoid cells expressing individual recombinant human cytochrome P450.

Reductive metabolism of o,p'-DDT by liver microsomes

o,p'-DDT, the isomer of *p,p'*-DDT, is known to contaminate technical-grade DDT, and to have a higher estrogenic activity than *p,p'*-DDT. In this study, the dechlorinating activity toward *o,p'*-DDT by rat liver microsomes was compared with that toward *p,p'*-DDT. The liver microsomes exhibited reductase activity toward *o,p'*-DDT in the presence of NADPH, NADH or both NADPH and NADH. The reductase activity in the presence of these cofactors toward *o,p'*-DDT was similar to that in the case of *p,p'*-DDT.

Metabolism of p,p'-DDD by liver microsomes

Metabolism of *p,p'*-DDD by rat liver microsomes was also examined. When *p,p'*-DDD was incubated with liver microsomes in the presence of NADPH under anaerobic conditions, two peaks corresponding to authentic *p,p'*-DDE and *p,p'*-DDMU were detected in the HPLC chromatogram of the extract of the incubation mixture. The metabolites isolated by HPLC were identified unequivocally as *p,p'*-DDE and *p,p'*-DDMU by comparison with authentic samples . When *p,p'*-DDE was incubated similarly with liver microsomes in the presence of NADPH, *p,p'*-DDMU was not detected.

In the present study, the dechlorination of *p,p'*-DDT was demonstrated to be mainly mediated by CYP 2B1, 3A1, 2B6 and 3A4. It is known that *p,p'*-DDT induces CYP 2B1 and 4A1 in rat livers [2]. This suggests that the dechlorinating activity toward *p,p'*-DDT would be enhanced by the pretreatment of rats with *p,p'*-DDT. It is reported that the major metabolite of *p,p'*-DDT remaining in tissues of mammalians is *p,p'*-DDE [5]. It remains to be determined whether *p,p'*-DDE is formed via *p,p'*-DDD as suggested above or by dehydrochlorinase, as has been demonstrated in insects. We are conducting a further study on this point.

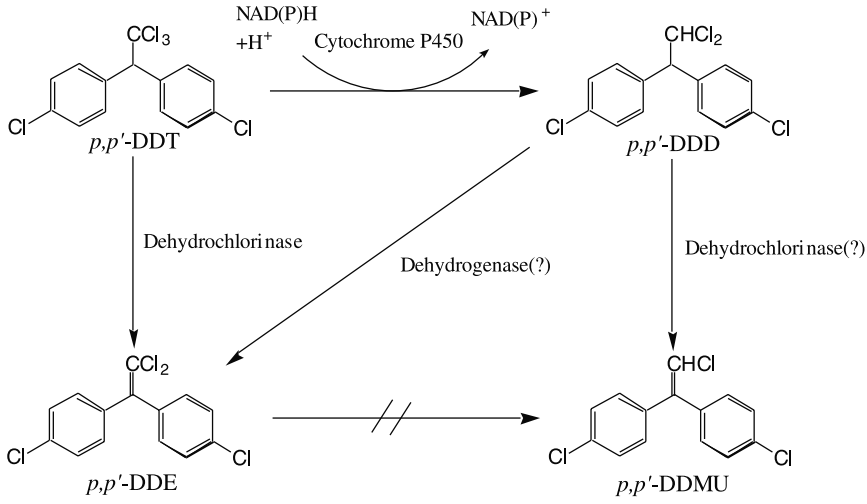

Figure 3. Postulated metabolic pathway of p,p'-DDT by rat liver microsomes

Acknowledgements

This work was supported by a Grant-in-Aid for Scientific Research on Priority Area (13027256) from the Japanese Ministry of Education, Science, Sports and Culture, and a Grant-in-Aid for Scientific Research (C13672343) from Japan Society for the Promotion of Science.

References

1. Lewis DFV and Lake BG (1997) *Xenobiotica* 27:443-478.
2. Nims RW, Luubet RA, Fox SD, Jones CR, Thomas PE, Reddy AB and Kocarek TA (1998) *J Toxicol Environ Health* 53:455-477.
3. Esaac EG and Matsumura F (1980) *Pharmac Ther* 9:1-26.
4. Kelce WR, Stone CR, Laws SC, Gray LE, Kemppainen JA and Wilson EM (1995) *Nature (London)* 375:581-585.
5. You L (2000) *CIIT Activities* 20:1-9.
6. Fox SD, Roman JM, Issaq HJ and Nims RW (1998) *Arch Environ Contam Toxicol* 35:104-108.
7. Baker MT and Van Dyke RA (1984) *Biochem Pharmacol* 33:255-260.
8. Kelner MJ, Mclenithan JC and Anders MW (1986) *Biochem Pharmacol* 35:1805-1807.