TOX (po)

Time-dependent Alterations of 1,3,6,8-TCDD Metabolites Formed by Mouse Liver Microsome

Osamu Aozasa, Teruyuki Nakao, Souichi Ohta and Hideaki Miyata
Faculty of Pharmaceutical Science, Setunan University 45-1, Nagaotoge-cho, Hirakata, Osaka 573-01, Japan

1. Introduction

At present, it is generally assumed the metabolic process of polychlorinated dibenzo-*p*-dioxins (PCDDs) plays a major role as a detoxification function, because of the acute toxicity of the excreted metabolites to the rodent is at least two order of magnitude less than that of their parent compounds[1,2]. PCDDs are metabolized to phenolic compounds in the liver in mammals[3,4]. The metabolic degradation of PCDDs to hydroxylated metabolites is catalyzed by cytochrome P-4501A1[5]. The hydroxylated metabolites are conjugated with glucuronic acid[6]. The formed glucronide conjugates are easily excreted in urine and bile. However, a enterohepatic circulation of metabolites extracted from bile in the dog has been observed in the rat[7]. Previously, it was appeared that metabolites in urine than in bile were more polar[8]. Therefore, the reabsorbed metabolites might be further metabolized to reactive electrophlic intermediates and metabolites in the liver.
The goal of this study is to reveal the biotransformation of several PCDD isomers to novel metabolites, such as quinones and sulfate conjugates, which have a potency of remaining in tissue of mammals. A low toxic 1,3,6,8-tetrachlorodibenzo-*p*-dioxin (1,3,6,8-TCDD) was initially selected, because this isomer was present as a major by-product in an agrochemical, chlornitrofen (CNP), used largely in Japan. In this study, we investigated on the *in vitro* metabolism of 1.3.6.8-TCDD in mouse liver microsome.

2. Methods

Male C57BL/6 mice weighing about 23g, 9 week old (Nihon SLC, Shizuoka, Japan), were treated with an oral administration of 2,3,7,8-TCDD in corn oil at a single dose of 10 μg/kg body weight. Mice were sacrificed by cervical dislocation 24 hr. after dosing, and hepatic microsomes were prepared by the method of Guenderich[9]. The activity of cytocrome P4501A1 dependent ethoxyresorufin O-deetylase (EROD) in hepatic microsome was determined fluorimetrically according to the procedures of Pohl and Fouts[10]. The hepatic microsomes were used for the *in vitro* metabolism of 1,3,6,8-TCDD. The *in vitro* experiment was carried out according to the method reported by Isida et all[11].

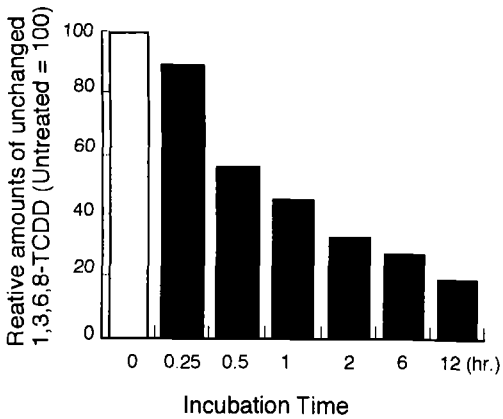

Fig. 1 The amounts of unchanged 1.3.6.8-TCDD after incubation in *in vitro* metabolism using the hepatic microsome from mice treated with 2,3,7,8-TCDD

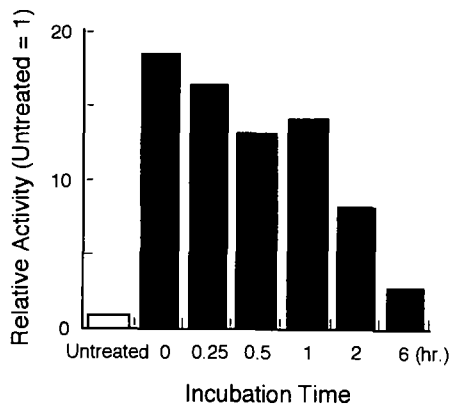

Fig. 2 EROD activity throughout incubation with hepatic mocrosome from mice treated with 2,3,7,8-TCDD

1,3,6,8-TCDD of 10 μg was incubated with microsome of 1 mg protein for 12 hr. The analytical procedures of formed metabolites were described elsewhere[12].

3. Results and Discussion

We have already reported that microsomal enzymes induced by 2,3,7,8-TCDD transformed 1,3,6,8-TCDD to eight hydroxylated metabolites, which were composed of one monohydroxylated trichlorodibenzo-*p*-dioxin (TrCDD), two dihydroxylated TrCDDs, three monohydroxylated TCDDs and two dihydroxylated TCDD isomers. In addition, a fragment ion cluster of $[M-15]^+$ was confirmed in mass spectra of all metabolites. The results indicated the hydroxylation of 1,3,6,8-TCDD caused at either 2-, 3-, 7- or 8-position of thier molecules. Therefore, in this study, the behavior of metabolites was investigated during a period of longer incubation time.

Fig. 1 shows the time-dependent alteration of amount of unchanged 1,3,6,8-TCDD in suspension medium during a period of 12 hr. incubation time in *in vitro* metabolism using liver microsomes from mice treated with 2,3,7,8-TCDD at a single dose of 10 μg/kg body weight. Compared to the no incubation suspension (control), the decreased to 56% at the first 0.5 hr. incubation. However, the decrease rate for the consecutive 0.5 hr. incubation was only 10%, that is, the metabolic rate of 1,3,6,8-TCDD reduced with an increase of incubation time. At the 12 hr. incubation, the residue amount deceased to 19% of the control, that is, 81% of 1,3,6,8-TCDD was metabolized during a period of 12 hr. incubation time with the hepatic microsome.

Fig. 2 shows the time-dependent alteration of EROD activity in suspension medium throughout incubation with the hepatic microsome from mice treated with 2,3,7,8-TCDD.

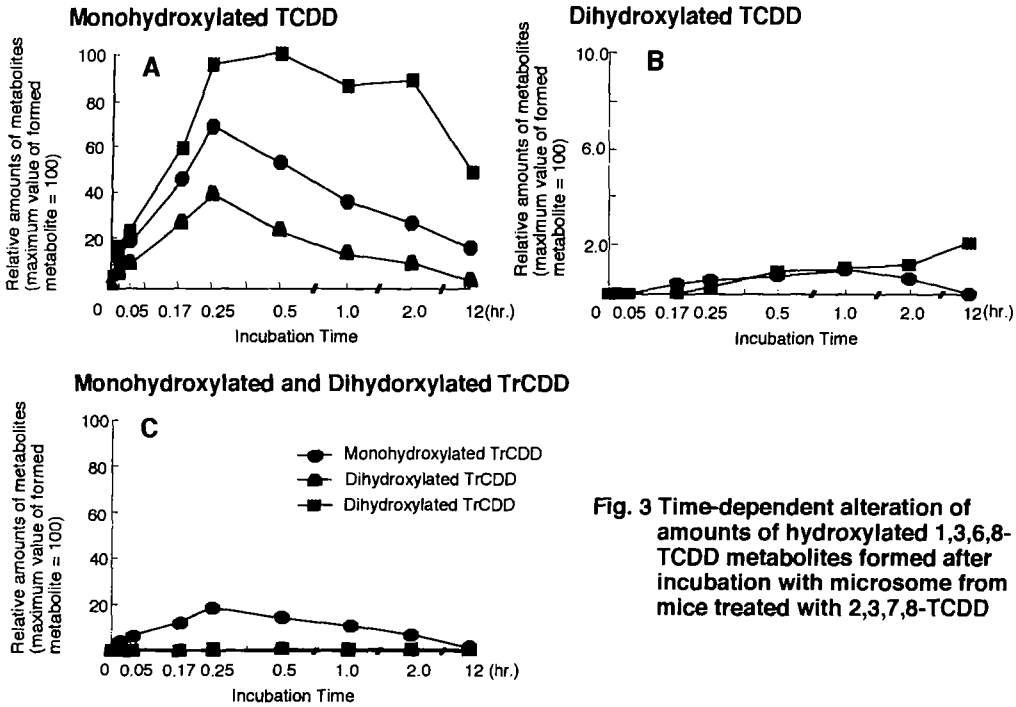

Fig. 3 Time-dependent alteration of amounts of hydroxylated 1,3,6,8-TCDD metabolites formed after incubation with microsome from mice treated with 2,3,7,8-TCDD

A slight decrease of hepatic microsomal EROD activity was observed at the incubation for the first 1 hr. indicating the microsomes to have an active metabolic potency for 1,3,6,8-TCDD. However, the enzymatic potency decreased remarkably after the incubation of 2 hr.

Fig. 3 illustrates the time-dependent alteration of amounts of hydroxylated 1,3,6,8-TCDD metabolites formed after incubation with the hepatic microsome from mice treated with 2,3,7,8-TCDD. Fig. 3-A shows the generation amounts of formed three monohydroxylated TCDDs. The amount of the major metabolite (illustrated as a mark of ■) was the maximum at 0.5 hr. throughout the 12 hr. incubation time. However, the maximum formation of other metabolites was observed at a shorter time of 0.25 hr. The generation of all three monohydroxylated TCDDs decreased with an increase of incubation time. Regard other five metabolites of two dihydroxylated TCDDs, one monohydroxylated TrCDD and two dihydroxylated TrCDD, the maximum generation amounts of four metabolites except for one monohydroxylated TrCDD were all one order lower level than those of monohydroxylated TCDDs (Fig. 3-B, C). On the other hand, the last one was largely formed (Fig. 3-C). The maximum level was 18 % of that of the major hydroxylated TCDD (■ in Fig. 3-A). As well as cases of monohydroxylated TCDDs, the generation of this metabolite decreased also with an increase of incubation time. Therefore, the total amounts of eight hydroxylated metabolites decreased with an increase of incubation time, although 80% of 1,3,6,8-TCDD was metabolized for the 12 hr. incubation time. From the above results, it is suggested that the hydroxylated

metabolites might be further biotransformed to other metabolites, such as quinones, sulfate conjugates and/or small molecular compounds, by hepatic enzymes for incubation with the liver microsome. Current studies are focused on identifying novel metabolites using the several methods of extraction, clean-up and isolation for TCDD metabolites in suspension.

4. References

1) Weber H., H. Poiger and Ch. Schalatter (1982) Acute Oral Toxicity of TCDD-Metabolites in Male Guinea Pigs. Toxicol. Lett. 14, 117-122
2) Mason G. and S. Safe (1986) Synthesis, Biologic and Toxic Effects of the Major 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin Metabolites in the Rats. Toxicology 41, 153-159
3) Tulp M.Th.M. and O. Hutzinger (1978) Rat Metabolism of Polychlorinated Dibenzo-*p*-dioxins. Chemosphere 9, 761-768
4) Poiger H. and H.-R. Buser (1984) The Metabolism of TCDD on the Dog and Rat. Biological Mechanisms of Dioxin Action vol. 18, pp. 39-47
5) Olson J.R., B.P. McGarrigle, P.J. Gigliotti, S. Kumar and J.H. McReynolds (1994) Hepatic Uptake and Metabolism of 2,3,7,8-Tetrachlodibenzo-*p*-dioxin and 2,3,7,8-Tetrachlorodibenzofuran. Fumdam. Appl. Toxicol. 22, 631-640
6) Poiger H. and Ch. Schlatter (1979) Biological degradation of TCDD in rats. Nature 281, 706-707
7) Weber H., H. Poiger and Ch. Schlatter (1982) Fate of 2,3,7,8-tetrachlorodibenzo-p-dioxin metabolites from dogs in rats. Xenobiotica 12, 353-357
8) Gasiewics T.A., L.E. Geiger, G. Rucci and R.A. Nael (1983) Distribution, excretion, and metabolism of 2,3,7,8-tetrachlorodibenzo-p-dioxin in C57Bl/6J, DBA/2J, and B602F/J mice. Drug Metab. Disp. 11, 397-403
9) Guengerich F.P., (1982) Microsomal enzymes involved in toxicology-analysis and separation. Principles and Methods of Toxicology, pp. 609-634
10) Pohl R.J. and J.R. Fouts (1980) A Rapid Method for Assaying the metabolism of 7-Ethoxyresorufin bynMicrosomal Subcellar Fractions. Analytical Biochemisty 107, 150-155
11) Isida C., N. Koga, N. Hanioka, H. Saeki and H. yoshimura (1991) Metabolism *in vitro* of 3,4,3',4'- and 2,5,2',5'-Tetrachlorobiphenyl by Rat Liver Microsomes and Cytochlrome P-450. J. Pharmacobio Dyn. 14, 276-284
12) Aozasa O., S, Ohta and H. Miyata (1995) PCDD Metabolites Formed by Mouse Liver Microsomes. Organohalogen Compounds 25, 327-330