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Increase of DT-diaphorase Activity and Atrophy of Thymus by Organotin Compounds

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Organotin compounds of differing structure are widely used as polyvinyl chloride stabilizer, antifouling paints and biocides such as fungicides and bactericides in agriculture. Since these uses are dispersive, it has been noted that elevated levels of organotin compounds exist in aqueous environment and marine products. Although inorganic tin compounds are basically not harmful, some organotin compounds have different biological and toxicological effects. In general, trialkyltin and triaryltin compounds appear to be more toxic than the mono-, di-, or tetra-compounds of the same chain length(Blunden and Chapman 1986). Alkyltin compounds are more toxic than aryltins, and acute toxicity of alkyltins is known to decrease with an increase in the number of carbon atoms(Blunden and Chapman 1986).

Rosenberg et al.(1980) reported that the administration of certain organotin compounds in single doses can produce a significant and prolonged induction of heme oxygenase, the rate-limiting enzyme of heme degradation, and a sustained decrease in cytochrome P-450 content in the liver of rats. Our preliminary study in rats also noted that organotin compounds, such as trimethyltin chloride(TMTC), tributyltin chloride(TBTC), triphenyltin chloride(TPhTC), dimethyltin dichloride(DMDC), dibutyltin dichloride (DBDC) and diphenyltin dichloride(DPhDC) led to a specific induced pattern in heme oxygenase activity with time and diminished cytochrome P-450 content in the liver(Arizono et al. 1989). However, we found a significant increase in DT-diaphorase activity in some organotins-treated rats(unpublished data), an effect which has not been described in the literature.

DT-diaphorase is a flavoprotein, which catalyzes the oxidation of NADH and NADPH by various redox dyes and quinones(Ernster et al. 1962). In addition, this enzyme is known to be induced by an inducers of the microsomal aryl hydrocarbon hydroxylase system, such as 3-methylcholanthrene(Lind and Ernster 1974), 2,3,7,8-tetrachlorodibenzo-p-dioxin(Beatty and Neal 1976), 2,3,7,8-tetrachlorodibenzofuran(Yoshihara et al. 1981) and β-naphthoflavone(Kumaki et al. 1977). In this study, we investigated in the

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effects of organotins on the activities of hepatic DT-diaphorase, P-450 enzymes and on their effect on the thymus.

MATERIALS AND METHODS

Enzyme, cofactor and chemical sources were as follows:D-glucose-6-phosphate, D-glucose-6-phosphate dehydrogenase and NADPH from Boehringer-Mannheim-Yamanouchi Co.,Ltd.,(Tokyo,Japan);NADP from Oriental Yeast Co.,Ltd.,(Tokyo, Japan);bovine serum albumin from Sigma Chemical Co.,(St.Louis,USA);TMTC, TBTC, TPhTC, DMDC and DBDC from Tokyo Kasei Co.,Ltd.,(Tokyo,Japan);DPhDC and 7-ethoxycoumarin from Aldrich Chemical Co.,Inc.,(Milwaukee,USA);2,6-dichlorophenolindophenol(DCPIP) from E.Merck A.G.(Darmstadt, W. Germany) and other chemicals were of highest grade available.

Male Wistar rats were used in all experiments. Animals received normal rat chow and water ad libitum. Rats were generally given a single subcutaneous injection with organotin compounds which dissolved in corn oil at 1 ml per dose per kg of body weight. Control rats were subcutaneously injected with corn oil alone at 1 ml/kg. A single dose of organotins was 50 μmole/kg body weight except TMTC which is high toxicity used a dose of 25 μmole/kg. After treatment with organotins animals were killed by decapitation, and thymus and spleen were dissected out, washed with saline, blotted, and weighed. The liver was perfused in situ with a cold 0,9% saline and then removed, washed, blotted and weighed. Subsequent procedures were carried out in a similar manner described previously(Ariyoshi et al. 1990).

Heme oxygenase activity was calculated from the amount of bilirubin formed by using an extinction coefficient of 40 $mM^{-1}cm^{-1}$ between 464 and 530nm as described by Maines and Kappas(1976). Cytochrome P-450 was estimated by the method of Omura and Sato(1964) using an extinction coefficient of 91$mM^{-1}cm^{-1}$ between the absorbance spectra at 450 and 490nm following carbon monoxide bubbling. DT-diaphorase activity was measured spectrophotometrically by the reduction of DCPIP using procedure of Ernster(1967). P-Aminophenol formed by aniline hydroxylase was determined by the method of Imai et al.(1966), and formaldehyde formed by aminopyrine N-demethylase was determined according to Nash(1953). 7-Ethoxycoumarin O-deethylase(7-EC) activity was measured by recording the fluorescence increase due to the formation of 7-hydroxycoumarin as reported by Ullrich and Weber(1972). Protein concentration was determined according to Lowry et al.(1951) using bovine serum albumin as a standard.

RESULTS AND DISCUSSION

Fig. 1 shows the time course of six organotin compounds effects on heme oxygenase activity and cytochrome P-450 content in the liver of rats. All compounds tested markedly induced heme oxygenase, and that activity reached peak values at 48 hr after treatment, and then gradually decreased except the activity induced by TPhTC. Five organotins except DMDC produced significant losses in cytochrome P-450 content at 48 or 96 hr after administration.

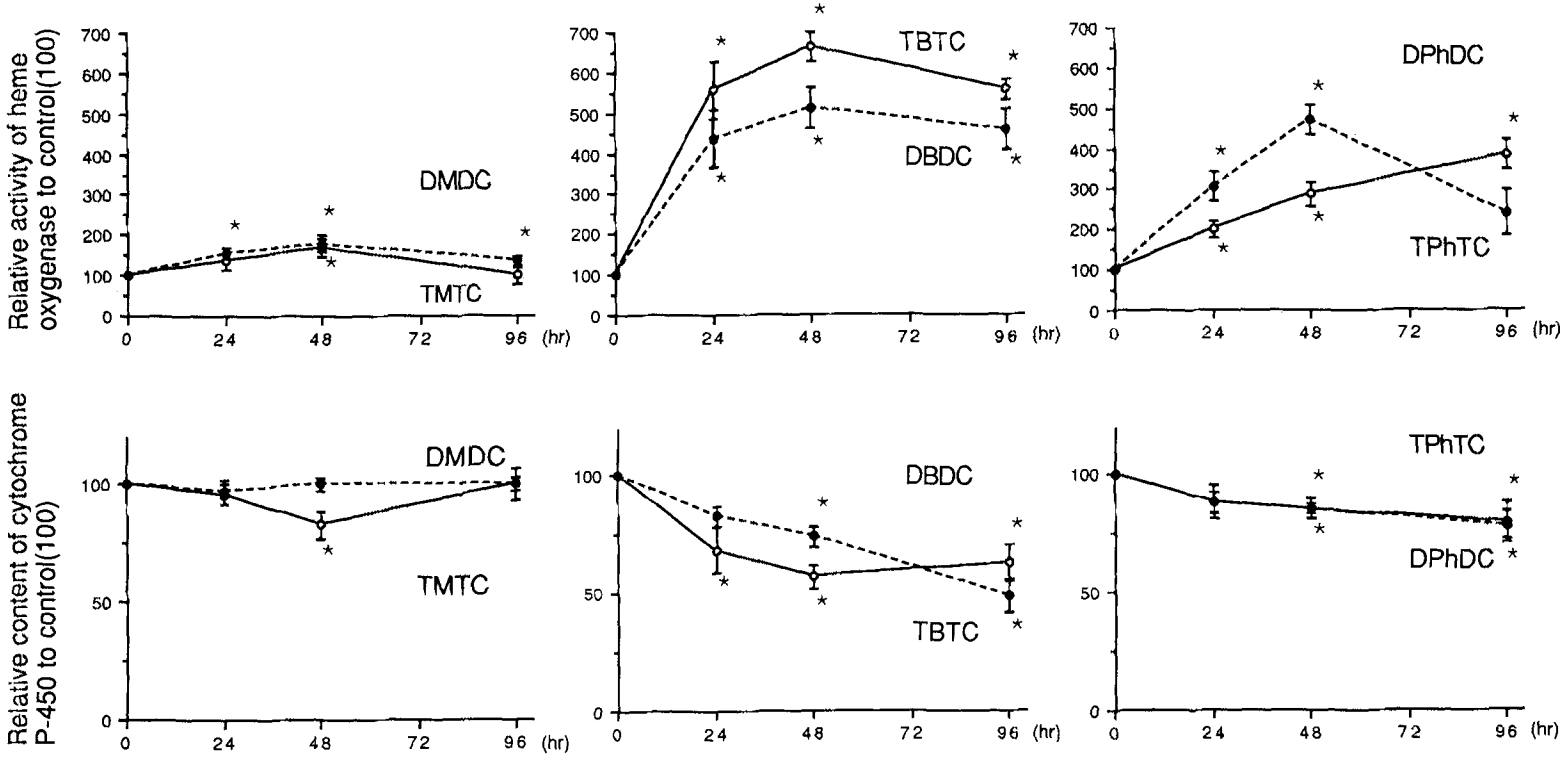

Figure 1. Time course of induction of heme oxygenase activity and of loss of cytochrome P-450 content after organotins administration. The average activity and content in the controls(mean ± S.E.) were : heme oxygenase,1.10 ± 0.12 nmole / mg protein / hr; cytochrome P-450, 0.85 ± 0.03 nmole / mg protein. 4 rats / group, * $P<0.05$.

These results agree with the observation of Rosenberg et al.(1980). They demonstrated that highly water-soluble compound diethyltin dichloride(DEDC) did not produce marked induction of hepatic heme oxygenase at 72 hr, but DEDC markedly increased heme oxygenase activity at 16-24 hr after its administration. In contrast, the least water-soluble organotins produced the greatest induction of hepatic heme oxygenase at 72 hr. Their observation and our findings suggest that such prolonged induction of heme oxygenase and continued reduction of cytochrome P-450 content may be based upon the differences in the lipid solubility, organ distribution or metabolism of each organotin compounds.

In a single dose of 50 μmole/kg(except TMTC dose of 25 μmole/kg), DT-diaphorase activity and thymus atrophy were examined at 24, 48 and 96 hr after treatment of rats with organotins, and the results are summarized in Fig. 2. The increase in DT-diaphorase activity was noted 48 or 96 hr after treatment with TBTC, DBDC, TPhTC and DPhDC, whereas TMTC(except at 24 hr) and DMDC did not significantly change the enzyme activity at any time after injection. This enhanced effect on DT-diaphorase activity produced by organotin treatment has not been described in the literature.

On the other hand, thymus weight per 100g body weight was considerably depressed by TBTC, DBDC and TPhTC treatments. Seinen and Willems(1976) observed the thymus atrophy in rats fed diets containing dioctyltin dichloride (DODC) for 6 weeks. In addition, Seinen et al.(1977a) reported that DEDC and dipropyltin dichloride showed similar but less pronounced effect on thymus atrophy, and that mono-, di-, tri- and tetra-alkyltin compounds did not cause that atrophy. Our results obtained from DMDC or TMTC treatment, despite a single treatment, partly agree with their observations. However, we found that there was a good reciprocal correlation between DT-diaphorase activity and thymus weight (TBTC:r=0.74,n=12; DBDC: r=0.73,n=12).

It is well known that DT-diaphorase is induced by certain methylcholanthrene(MC)-type inducer which specifically induced aryl hydrocarbon hydroxylase(AHH). Yoshimura et al.(1979) reported that thymus atrophy by highly chlorinated biphenyl congeners correlated with their MC-type inducing abilities for the hepatic microsomal mixed function oxidase and cytosolic DT-diaphorase. In our experiments, DT-diaphorase was induced by some organotins, especially, that activity was still enhanced at 96 hr after a single injection of TBTC. Therefore, we investigated further time course of TBTC effects on organ weights, cytochrome P-450 content, heme oxygenase and drug-metabolizing enzymes activities as well as DT-diaphorase. The results are summarized in Table 1.

Thymus weight was restored to the control level on day 7 after treatment, although a significant decrease was observed 4 days after injection as shown in Fig.2. However, we observed an increase of spleen weight on day 7 or 12 after a single injection of TBTC. The means of spleen enlargement is not clear at the present time, but that may be due to the adaptive increase of reticuloendothelial system or phagocytosis in this organ which

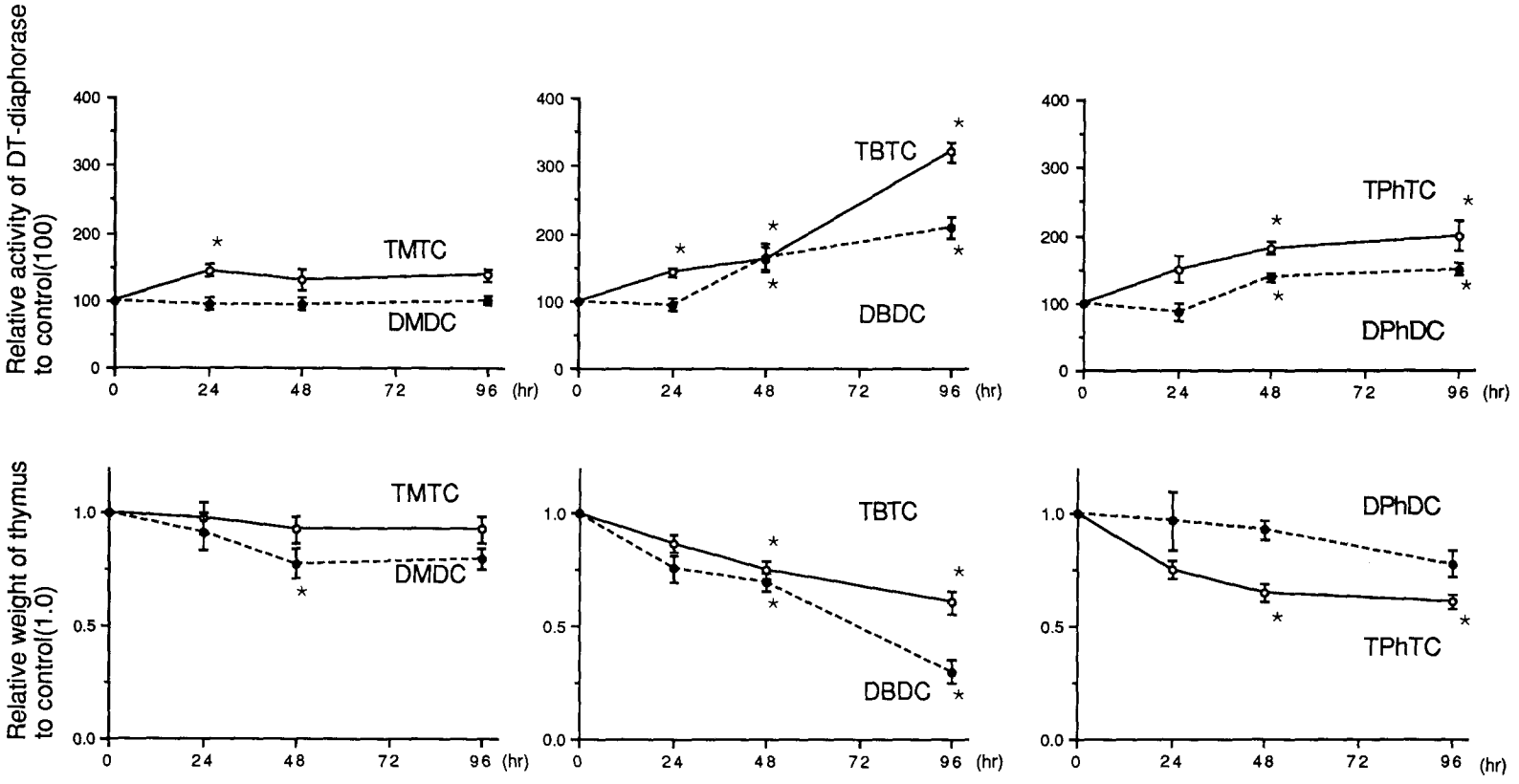

Figure 2. Time course of induction of DT-diaphorase activity and of thymus atrophy after organotins administration. The average activity and organ weight in the controls(mean ± S.E.) were : DT-diaphorase, 0.264 ± 0.021 μmole DCPIP reduced / mg protein / min ; thymus, 0.352 ± 0.022 g / 100g body weight. 4 rats / group, *P<0.05.

Table 1. Time course of tributyltin chloride(TBTC) effects on organ weights, microsomal cytochrome P-450 content and hepatic enzymes activities after administration

	Days after treatment			
	Control	7 d	Control	12 d
Organ weight[a)]				
Liver	5.09 ± 0.04	4.86 ± 0.08	4.68 ± 0.06	4.53 ± 0.12
Thymus	0.39 ± 0.02	0.40 ± 0.02	0.37 ± 0.02	0.41 ± 0.02
Spleen	0.38 ± 0.02	0.56 ± 0.02***	0.35 ± 0.02	0.59 ± 0.03***
Cytochrome P-450[b)]	0.90 ± 0.01	0.75 ± 0.03***	0.87 ± 0.05	0.80 ± 0.03
Enzymes activity				
Heme oxygenase[c)]	1.11 ± 0.14	1.94 ± 0.18*	1.04 ± 0.13	1.63 ± 0.19
DT-diaphorase[d)]	0.185 ± 0.015	0.363 ± 0.024*	0.222 ± 0.045	0.237 ± 0.023
Aniline hydroxylase[e)]	0.83 ± 0.05	0.65 ± 0.03***	0.84 ± 0.03	0.68 ± 0.03**
Aminopyrine N-demethylase[e)]	8.94 ± 0.39	6.28 ± 0.48*	9.27 ± 0.80	7.42 ± 0.49
7-Ethoxycoumarin O-deethylase[e)]	0.57 ± 0.02	0.57 ± 0.03	0.65 ± 0.02	0.62 ± 0.02

Animals were subcutaneously injected with TBTC at a single dose of 50 μmole/kg. Each value represents the mean ± S.E. of 6 rats. Significantly different from corresponding mean of contrtol(*P<0.05, **P<0.02, ***P<0.01). a): g/100g body weight; b):nmole/mg protein; c):nmole/mg protein/hr; d): μmole DCPIP reduced/mg protein/min; e)nmole/mg protein/min.

take a destroyed red blood cell, heme or hemoproteins caused by sustained induction of heme oxygenase after treatment with organotins.

The activities of heme oxygenase and DT-diaphorase increased after treatment, whereas cytochrome P-450 content decreased, accompanied by a decrease in the activities of drug-metabolizing enzymes except 7-ethoxycoumarin O-deethylase, this is, the continuation in activity of aniline hydroxylase up to day 12. These findings are apparently distinct from induction profile of DT-diaphorase and AHH produced by the conventional MC-type inducers.

However, previous studies have shown that lead acetate-, 3,5-di-tert-butyl-4-hydroxytoluene(BHT)- and 2(3)-tert-butyl-4-hydroxyanisole(BHA)-feeding appreciably induced DT-diaphorase, whereas these treatments did not increase AHH activity (Iannaccone et al 1976; Suzuki et al.1979; Benson et al.1980). More detailed works on the thymus by these chemicals are needed.

Seinen et al.(1977a) reported that thymus atrophy caused by organotins is shown only in rats, while that atrophy did not occur in mice, guinea pig or Japanese quail fed DODC or DBDC, and further observed that DODC or DBDC induced immune suppression in rats by a selective inhibition of T-lymphocyte activity(Seinen et al. 1977b).

As the lipid-soluble organotin compounds would be induced the unexpected effects on the living animals, further studies will be needed from various viewpoints.

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