AH-RECEPTOR MEDIATED INDUCTION OF LIVER XANTHINE OXIDASE / XANTHINE DEHYDROGENASE BY 2,3,7,8-TCDD

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Introduction

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is one of the most toxic polychlorinated organic pollutants that have been studied. Most of the effects of TCDD are mediated by a cytosolic receptor known to as the aryl hydrocarbon receptor (AhR), which exists as cytoplasmic aggregates bound to heat shock protein 90 (HSP 90). Upon TCDD binding, AhR dissociates from HSP 90 and the ligand-receptor complex translocates to the nucleus. Then, AhR binds to the AhR nuclear translocator protein (ARNT), and this complex recognizes and binds to the xenobiotic response element (XRE) sequence upstream of the target genes to activate their transcription. Several xenobiotic-metabolizing enzymes, such as cytochrome P450 1A1 (CYP1A1), 1A2 (CYP1A2), UDP-glucuronosyltransferase, NAD(P)H-quinone oxidoreductase, and aldehyde dehydrogenase-3, have the XRE sequence in the 5'-upstream region of their genes and AhR-mediated induction of gene expression by TCDD is observed. However, the role of the induced xenobiotic-metabolizing enzymes in the toxic effects of TCDD is poorly understood. Recently, it has been reported that AhR-null (*Ahr*$^{-/-}$) mice do not exhibit TCDD-induced liver toxicity (1) or teratogenicity (2). In this study, we examined other enzymes that might be relevant to the toxicity of TCDD. Xanthine dehydrogenase (XDH) and xanthine oxidase (XO) are molybdenum-containing flavoenzymes and catalyze oxidation of hypoxanthine to xanthine and xanthine to uric acid with concomitant reduction of NAD^+ or molecular oxygen. The reaction generates reactive oxygen species, and XO is involved in lipid peroxidation (3) and reperfusion injury (4). Though XDH/XO are induced by bacterial lipopolysaccaride (5) and hypoxia (6), there is no report about the induction of them by chemicals. In this study, we showed that XDH/XO activities are induced by TCDD and 3-methylcholanthrene (MC), and the inductions are mediated by AhR.

Materials and Methods

TCDD was purchased from Cambridge Isotope Laboratories Japan. Male C57BL/6J:Jcl mice (8-9 weeks) were housed in the cages. The generation of *Ahr*$^{-/-}$ and checking of genotypes of mice were done as previously reported (2). Male C57BL/6J mice (8 weeks old) were given TCDD by gavage with a single dose of 40 μg / kg body weight dissolved in corn oil. MC was given by intraperitoneal injection (25 mg / kg body weight). Vehicle mice were given the same volume of

corn oil. The TCDD-treated mice were killed 3 days and 1, 2 and 4 weeks later and the MC-treated mice 5 days later.

The livers were quickly removed, and microsomes and cytosol were prepared according to usual methods. The ethoxyresorufin-*O*-dealkylase (EROD), methoxyresorufin-*O*-dealkylase (MROD) and pentoxyresorufin-*O*-dealkylase (PROD) activities in liver microsomes were assayed by a fluorophotometric method (7). Xanthine dehydrogenase (XDH) and xanthine oxidase (XO) activities in liver cytosol were assayed using 1-methylxanthine as a substrate with or without NAD^+. The oxidative product, 1-methyluric acid, was measured with HPLC. Male $Ahr^{-/-}$ mice (10 weeks old) were treated with TCDD in the same manner as $Ahr^{+/+}$ (wild) mice.

Results and Discussion

The microsomal alkylresorufin-*O*-dealkylase activities, EROD, MROD and PROD, of male C57BL/6J mice a week after treatment with TCDD or MC are shown in Fig. 1, (A). EROD and MROD activities were increased 59- and 47-fold, respectively, by a single dose of TCDD compared with the control mice given the vehicle only. MC also induced EROD and MROD activities by 28- and 16-fold, respectively. However, PROD activity, which is catalyzed by the phenobarbital-inducible cytchrome P450 isozyme CYP2B1, was not induced by TCDD or MC. The activities of XDH and XO in liver cytosol of mice were enhanced 2.5-2.3 times by TCDD and MC (Fig. 1, (B)).

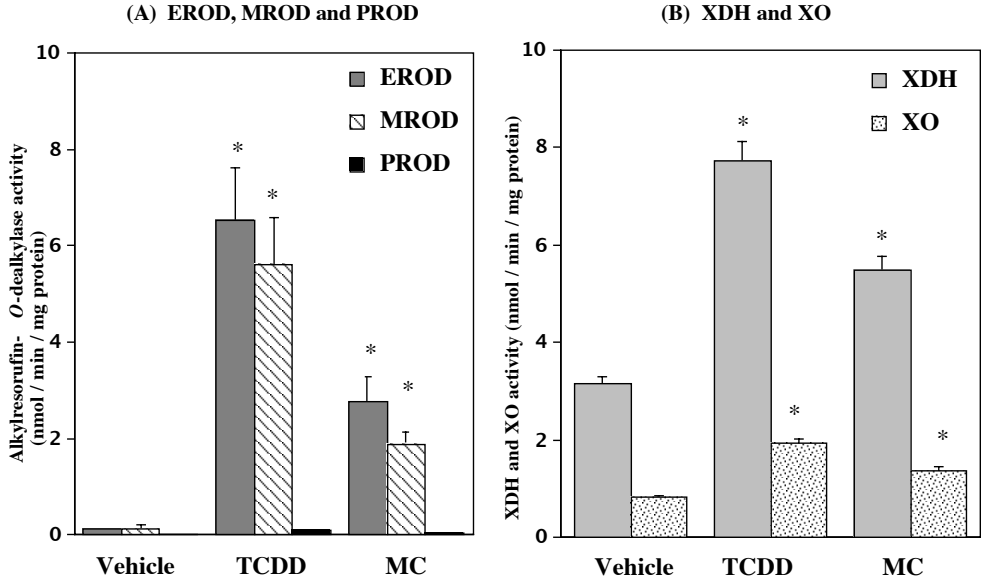
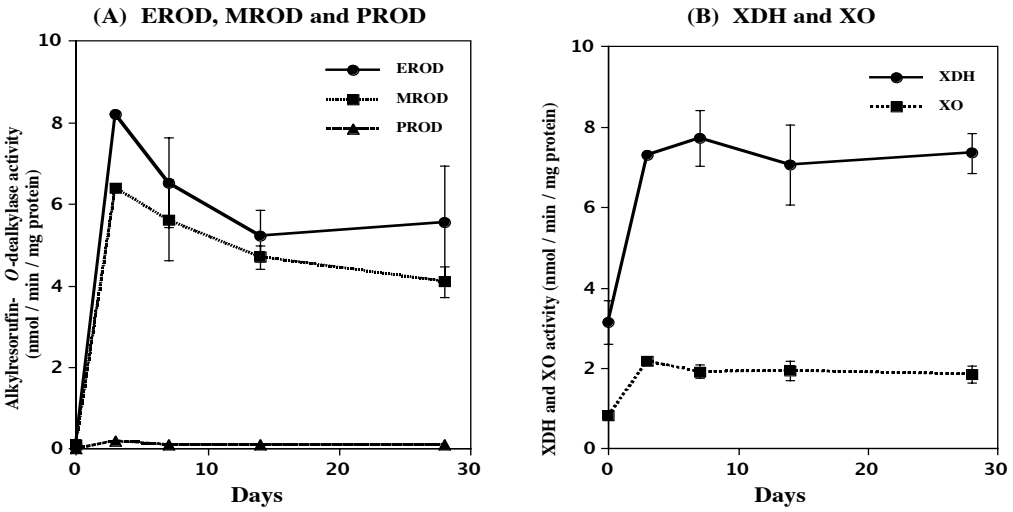


Fig. 1. Effects of TCDD and MC on EROD, MROD, PROD, XDH, and XO activities in mice.

(A): The activities of EROD, MROD and PROD (nmol / min / mg protein) in liver microsomes of C57BL/6J mice dosed with vehicle, TCDD or MC. (B): XDH and XO activities (nmol / min / mg



protein) in liver cytosol of the same mice. Treatments were described in Materials and Methods. Each bar is the mean + s.d. of four individual mice. *P<0.001 compared with vehicle (Welch's t test).

Fig. 2. Time courses of liver enzyme activities in TCDD-treated mice (A): The activities of EROD, MROD and PROD (nmol / min / mg protein) 3 days and 1, 2 and 4 weeks after TCDD. (B): The activities of XDH and XO (nmol / min / mg protein) 3 days and 1, 2 and 4 weeks after TCDD. Each dot is the mean ± s.d. of three individual mice.

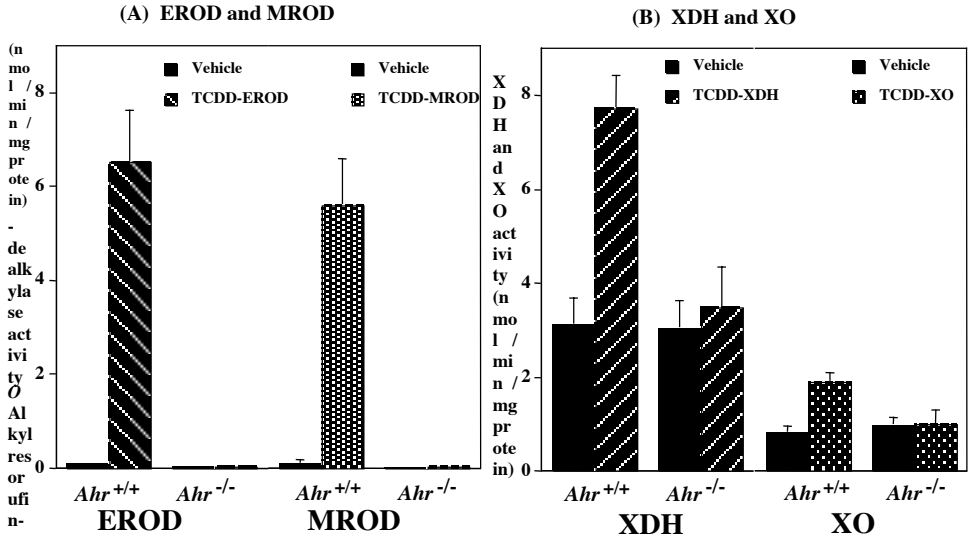


Fig. 3. Effects of TCDD on EROD, MROD, XDH and XO activities in liver preparations of *Ahr*$^{+/+}$ and *Ahr*$^{-/-}$ mice. (A): EROD and MROD activities in liver microsomes of *Ahr*$^{+/+}$ and *Ahr*$^{-/-}$ mice with or without TCDD treatment. (B): XDH and XO activities in liver microsomes of *Ahr*$^{+/+}$ and *Ahr*$^{-/-}$ mice with or without TCDD treatment. Each bar is the mean + s.d. of four individual mice.

The activities of these enzymes in liver microsomes and cytosol were assayed at various times after TCDD. The activities were increased within 3 days and the induced activities were well maintained for 4 weeks (Fig. 2). The inductions of EROD and MROD activities by TCDD were proved to be mediated by AhR, since TCDD treatment of *Ahr*$^{-/-}$ mice, which lack the AhR gene, produced no enhancement of EROD and MROD activities in liver microsomes (Fig. 3, (A)). XDH and XO activities of *Ahr*$^{+/+}$ (C57BL/6J) mice were also increased by TCDD. However, no induction was observed in *Ahr*$^{-/-}$ mice. Thus, induction of XDH and XO activities was also mediated by AhR (Fig. 3, B). But, there has been no report about the existence of an XRE in the 5'-upstream region of the XDH gene (8).

This study demonstrated for the first time that XO and XDH are induced by TCDD in liver cytosol of mice. Reactive oxygen species, which have been implicated in a broad spectrum of pathologies, are generated as by-products of the action of XO/XDH. Therefore, the induction of these enzymes by TCDD may contribute significantly to the various toxicities of TCDD.

Acknowledgements

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