INDUCTION OF LIVER XANTHINE DEHYDROGENASE / XANTHINE OXIDASE BY 2,3,7,8-TCDD AND COBALT CHLORIDE

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Introduction

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is one of the most toxic polychlorinated organic pollutants, which pose a potential risk to human health. The biological effects of TCDD include the tumor promotion and several toxicities to the endocrine development and immune systems. Most of the effects of TCDD are mediated by a cytosolic receptor known to as the aryl hydrocarbon receptor (AhR). It has been reported that AhR-null (*Ahr*$^{-/-}$) mice do not exhibit TCDD-induced liver toxicity or teratogenicity [1,2]. 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 xenobiotic response element (XRE) sequence in the 5'-upstream region of their genes and AhR-mediated induction of gene expression by TCDD is observed.

In previous study, we showed that liver Xanthine dehydrogenase (XDH) and xanthine oxidase (XO) activities in mice are induced by TCDD. The induction was proved to be mediated by AhR using *Ahr*$^{-/-}$ mice [3]. XDH/XO are molybdenum-containing flavoenzymes and catalyze oxidation of hypoxanthine to xanthine and subsequently to uric acid with concomitant reduction of NAD^+ or molecular oxygen. The reaction generates reactive oxygen species, which have been implicated in a broad spectrum of pathologies, and XO is involved in lipid peroxidation and reperfusion injury [4,5]. Therefore, the induction of these enzymes by TCDD may contribute significantly to the various toxicities of TCDD.

In this study, the effect of TCDD on the induction of lipid peroxidation in *Ahr*$^{+/+}$ and *Ahr*$^{-/-}$ mice liver has been investigated. Additionally we have demonstrated cobalt chloride, which induces hypoxia inducible factor-1α (HIF-1α), increased XDH/XO in mice liver. It is regarded that AhR and HIF-1α play a key role in the regulation of XDH/XO.

Materials and Methods

Chemicals. TCDD was purchased from Cambridge Isotope Laboratories Japan. Cobalt chloride, alkylresorufins, 1-methylxanthine and thiobarbituric acid were purchased from Sigma Chemical Co.

Animals. Male C57BL/6J:Jcl mice (8-9 weeks) were obtained from Clea Japan, Tokyo. The generation of $Ahr^{-/-}$ and $Ahr^{+/-}$ mice and checking of genotypes were done as previously reported [1]. Mice were housed in plastic cages and given food and water *ad libtum*.

Mice were given TCDD by gavage with a single dose of 40 μg / kg body weight dissolved in corn oil. Cobalt chloride ($CoCl_2$) was injected subcutaneously with a single dose of 40 mg / kg body weight dissolved in saline solution. Vehicle mice were given the same volume of corn oil or saline solution. Male $Ahr^{+/-}$ and $Ahr^{-/-}$ mice (8-10 weeks) were treated with TCDD in the same manner as $Ahr^{+/+}$ (wild) mice.

Assays of enzyme activities. The TCDD-treated mice were killed 3 days and 1, 2 and 4 weeks later and the $CoCl_2$-treated mice were 24 hr 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 the fluorophotometric method [6]. 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.

Assay of TBARS. Thiobarbituric acid-reacting substances (TBARS) in liver was measured spectrophotometrically described previously [7].

Results and Discussion

The xanthine dehydrogenase (XDH)/xanthine oxidase (XO), which generates the superoxide anion as a by-product of action on endogenous substrates, is believed to play a role in mediating pathophysiological changes through its contribution to superoxide production.

In previous study, we demonstrated that XDH/XO were induced by TCDD in liver cytosol of mice. As a microsomal drug-oxidase activity, ethoxyresorufin-*O*-dealkylase, EROD, was compared among male $Ahr^{+/+}$, $Ahr^{+/-}$, and $Ahr^{-/-}$ mice a week after treatment with TCDD. The activities were increased in liver microsomes of $Ahr^{+/+}$ and $Ahr^{+/-}$ mice by a single dose of TCDD compared with the control mice given the vehicle only. However, in $Ahr^{-/-}$ mice, which lack the Ah receptor gene, this activity was not induced by TCDD (Fig. 1, (A)).

The inductions of EROD activities by TCDD were proved to be mediated by AhR, since TCDD treatment of $Ahr^{-/-}$ mice produced no enhancement of EROD activities in liver microsomes. The activities of XDH / XO in liver cytosols of $Ahr^{+/+}$ and $Ahr^{+/-}$ mice were enhanced 2.3-2.5 times by TCDD. However, no induction was observed in $Ahr^{-/-}$ mice (Fig. 1, (B)). Thus, the induction of XDH/XO activities were also demonstrated to be mediated by AhR. But, there has been no report about the existence of an XRE in the 5'-upstream region of the XDH gene.

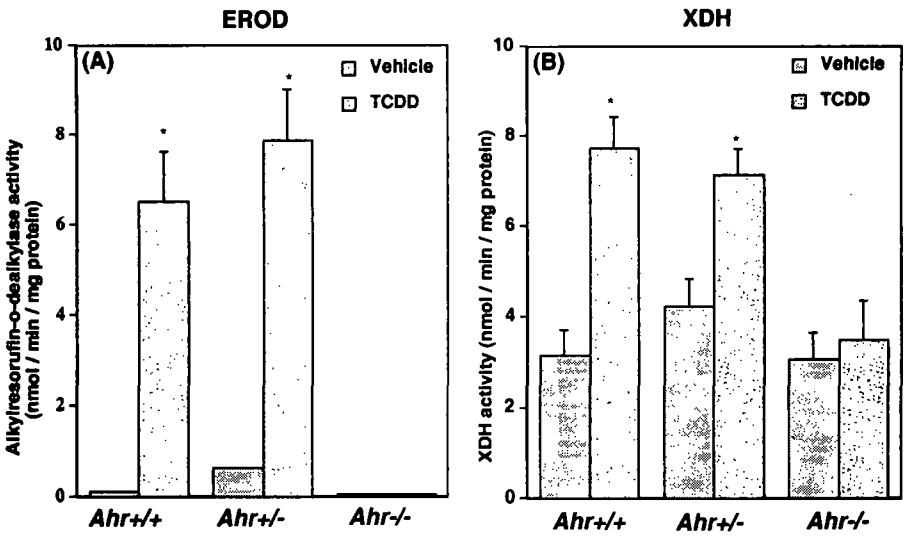
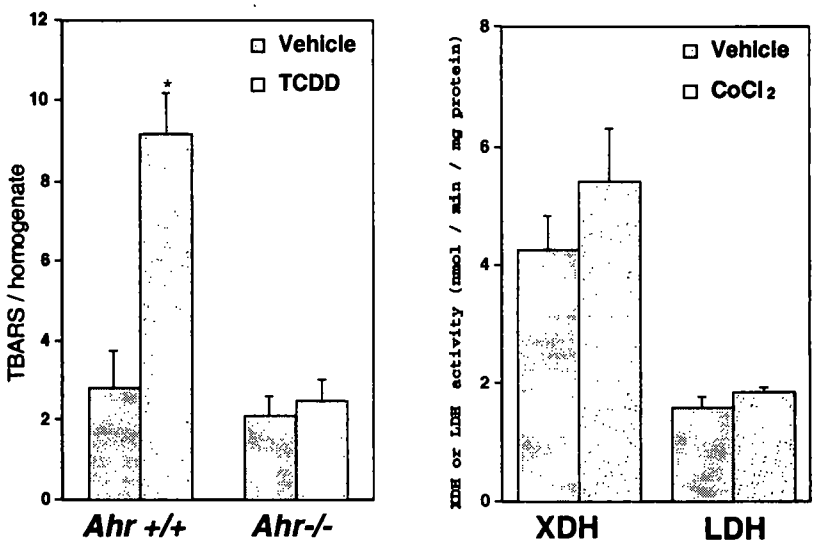


Fig. 1. Effects of TCDD on EROD, and XDH activities in liver preparations of *Ahr*$^{+/+}$, *Ahr*$^{+/-}$ and *Ahr*$^{-/-}$ mice. (A): The activities of EROD (nmol / min / mg protein) in liver microsomes of *Ahr*$^{+/+}$, *Ahr*$^{+/-}$ and *Ahr*$^{-/-}$ mice dosed with vehicle or TCDD. (B): XDH activities (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 (Student's t test).



(Left) **Fig. 2. TBARS in liver homogenates of vehicle and TCDD-treated mice.** TBARS in liver homogenates of *Ahr*$^{+/+}$ and *Ahr*$^{-/-}$ mice 1 week after TCDD-treatment. Each bar is the mean + S.D. of three individual mice. *P<0.001 compared with vehicle (Student's t test).

(Right) **Fig. 3. Effects of cobalt chloride on XDH /XO and LDH activities in liver preparations of mice.** XDH/XO and LDH activities in liver cytosols of mice with or without cobalt chloride treatment. Each bar is the mean + S.D. of four individual 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 lipid peroxidation (TBARS) were measured in $Ahr^{+/+}$ and $Ahr^{-/-}$ mice liver after administration of TCDD. TBARS were increased 3.2 times by TCDD in $Ahr^{+/+}$ mice, but no significant effect was detected in $Ah^{-/-}$ (Fig. 2). It is considered that the increase of TBARS is due to the enhancement of XDH/XO activities.These results indicate that XDH/XO are induced by xenobiotics including TCDD via the AhR/ARNT pathway, and the effect enhances the oxidative stress.

In the previous report, XO was expressed under hypoxia in hepatoma cells[8]. HIF-1α dimerizes with ARNT and enhances expression of the hypoxia-responsive genes. To confirm that HIF-1α plays a role in regulating XDH/XO, we studied the effects of cobalt chloride, which induces HIF-1α and mimic hypoxia *in vivo*, on XDH/XO activities. These enzymes activities were increased in cobalt chloride-treated mice liver, and LDH which was known to be up-regulated by hypoxia and cobalt chloride-treatment showed slight increase (Fig. 3).

In this study, we demonstrated that XO/XDH were induced by TCDD and cobalt chloride in mice. The AhR/ARNT and HIF-1α/ARNT complexes were considered to play a key role in the regulation of XDH/XO.

Acknowledgements

This study was supported by Health Science Research Grants for Research on Environmental Health from the Ministry of Health and Welfare of Japan, and Core Research for Evolutional Science and Technology (CREST) of Japan Science and Technology Corporation (JST).

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