# MICROSCOPIC FINDINGS OF THE LIVER OF C57BL/6J AND ARYL HYDROCARBON RECEPTOR-NULL MICE AFTER A SINGLE ADMINISTERATION OF TCDD

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## Introduction

The aryl hydrocarbon receptor (AhR) has been considered to be one of the key molecules to mediate toxicity of dioxins[1, 2], benzo[a]pyrene[3] and 3-methylcholanthrane. Dioxins induce drug-metabolizing enzymes in the liver. It is revealed, by using *Ahr*-deficient mice, that this induction is mediated by AhR[1, 4].

The present study aimed at evaluating microscopic findings of liver after administration of TCDD in C57BL/6J and *Ahr*-deficient homozygous mice.

## Materials and Methods

*Animals.* Sexually mature C57BL/6J male and female mice from CLEA Japan, Inc. (Tokyo) were used. *Ahr*-deficient homozygous males and females were maintained in Research Facilities for Laboratory Animal Science, Hiroshima University School of Medicine[3].

*Dioxin and Dosing.* 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (product No. ED-901) was purchased from Cambridge Isotope Laboratories Japan (Osaka, Japan) and solved in corn oil. Male or female mice were given a single oral dose of 0 (vehicle control) or 40 μg TCDD/kg body weight by gavage. The volume of dosing was 5,000 μl/kg b.w.

*Sample collection.* Under anesthesia with diethylether, male mice (C57BL/6J strain) were sacrificed 1, 3, 7, 14, 28 days after administration of vehicle or TCDD (n = 5 for each group). Female mice (C57BL/6J or *Ahr*-null) were sacrificed 7 days after administration of TCDD (n = 5 for each group). The liver was removed and weighed.

*Histology.* For histological analysis mice were sacrificed 7 days after administration of TCDD. The thorax was opened and 10% neutralized formalin in 0.1 M phosphate buffered solution was perfused through the left ventricle with the right atrium cut for drainage of the blood. Liver was removed and minced into small pieces. Cryosections cut in a Leica cryomicrotome (CM3050 type) were stained for sudan black B.

*Phenotypic aspects of the liver in* Ahr-*null mice.* In comparing changes of liver caused by TCDD between C57BL/6J and *Ahr*-null mice, we evaluated phenotypic aspects of the liver in *Ahr*-null mice.

*Statistical analysis.* Data were analyzed with Student's *t* test.

**Results and Discussion**

*Liver swelling by TCDD*

Figure 1 shows the time-course of the liver/body weight ratio 1, 3, 7, 14 and 28 days after a single administration of vehicle or TCDD. In the TCDD-treated mice liver swelling was noticed compared with controls, and this swelling was most prominent 7 days after administration. Liver swelling was observed in the treated females as well as in the treated males.

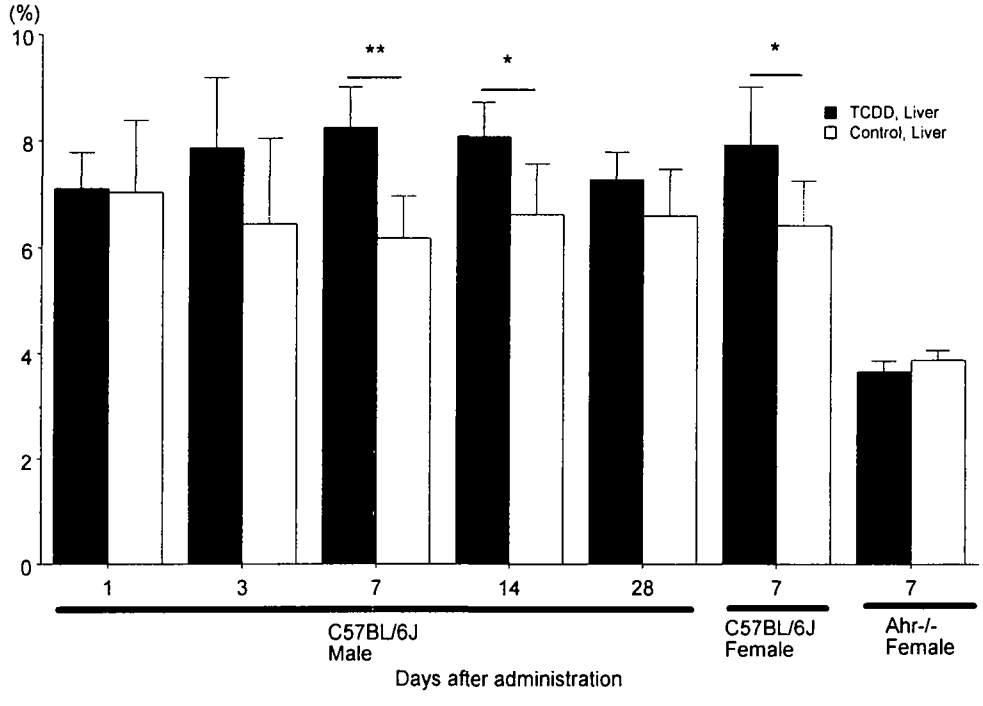


**Figure 1.** Time-course of the liver/body weight ratio 1, 3, 7, 14 and 28 days after a single oral administration of vehicle or TCDD. Data were analyzed between TCDD-treated and control mice in each time point. **: $P < 0.01$, *: $P < 0.05$.

However, in the TCDD-treated *Ahr*-null mice, no liver swelling was seen. In addition, the liver /b.w. ratio is smaller in the *Ahr*-null females (vehicle-treated) than in C57BL/6J females (vehicle-treated).

*Histological changes of the liver by TCDD*

A large number of lipid droplets was deposited in hepatocytes of C57BL/6J mice 7 and 14 days after administration of TCDD, as revealed by sudan black staining. Fat-laden hepatocytes were located around central veins in hepatic lobules. Although corn oil was injected as vehicle, no accumulation of fat was observed in vehicle-treated mouse livers. Seven days after administration of TCDD, no lipid droplets were seen in hepatocytes in *Ahr*-null mice. No other changes were noticed in the liver of *Ahr*-null mice given TCDD. The size of hepatic lobules was not uniform from lobule to lobule in vehicle-treated *Ahr*-null mice, when compared with C57BL/6J mice. This

observation implies that accumulation of lipid droplets in the liver is mediated by AhR and that no AhR-independent changes were seen as dioxin effect on the liver of *Ahr*-null mice.

*Phenotypic aspects of the liver in* Ahr-*null mice*

Figure 1 shows Ahr-null mice have smaller liver than C57BL/6J mice. This is because the liver is poorly developed. A similar phenotype is reported by Lahvis et al.[5], who concluded that the difference may be attributed to the postosystemic shunting.

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**References**

1. Sugihara K., Kitamura S., Yamada T., Ohta S., Yamashita K., Yasuda M. and Fujii-Kuriyama Y. (2001) Biochem Biophys Res Comm. 281, 1093.
2. Mimura J., Yamashita K., Nakamura K., Morita M., Takagi T.N., Nakao K., Ema M., Sogawa K., Yasuda M., Katsuki M. and Fujii-Kuriyama Y. (1997) Genes Cells. 2, 645.
3. Shimizu Y., Nakatsuru Y., Ichinose M., Takahashi Y., Kume H., Mimura J., Fujii-Kuriyama Y. and Ishikawa T. (2000) Proc Natl Acad Sci USA. 97, 779.
4. Fernandez-Salguero P.M., Hilbert D.M., Rudikoff S., Ward J.M. and Gonzalez F.J. (1996) Toxicol Appl Pharmacol. 140, 173.
5. Lahvis G.P., Lindell S.L., Thomas R.S., McCuskey R.S., Murphy C., Glover E., Bentz M., Southard J. and Bradfield C.A. (2000) Proc Natl Acad Sci USA. 97, 10442.