

[P-77]

Tissue-Specific Splicing and Expression of Cyp1A1 in the Liver and Brain of Offspring Rats after Gestational Exposure to 2,3,7,8-Tetrachlorodibenzo-P-Dioxin

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We investigated the effects of gestational and lactational exposures to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on the differential induction of CYP1A1 in the levels of protein and gene expression in the liver and brain regions of offspring rats. For this study, pregnant Sprague Dawley rats were orally exposed to TCDD (1 or 10 ng/kg body weight/day) starting at Day 1 of gestation up to Day 20 of postpartum. The activity of ethoxyresorufin O-deethylase (EROD), induction of CYP1A1, and expression of CYP1A1 mRNA were measured in the liver and brain of fetuses and dams from Day 20 of gestation, of male and female neonates from Day 1 of postpartum, and of lactational offsprings 20 days after birth. EROD activity in the liver microsomes of fetal, post-natal, and lactational group increased 0, 5, and 15-fold compared to control group, respectively. Results of immunoblot analysis showed that CYP1A1 was strongly induced in the liver of lactational group, but not in the fetal group. These results suggest, even though fetuses are somewhat protected from the TCDD exposure by the placenta, that TCDD exposed to dams is directly transferred to their offsprings via lactation. However, there were not detectable in the activity of EROD and induction of CYP1A1 in the brains of all groups. Induction of CYP1A1 mRNA expression, quantitatively analyzed by the competitive reverse transcription-polymerase chain reaction (QC RT-PCR) method was detectable at extremely low amounts in the liver of control fetuses, neonates, and lactational offsprings. The mRNA in the livers of offsprings after birth from dams pre-exposed to TCDD was age-dependently increased. Expressed level of mRNA was 1, 5, and 10 pg/3 mg total RNA in the liver of fetal, postnatal, and lactational offsprings, respectively. Induction of

CYP1A1 mRNA expression in the brains of offspring rats by TCDD exposure was also detectable, but at extremely low levels compared to those in the livers of corresponding groups. Representative ethidium bromide-stained gel, in which the amplified mRNA bands were electrophoretically separated, showed that RT-PCR product for CYP1A1 in the brain was 1161 bp, which was 462 bp longer in size than in the liver of offspring rats. DNA sequencing analysis unraveled this difference, i.e., RT-PCR product for CYP1A1 in the brain of offspring rat includes 4 introns, suggesting incomplete splicing out of introns. Whether the incomplete splicing out of introns of CYP1A1 gene in the brain is tissue-specific or TCDD-responsible is not known, which remains for further study.

Keyword : tcdd, cyp1a1, gestation, splicing