GENE EXPRESSION DISORDER IN VARIOUS TISSUES IN RHESUS MONKEYS TREATED WITH 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN VIA SUBCUTANEOUS SINGLE INJECTION

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Introduction

In Dioxin isomers, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is the most toxic and contaminating in an environment and bio-life. Human populations exposed with highly TCDD were reported to be caused immunological dysfunctions, carcinogenesis, and developmental and reproductive dysfunctions. The effects of TCDD on monkeys have been investigating as a model for assessment of TCDD exposure on human health [1-4]. Because of species specificity, monkey model could expect to clear TCDD events on humans adding to many reports in other experimental animals[5-8]. In this study TCDD was subcutaneously administrated to female rhesus monkeys. At 49 days after the exposure, TCDD was remained in monkeys and caused the disorder of gene expression in many organs, especially in mammary gland in the monkeys.

Methods and Materials

Chemicals. ^{3}H-2, 3,7,8-TCDD (3.84 GBq/mg) dissolved in toluene and DMSO (1:2, V/V) was purchased from Daiichi Pure Chemicals Co., Ltd. Tokyo, Japan.

Animals. Rhesus monkeys were purchased from China National Scientific Instruments & Materials Import/Export Corporation (Beijing, China). The monkeys (6-9 years old and 4.5-6.5 kg in body weight) were kept in Shin Nippon Biomedical Laboratories, Ltd, Kagoshima, Japan. The breeding conditions were described previously[9]. ^{3}H-2, 3,7,8-TCDD (30-300 ng/kg of body weight) was administrated subcutaneous to monkeys.

Measurement of TCDD in blood and tissues. The concentration of TCDD in various tissues of the monkeys after 7 and 49 days from the exposure was measured by the radioactivity counting by the methods described previously[2].

Measurement of gene expression in various tissues. Total RNA was extracted with Trizsol from the tissues froze at –80 until use. For RT-PCR, an aliquot of the RNA was reversed to cDNA with super script reverse transcriptase and followed by PCR which was performed in the previous tube with primers adjust to each genes. For the microarray analysis, the reverse

transcription of the RNA was performed with dCTP labeled Cy3 or Cy5 and hybridization was done on a glass microarray, AceGene (Hitachi Software Engineering Co., Ltd, Tokyo, Japan).

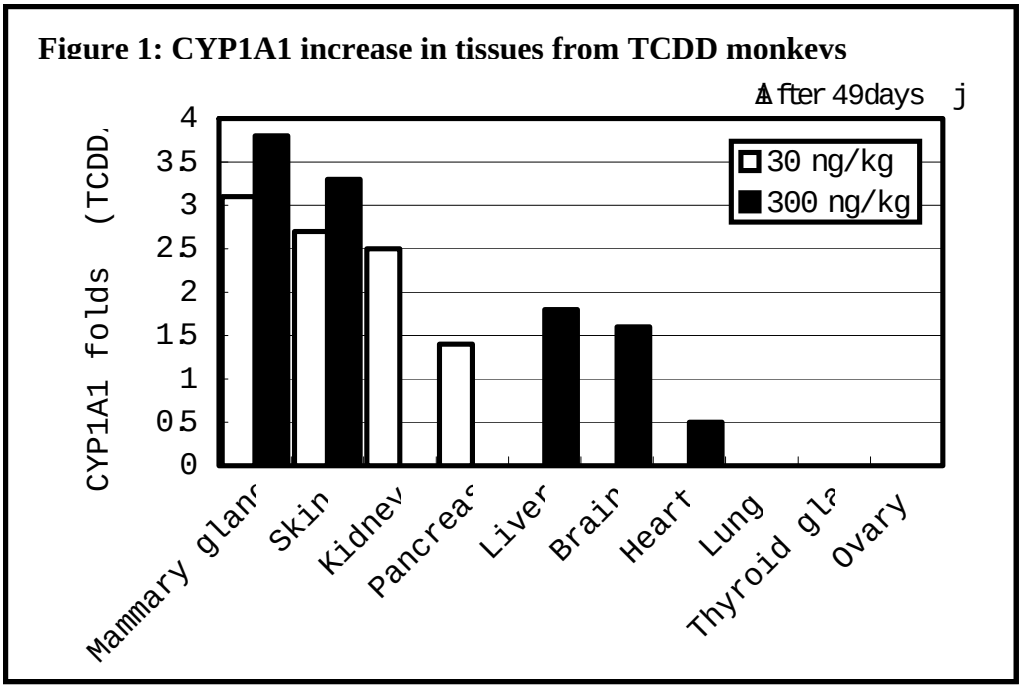
Results and Discussion

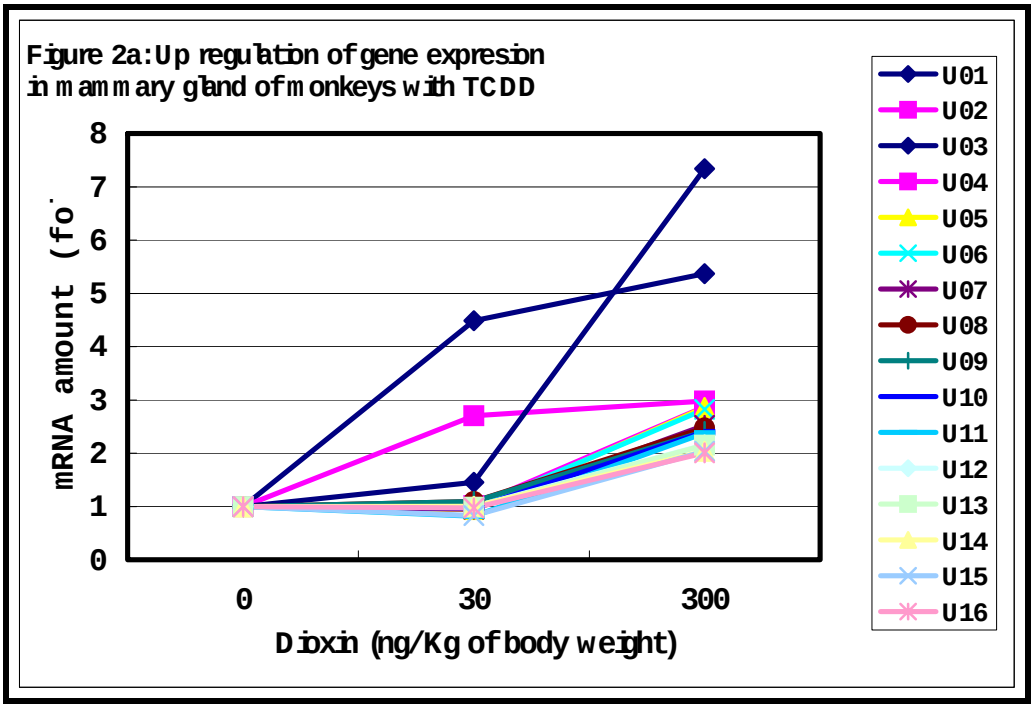
RT-PCR showed CYP1A1 content was increased in many tissues in monkeys treated with TCDD at 49 days after the single exposure than that in the tissues treated with no-TCDD, as shown in Fig 1. The higher increasing was detected in the order from the mammary gland, skin, kidney, pancreas, liver and brain than their control. The increased amount was a slightly in heart and could not detect in the ovary, thyroid gland and lung. The increasing folds of CYP1A1 amount in the tissues were depending on the TCDD amounts exposed to monkeys. The increased CYP1A1 amount in the tissues exposed with 30 ng TCDD /kg of body weight was shown almost saturate to be compared with that of 300 ng TCDD /kg of body weight.

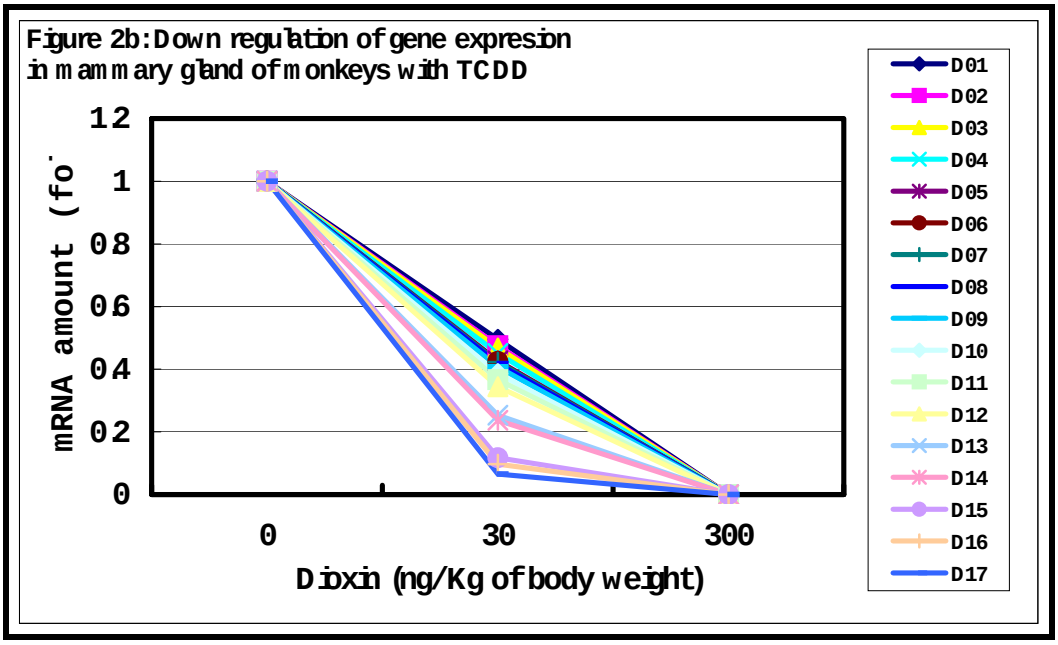
CYP1A1 plays as detoxifying enzyme acting on environmental chemicals but the over-activation of CYP1A1 might cause dysfunction of normal metabolites in life and attach to DNA, which is known in vitro, and cell experiments. Therefore, the increasing of CYP1A1 in the tissues of the monkey exposed with TCDD indicates the disorder of biological functions in the monkeys exposed with TCDD. Even though the mortality event was not affected in the monkey, some tissues of the monkeys were detected in anatomical disorders, in the liver (accentuated lobular pattern), kidney (bilateral heteroplasia), and intestine (dark red mucosa) by the levels of the administration of 30-300 ng TCDD / kg of body weight.

Microarray analysis – One hundred genes were analysed with the microarray using the RNA from the mammary gland which was the most affected in the CYP1A1 increase. Among a lot of genes, about 16 genes were increased depend on the amount exposed with TCDD to monkey and about 17 genes were decreased, as shown in Fig 2a and 2b. Those 33 genes may be candidate to detect the TCDD affections on monkeys and humans. In this analysis, some mismatch information of the genes in monkey might be given because the microarray glass is prepared to adjust for human gene analysis. Even though the lower sensibility and the gene name mistake for the monkey genes, some genes that were hybridized in the microarray were useful to evaluate the TCDD affection on the monkey. The number and the amount changing in the genes reacted on the glass were different profiles depending on the treated TCDD amount to monkeys. So the profile of each gene could give us the evaluation methods to estimate and compare the TCDD affection levels on monkey and humans. In the present results show the 14/33 genes were a non-sensitive in the 30 ng TCDD /kg administration to the monkey. This value might be near to affection level on humans with TCDD because of the physiological resemblance between human and monkeys.

Figure 1: CYP1A1 increase in tissues from TCDD monkeys







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