

## TOXICITY DEVELOPMENT OF 2,3,7,8-TCDD DEPENDS ON THE XENOBIOTICAL INDUCTION OF ROS, AND ON THE PEROXIDATION OF THE TARGET ORGAN BY THE ROS AND OXYGEN SUCCESSIVELY

Fuminori Hyodo<sup>1,4</sup>, Kazuaki Kawai<sup>1</sup>, Kiyoshi Nakazawa<sup>1</sup>, Tsunemasa Nonogaki<sup>2</sup>, Yoshito Masuda<sup>3</sup>, and Hideyuki Furukawa<sup>1,5</sup>

<sup>1</sup>Faculty of Pharmaceutical Sciences, Meijo University, 150 Yagotoyama, Tempaku-ku, Nagoya 468-8503, <sup>2</sup>International Institute of Medical Technology, 2-16-1, Meieki, Nishi-Ku, Nagoya 451-0045 and <sup>3</sup>Daiichi College of Pharmaceutical Sciences, 22-Itamagawa-cho, minami-ku, Fukuoka 815-8511, <sup>4</sup>Present address, Faculty of Pharmaceutical Sciences, Kyushu University, 3-1-1, Maidashi, Higashi-ku, Fukuoka 812-8582, <sup>5</sup>Research Laboratory on Oxidative Stress, 2-219, Umemorizaka-nishi, Meito-ku, Nagoya 465-0066, Japan

### Introduction

2,3,7,8-Tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) is the most toxic environmental contaminant<sup>1,2</sup> which produce in combustion of organo-halogen compounds. But it is unclear the way to lesion by 2,3,7,8-TCDD. The purpose of this study is to discover the mechanism in the target tissue especially in the liver. We would like to be clear the reactive oxygen species (ROS) induction by 2,3,7,8-TCDD, and ROS-mediated peroxidation at target tissue in the early periods after injection by 2,3,7,8-TCDD in the rat.

### Methods and Materials

2,3,7,8-TCDD was dissolved in corn oil at concentration of 200 ng/ml, and it was injected caudal vein of Sprague-Dawley male rat at the dose of 100 ng/kg body weight. After 3, 8 hr, the rats were euthanized using ether. Livers were removed, snap-frozen in liquid nitrogen, and stored at -80 °C until measurement of oxidative stress.

We employed the histochemical detection for the peroxidized tissue<sup>3</sup>. The sections from frozen tissue were treated with 2', 7'-dichlorofluorescein (DCFH) as the probe of peroxidized tissue. The peroxidized tissue oxidize DCFH to DCF on the section of tissue. The peroxidized tissue was characterized by the increase in fluorescence of histological sections as assessed with a fluorescence microscope.

Furthermore, the increase in thiobarbituric acid reactive substances (TBARS) of the liver homogenate, glutamate pyruvate transaminase (sGPT) in the serum and 8-hydroxy-2'-deoxyguanosine (8-OHdG) in the liver were measured.

### Results and Discussion

Measurements of the fluorescent product DCF in the liver tissue showed significant increase in 2,3,7,8-TCDD treated rat liver after exposure of 8hrs compared with control (corn oil treated) rat. Especially, strong fluorescence was observed near the Glisson Sheath. No significant increases in TBARS, sGPT and 8-OHdG were observed compared with control. Therefore, it was considered that xenobiotical induction of ROS by 2,3,7,8-TCDD should occurred in few hours after exposure to 2,3,7,8-TCDD, and peroxidized tissues should alter from ordered tissues to disordered tissues.

## References

1. Alan Poland and Joyce C. Kuntson(1982), 2,3,7,8-Tetrachlorodibenzo-p-dioxin and related aromatic hydrocarbons: Examination of the mechanism of toxicity., *Annu. Rev. Pharmacol. Toxicol.*, 22, 517-554.
2. James P. Whitlock Jr(1990), Genetic and molecular aspects of 2,3,7,8-tetrachlorodibenzo-p-dioxin action., *Annu. Rev. Pharmacol. Toxicol.*, 30, 25-77.
3. J. Frank, H.K. Biesalski, S. Dominici and A. Pompella(2000), The visualization of oxidant stress in tissues and isolated cells., *Histol. Histopathol.*, 15, 173-184.