POSSIBLE MODIFICATION OF DIOXIN RISK IN THE PRESENCE OF ENDOGENOUS LIGANDS FOR ARYLHYDROCARBON RECEPTOR

Jun Sekizawa[1] Shinichi Miyairi[2], Makoto Ema[3]

1 Faculty of Integrated Arts and Sciences, The University of Tokushima, 1-1 Minamijosanjima, Tokushima 770-8502 Japan

2 College of Pharmacy, Nihon University, 7-7-1 Narashinodai, Funabashi, Chiba 274-8555 Japan

3 National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagayaku, Tokyo 158-8501 Japan

Introduction

The World Health Organization re-evaluated health risk of exposure to dioxins and re-established a tolerable daily intake (TDI) of 1-4 pg WHO TEQ/kg in 1998 [1]. This was based on assumptions that body burden can be a basis for extrapolation of animal data to humans, and humans are equally sensitive to or less sensitive than experimental animals with regard to critical effects. In 2001, Scientific Committee on Food of the European Commission has established temporary tolerable weekly intake (t-TWI) of 7 pg/kg based on basically similar assumptions.

However they did not take into considerations of the possibility of the presence of endogenous ligands of the arylhydrocarbon receptor (AhR) which is assumed to be mediating toxic effects of dioxins. Indirubin was identified as a novel ligand for AhR by Adachi et al (2001) in human urine[2]. Indirubin was 50 times more potent than 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in inducing ligand specific LacZ response in the yeast reporter assay constructed on human AhR. Based on this finding, we started to estimate concentrations of indirubin in urine of humans and rats and various organs of rats in expectation of re-examining dioxin risk in the presence of a putative endogenous ligand.

Methods and Materials

A sensitive and specific assay method for indirubin in biological samples was developed by Miyairi et al (2003)[3] using an enzyme-linked immunosorbent assay (ELISA) technique.

Antiserum was raised against 5-carboxypropylindirubin-KLH conjugate in a rabbit and an enzyme labeled antigen was prepared from 5-carboxymethylindirubin with β-galactosidase. Human urine collected from 12 college students, was assayed directly for concentrations of indirubin. Rat urine was collected from 30 male BrlHan:WIST@Jcl (GALAS) rats (11-week old) for 7days and assayed for indirubin concentration similarly. Animals were sacrificed by exsanguination at the end of urine collection, and the blood and organs (liver, kidney, spleen, testis and epididymis) were collected for the assay of indirubin concentration.

Results and Discussion

Human urine was found to contain 2.1 nM (± 1.1 nM) of indirubin in this study. Adachi et al (2001) reported average concentrations of 0.2 nM (0.03-0.25 nM) in urine of ten college students[2]. Matsuda and Matsui (2002)[4] found 0.07 nM concentration of indirubin in human serum. These findings suggest that indirubin is present in the human body in appreciable amounts. Estimated average human intakes of dioxins in Japan was 2.6 pg/kg bw/day[5] which would produce a body burden of 5.2 ng WHO TEQ/kg bw[1)]. Blood dioxin levels of 467 people from 15 areas in Japan were surveyed in 1998-2000 and found to be 25.5 pg/g lipid in average, which corresponds to around 2.3 pM in blood[6]. This value was about twenty to thirty times lower than the concentrations of indirubin as estimated by Matsuda and Matsui (2002)[4].

Adachi et al (2002) reported indirubin could induce CYP1A1 mRNA at as low as 1 pM in HepG2 cells, whereas TCDD could induce at 100 pM level[7]. Studies show that animals and humans have similar sensitivity with regard to biochemical effects such as CYP1A1 induction. Combined with the above finding, it is suggested that indirubin was at physiologically workable concentrations in human body, while dioxin level in blood of Japanese was not as high as to induce CYP1A1 mRNA. Concentrations in the urine, blood and organs are currently under investigation in rats. These values can be compared with those in humans, which may explain species differences in dioxin toxicities between humans and experimental animals, as one of the possible factors.

Not details of physiological roles of AhR in human body were known, and exact mechanisms of

toxicities of dioxin were not elucidated yet although AhR is known to be mediating toxicities. Indirubin can be a good substrate of CYP1A1 which it induces, and could be degraded easily, while dioxin is not. This also suggest physiological roles of indirubin in the human body, while dioxin may disturb it, because the latter persists in the body. Indirubin is shown to be working via AhR not only to induce CYP1A1 mRNA etc., but also in cell cycle regulation through $p27^{Kip1}$ and RB etc. Since expression of AhR is known to be organs and time dependent, AhR and indirubin may be playing important roles in regulation of cell's physiological functions, and dioxin may disturb these functions at rather high concentrations compared to indirubin[8]. In order to elucidate the mechanisms of toxicity of dioxin and also physiological roles of AhR and indirubin, we started to determine the indirubin concentrations in the urine, blood and organs in male and female rats using our sensitive and specific immunoassay method and to compare with the specific concentrations of indirubin in urine and blood of humans. These data may also explain parts of species differences in dioxin toxicities.

Acknowledgements

Financial support on Research on Food and Chemical Safety from the Ministry of Health, Labor and Welfare Japan is appreciated.

References

1. WHO (1998) Executive summary Assessment of the health risk of dioxins: reevaluation of the tolerable daily intake, European Centre fror Environment and Health, and International Programme on Chemical Safety, World Health Organization, http://www.who.int/pcs/docs /dioxin- exec-sum/exe-sum/final.html
2. Adachi J, Mori Y, Matsui S, Takigami H, Fujino J, Kitagawa H, Miller CA III, Kato T, Saeki K. & Matsuda T (2001) J Biol. Chem., 276, 31475-31478
3. Mitsui S, Takei A, Kojima N, Takabatake T, Matsuda T, Sekizawa J. & Miyairi S (2003) Proceedings of the 123th Annual Meeting of the Pharmaceutical Society of Japan, Japan, 28, P2, II-473
4. Matsuda T. & Matsui S (2002) Indirubin, a potent ligand for arylhydrocarbon receptor in the human urine, Progress in Medical Sciences (Igaku-no-Ayumi), 201, 157-158
5. MHW/EA (1999) Estimation of the tolerable daily intake of dioxins, Central Commission on

Pharmaceuticals, Ministry of Health and Welfare/Central Commission on the Environment, Environment Agency, Japan

6 Watanabe S, Kitamura K, Iida T, Ohtaki M. & Waechter G (2001) Organohalogen Compounds, 53, 136-139

7. Adachi J, Matsui S & Matsuda T (2002) Organohalogen Compounds, 56, 13-15

8. Sekizawa J. & Matsuda T (2002) Organohalogen Compounds, 59, 433-435