RELATION BETWEEN DIOXINS LEVELS IN HUMAN BREAST MILK SAMPLES AND SCE VALUES AMONG LACTATING FEMALES IN A DEFOLIANTS -SPRAYED AREA IN VIETNAM

Horikawa H [1,2], Suzuki H [3], Naganuma R [3], Tawara K [4], Nishijo M [4], Nakagawa H [4], Hung NN [5], Thom LTH [5], Dung PT [5], Nhu DD [5], Van PT [6], Binh NT [6], Watanabe T [2], Kido T [3], and Sato Y [1]

1. Division of Ultrafine Structure, Department of Pathology, Research Institute, International Medical Center of Japan, 1-21-1 Toyama, Shinjuku, Tokyo, Japan
2. Laboratory of Tumor Cell Biology, Department of Medical Genome Sciences, Graduate School of Frontier Sciences, the University of Tokyo, 4-6-1 Shirokanedai, Minato, Tokyo, Japan
3. Division of Health Sciences, Graduate School of Medical Sciences, Kanazawa University, 5-11-80 Kodatsuno, Kanazawa, Ishikawa, Japan
4. Department of Public Health, Kanazawa Medical University, 1-1 Daigaku, Uchinada, Ishikawa, Japan
5. Division for Mitigation of the Consequences of the Chemicals used during the War on Human Health (10-80 Division), Hanoi Medical University, Hanoi, Vietnam
6. Blood Transfusion and Hematology Hospital at Ho Chi Minh City, Ho Chi Minh, Vietnam

Introduction

During Vietnam War, the US military sprayed more than 80 million liters of defoliants mainly in the south part of Vietnam. The defoliants including Agent Orange contained a substantial amount of dioxins that are a kind of endocrine disruptors chemicals. Dioxins include more than 220 kinds of isomers which show different strength of toxicity, among which 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is the strongest in toxicity, and classified to be a carcinogen in humans[1]. It is estimated that 160-165kg of TCDD contaminated in defoliants were sprayed in southern Vietnam during Vietnam War. It is well known that TCDD causes a number of reproductive and developmental effects in both males and females in animal experiments. There were some studies on health and genetic effects of sprayed dioxins in Vietnam War, however most subjects for the studies were American or Korean veterans who were exposed to dioxins during the war[2,3]. The studies on Vietnamese subjects have been rarely published internationally so far. Moreover, in most former studies dioxins levels were analyzed by pooling samples, because measurement of dioxins requires a large amount of samples. This makes very difficult to estimate whether there exists a relation between an individual dioxins level and individual health and genetic effect. In previous study, we analyzed dioxins levels in lactating females individually, and have shown that nevertheless more than 30 years have passed since Vietnam War, internal dioxins levels in a sprayed area are 3-4 fold higher than those in a non-sprayed area[4]. This prompted us to investigate genetic effects of dioxins between

the sprayed area and non-sprayed area by checking sister chromatid exchange (SCE) which is a widely adopted sensitive tool to measure genetic damage of carcinogens or mutagens, as it is considered to reflect damages and repair systems of DNA.[5] In this study, we report that SCE values of individuals in the sprayed area are significantly higher than those in the non-sprayed area, and that individual SCE values in logarithm are substantially correlated with TEQ values in logarithm. This report, for the first time, suggests the genotoxicity of internal dioxins in Vietnamese sprayed during Vietnam War.

Materials and methods

Two villages in center part of Vietnam were selected for this study. They are separated by the demilitarized zone of latitude 17 degrees north line, a military boundary in Vietnam War: Cam Chinh Commune in Quang Tri Province (QT) as a dioxin-sprayed area and Cam Phuc Commune in Ha Tinh Province (HT) as a non-sprayed control area. These 2 villages were considered to be similar in economic-social, custom, ethnic group, and health system. In July 2003, breast milk samples were collected from lactating females aged 20-30 yrs-old in the 2 areas (61 in QT and 46 in HT). Measurement of dioxins concentration using high resolution gas chromatography with high resolution mass spectrometric detection showed mean 12.25 (range: 2.10~41.38) pg TEQ/ml in QT and mean 3.91 (range: 2.24~5.96) pg-TEQ/ml in HT. In August 2008, peripheral blood (PB) samples were collected among them (51 in QT and 20 in HT). PB (0.5 ml) was cultured in a bottle with 9.5 ml RPMI-1640 medium and 2% phytohemagglutinin-M at 37 ^{o}C in a humidified atmosphere of 5% CO_2 for 34 hr. Then the samples were exposed to 1 μg/ml BrdU for 38 hr, and 20 ng/ml colcemid for 2 hr. Cells were harvested and preparation slides were made with a standard method [6]. Next, fluorescence-plus-Giemsa-staining was performed on the slides according to a standard method[7]. After confirming existence of complete 46 chromosomes, consecutive second mitotic metaphase cells with differentially stained sister chromatids were analyzed for SCE blinded to dioxins levels in individuals.

Results

SCE values were scored on an average of 35.5 cells (range: 20-48) per individual among 45 individuals in QT and 19 in HT. Mean SCE values were 2.34 in QT and 1.53 in HT (Table 1-2) showing statistically significant ($P = 4.63 \times 10^{-12}$) difference (Table 2). QT was divided into 2 groups according to TCDD values; 22 individuals in QT with high TCDD (QT-high) and 23 in QT with middle TCDD (QT-middle:). Statistic analysis showed that mean SCE value of 2.50 in QT-high was not significantly higher than that of 2.19 in QT-middle (Table 1-2). Correlation analysis showed a substantial correlation (r=0.463) between SCE values in logarithm and TEQ values in logarithm (Fig. 2), although no significant correlation (r= 0.340) between TEQ values and SCE values were observed.

Discussion

In this study, for the first time, we have demonstrated that there is statistically significant difference in SCE values between individuals in QT (dioxin-exposed area) and HT (non-exposed control area) and that log SCE values are statistically correlated with log TEQ values in individuals. Although many animal studies or in vitro studies have proved that exposure to dioxins increases SCE values[8], no studies have shown the correlation between internal dioxins levels and SCE values on humans. Dioxins are considered to express their toxicities via aryl hydrocarbon receptor (AHR) pathway[9], and exposure to dioxins leads to negative feedback by expression of AHR repressor (AHRR) that inhibits AHR pathway[10]. Since the correlation between SCE and TEQ values was only observed in logarithm mode, but not in simple mode, a slowly increasing tendency of SCE values likely exists in a higher TEQ area (Fig. 2). This observation might be explained by negative feedback effect by induced AHRR expression. Of interest is that there are a substantial number of individuals in the sprayed area whose SCE and TEQ vales are as low as those in the non-sprayed area. AHR pathway consists of some factors including AHR, AHRR, AHR nuclear translocator, most of which have some genotypes[11]. Among them, the genotypes of AHRR are considered to be involved in susceptibility to dioxins[12]. Genotyping study of genes involved in AHR pathway may elucidate different susceptibility to dioxins in individuals.

References

1. McGregor D.B., Partensky C., Wilbourn J., and Rice JM., *Environmental Health Perspective* 1998; 106: 755-760.

2. Michalek J.E., Ketchum N.S., and Longnecker M.P., *Annals of Epidemiology* 2001; 11: 304-311.

3. Kim HA., Kim EM., Park YC., Yu JY., Hong SK., Jeon SH., Park KL., Hur SJ., and Heo Y., *Industrial Health* 2003; 41: 149-157

4. Kido T. Report of Grant-in-Aid for Scientific Research B 2002-2004 (in the literature)

5. Perry P. and Evans H.J., *Nature* 1975; 258: 121-125.

6. Kanda N. and Kato H., *Chromosoma* 1979; 74: 299-305.

7. Goto K., Akematsu T., Shimazu H., and Sugiyama T., *Chromosoma* 1975; 53: 223-230.

8. R.E.Rowland, L.A. Edwards, and J.V. Pobb, *Cytogenet Genome Res* 2007; 116: 248-251.

9. Arleen B. Rifkind, *Drug Metabolism Reviews* 2006; 38: 291-335.

10. Mimura J., Ema M., Sogawa K., and Fujii-Kuriyama Y., *Genes Dev* 1999; 13: 20-25.

11. Ave M., Margus P., Signe A., Kadri H, Tarmo T., Maire P., and Andres S, *Fertility and Sterility* 2007; 88: 854-859.

12. Watanabe M., Sueoka K. Sasagawa I., Nakabayashi A., Yoshimura Y., and Ogata T., *Fertility and Sterility* 2004; 82: 1067-1071.

Table 1: Summary of SCE values and dioxins concentrations among females in QT and HT

	Number of anlysis	SCE values/cell	(mean)	Concentrations of dioxins (pg/ml)			
				TEQ	(mean)	TCDD	(mean)
QT	45	1.24~3.37	2.34	2.11-41.38	12.25	0.08-4.06	1.11
(QT with high TCDD)	22	1.96~3.11	2.50	7.80-41.38	17.26	0.95-4.06	1.74
(QT with middle TCDD)	23	1.24~3.37	2.19	2.11-14.86	7.46	0.08-0.88	0.51
HT	19	1.05~2.00	1.53	2.24-5.96	3.91	0.32-0.88	0.57

Table 2: t-test and ANOVA in comparison among QT, QT-high, QT-middle and HT groups

Group 1	Group 2	P value	Analysis
QT	HT	$P = 4.63 \times 10^{-12}$	t-test
QT-high	QT-middle	$0.01 < P < 0.05$	ANOVA
QT-high	HT	$P < 0.001$	ANOVA
QT-middle	HT	$P < 0.001$	ANOVA

Figure 1: Comparison of SCE values individuals among QT-high, QT-middle and HT groups

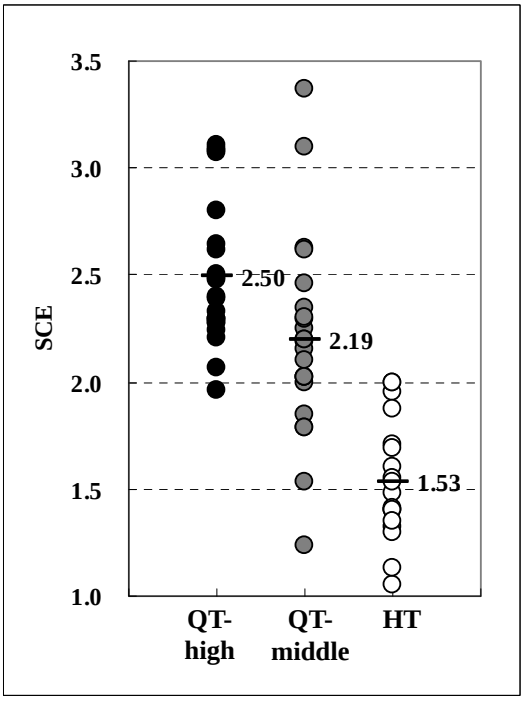
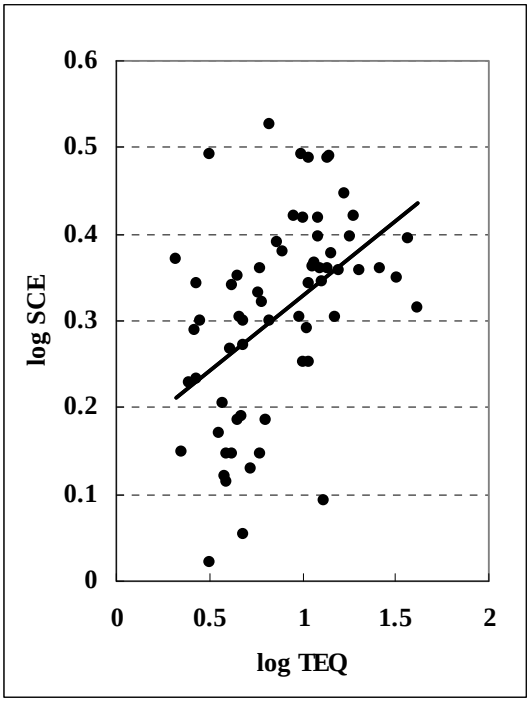


Figure 2: Correlation between SCE values and TEQ values in logarithm



(log SCE = 0.173 x logTEQ + 0.156, r = 0.463)