TERATOGENIC EFFECTS OF ORGANOHALOGEN CONTAMINANTS EXTRACTED FROM SMALL CETACEAN PRODUCTS ON *IN VITRO* RAT EMBRYOS DURING ORGANOGENESIS

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Introduction

Organohalogen compounds such as PCBs, DDTs and chlordanes (CHLs) are widely distributed throughout the ecosystem and readily accumulate in marine food chain, especially in odontoceti cetaceans - toothed whales and dolphins[1]. Small cetaceans have been traditionally hunted in the Japanese coastal water and some people have been consuming the cooked, fresh or frozen meat and blubber products. Recently we have surveyed the contaminant levels of organochlorines and heavy metals in the whale meat products marketed for human consumption in Japan[2-4], and found that lipid-rich products of cetaceans were highly contaminated with organochlorines, which may have the potential to cause adverse health effects on reproductive and development system in animals[5, 6].

In the present study, we examined the teratogenic effects of the contaminants extracted from whale bacon products on gestation day 11.5 rat embryos cultured for 48 hours in the *in vitro* whole embryo culture system.

Methods

Whale bacon products were purchased from a retail outlet from Fukuoka in 2000. The spieces of origin for the products used in this study was identified as pilot whale (*Globicephala macrorhynchus*) by DNA analysis[7] by Dr Cipriano, San Francisco State University. Percentage extractable lipid from the products was 55.1 %. Organohalogens in products were cleaned up by gel permeation chromatography and silicagel column as described elsewhere[8]. The extract was dissolved in DMSO (20 µL) for exposure to culture medium (4 mL). Serum was prepared immediately after exsanguinating from male Wistar-Imamichi rats and used as the culture medium in the rat whole embryo culture system [9]. Conceptuses were removed at gestational day 11.5 (plug day = 0) from dam and transferred into tyrode solution. Embryos were cultured for 48 hours at 38 °C with 95 % O_2 and 5 % CO_2 by rotating. A mixture of contaminants consisted of 2.5 ppm of PCBs (major congener, #153>#180>#138>#101>#118>#170), 2.0 ppm of DDTs (4,4'-DDE > 4,4'-DDT>4,4'-DDD) and 0.2 ppm CHLs (trans-nonachlor>cis-nonachlor) for high dose exposure, and of 0.30 ppm PCBs, 0.24 ppm DDTs, and 0.02 ppm CHLs for low dose exposure. The both exposure groups also contained hexachlorocyclohexanes, hexachlorobenzene, dieldrin and some brominated compounds as minor components. Control embryos were cultured in the culture medium with or without vehicle (DMSO).

Cultured rat embryos were evaluated for heart rate, embryonic blood circulation, crown-rump length, total number of somites and malformation as described previously[9, 10]. The concentrations of organochlorines in embryos after exposure were determined by GC/ECD and GC/MS as described previously [11].

Results and Discussion

After 48 hours from day 11.5 of gestation, the concentrations of PCBs in embryos were 13.4 and 2.44 ppm in high and low doses exposure, respectively, as shown in Table. 1. In both contaminated media, the contaminant transfer ratios to embryos from serum exposed were 6.2-7.7 for PCBs, 7.9-9.5 for DDTs and 7.7-8.7 for CHLs.

Compared to the control, high and low contaminated groups had no marked change on heart rate, embryonic blood circulation and total number of somites. However, crown-rump length of embryos reduced significantly in the both contaminated groups. Rat embryos in high contaminated medium showed malformations (cleft lip, hematoma, cacogenesis of fore or hind limbs) with incidence of 100% (in 12 out of 12 embryo compared to 0 of 6 for the control with vehicle), whereas cultured embryos in low contaminated medium showed the weak anomalies of cacogenesis to telencephalon or mandibla and hematoma of cranioface or fore limbs with incidence of 50% (in 6 out of 12 embryos). These results suggest that the organohalogen contaminants from whale products show the teratogenecity to rat embryos during organogenesis in a dose-dependent manner. The concentrations of contaminants (0.3 ppm PCB levels) that interfere with the normal embryonic development are within the range of blood levels measured in marine mammals [12]. Although the concentrations of PCBs in human blood[13] are about two orders of magnitude lower than those in this exposure medium, placental transfer of lipophilic contaminants to the embryo during gestation could result in localized concentrations to interfere with growth and development[14].

In conclusion, the organochlorine contaminants extracted from whale products was readily transferred to rat embryos in the whole embryo culture system. The teratogenic effects appear to occur at environmentally relevant concentrations of these compounds. These studies further should establish the dose-effect relationship of these contaminants.

Table 1. Concentration of contaminants of major organochlorine compounds and their transfer ratio from culture medium to embryos

	High dose exposure			Low dose exposure		
	enbryo	serum	ratio*	embryo	serum	ratio*
SPCB	13.4 ± 1.95	2.15 ± 0.21	6.23	2.44 ± 0.78	0.316 ± 0.055	7.72
SDDT	15.7 ± 3.12	1.99 ± 0.36	7.89	1.48 ± 0.22	0.156 ± 0.033	9.49
SCHL	1.74 ± 0.46	0.20 ± 0.03	8.70	0.17 ± 0.02	0.022 ± 0.006	7.73

Values are expressed as ppm on wet weight basis, mean ± S.D. for four embryos. *Concentration ratio of embryos relative to serum. SPCB; sum of 14 isomers,SDDT; sum of 4,4'-DDE, 4,4-DDT and 4,4'-DDD,SCHL; sum of trans-/cis-nonachlor, trans-/cis-chlordane, and oxychlordane.

Table 2. Effects of growth and differentiation on cultured rat embryos

	No. of embryos cultured	Crown-rump length (mm)		Total number of somites	
		24 h	48h	24h	48h
Control without vehicle	10	5.68 ± 0.07	7.27 ± 0.06	38 ± 0.4	43 ± 0.6
Control with vehicle	6	5.66 ± 0.05	7.25 ± 0.04	39 ± 0.3	43 ± 0.5
Low dose exposure	12	5.63 ± 0.03	7.06 ± 0.05*	38 ± 0.4	43 ± 0.3
High dose exposure	12	5.63 ± 0.08	6.82 ± 0.07**	38 ± 0.5	42 ± 0.4

Rat embryos were cultured for 48 hours from gestational day of 11.5 (plug day = 0).
Values are expressed as mean ± S.E. *p<0.05, **p < 0.01; significantly different from control with vehicle.

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References

1. Minh, T.B., Nakata, H., Watanabe, M., Tanabe, S., Miyazaki, N., Jefferson, T.A., Prudente, M., and Subramanian, A. (2000*) Arch. Environ. Contam. Toxicol*., 39, 398-410.
2. Haraguchi, K., Endo, T., Sakata, M., Masuda, Y., and Simmonds, M. P. (2000) *J. Food Hyg. Soc., Japan*, 41, 287-96.
3. Haraguchi, K., Simmonds, M., Endo, T., and Masuda, Y. (2000) *Organohalogen Compounds*, 47, 342-344.
4. Simmonds M. P., Haraguchi, K., Endo, T., Cipriano, F., Palumbi, S.R., and Troisi, G.P. (2002*) J. Toxicol. Environ. Health*, in press.
5. Mayura, K., Spainhour, C.B., Howie, L., Safe, S., and Phillips, T.D. (1993) *Toxicol.*, 77, 123-131.
6. Marks, T.A., Kimmel, G.L., and Staples, R.E. (1981) *Toxicol. Appl. Pharmacol*., 61, 269-276.
7. Cipriano, F., and Palumbi, S. R. (1999) *Report to the International Whaling Commission (IWC)*, SC/51/E13.
8. Haraguchi, K., Kato, Y, Kimura, R., and Masuda, Y. (1999) *Arch. Environ. Contam. Toxicol.*, 37, 135-142.
9. New, D.A.T. (1978) *Biol. Rev.*, 53, 81-122.
10. Akita, M., Yokoyama, A., and Kuroda, Y. (1998) *Alternative to animaltesting and experimentation*, 4, 132-133.
11. Mimura, K., Tamura, M., Haraguchi, K., and Masuda, Y. (1999) *Fukuoka Acta Medica*, 90, 192-201.
12. Bergman, A., Klasson-Wehler, E., and Kuroki, H. (1994) *Environ. Health Perspect*., 102, 464-469.
13. Noren, K., Weistrand, C., and Karpe, F. (1999) *Arch. Environ. Contam. Toxicol*., 37, 408-414.
14. Jacobson, J.L., Jacobson, S.W., and Hymphrey, H.E.B., (1990) *J. Pediatr.*, 116, 38-45 .