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Effects of *Chlorella* Supplementation on Decreasing Concentrations of Dioxins in the Blood of Pregnant Japanese Women

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Abstract

Adverse health consequences from prenatal, perinatal and postnatal exposure to background levels of dioxins in the environment, including polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), have been reported. In order to prevent or reduce such health risks, it is important to decrease maternal exposure to dioxins. This study investigated the effect of maternal supplementation with *Chlorella* on dioxin concentration levels in the blood during pregnancy. Twenty healthy pregnant women participated in the study. Ten received 6 g of *Chlorella* daily from gestational week 16-20 until the day of delivery (*Chlorella* group); ten others did not (control group). The blood PCDFs and PCDD/DFs concentrations in the *Chlorella* group significantly decreased during the supplementation period, from 2.20 ± 1.66 to 1.00 ± 0.61 pg-TEQ/g lipid ($p<0.05$) and from 5.48 ± 4.01 to 3.38 ± 1.96 pg-TEQ/g lipid ($p<0.05$), respectively. The levels of dioxins in the control group did not change significantly. These results suggest that *Chlorella* supplementation may be effective in reducing dioxin exposure in pregnant woman.

Keywords: Persistent organic pollutants; Pregnant women; Japan

Introduction

Dioxin is the common name for polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). Dioxin (PCDD/DFs) and dioxin-like-polychlorinated biphenyls (DL-PCBs) are highly fat-soluble and bioaccumulate through the food chain [1,2]. As a result, they enter the human body mainly through food, and accumulate in the fatty tissues. They are eliminated slowly from the body by metabolic degradation and excretion, primarily in the faeces. They may also concentrate in human milk, both an important route of excretion and a primary source of nutrition for newborns. Pregnant and nursing women thus transfer dioxins to their babies transplacentally and by breast-feeding, i.e., by both prenatal and postnatal exposure.

Humans are most sensitive to dioxins during early gestation, when organogenesis is occurring, and those most affected by these toxins are the descendants of individuals originally exposed. With high-dose exposure, such as in the Yusho accident, a mass food poisoning that occurred in western Japan in 1968 [3], PCDFs are known to be a causal agent of foetal Yusho disease [4]. Prenatal exposure to background levels of dioxin and DL-PCBs in the environment influences the hypothalamic-pituitary-gonadal axis of newborn babies [5], foetal brain development [6], and mental and motor development in Japanese infants [7]. Such exposure also enhances the risk of congenital hypothyroidism in Japan [8]. In addition, perinatal and postnatal exposure to background levels of environmental dioxins and DL-PCBs exerts an adverse effect on thyroid hormone status [9,10] and on immune surveillance [11] in Japanese breast-fed infants. Finally, perinatal exposure of children to relatively low dioxin and DL-PCB doses can permanently reduce sperm quality [12].

One effective approach to lowering or preventing the above-mentioned adverse health risks of dioxins is to eliminate them from the maternal body, thus reducing the body burden. Several food materials such as dietary fibre, olestra, chitosan and fermented brown rice have been reported to possibly eliminate dioxins [13-16]. Increasing dietary chlorophyll prepared from *Chlorella* 0.01 to 0.5% has been shown to inhibit dioxin absorption from the gastrointestinal tract, accelerate both PCDD and PCDF excretion, and significantly and efficiently decrease the body burden from 3.5-50% in rats [17]. Indeed, administration of 10% *Chlorella* in the diet of an experimental vs. a control group of rats was found to inhibit the absorption and reabsorption of dioxins from the digestive tract, increasing faecal excretion 1.3-4.4 times [18]. Therefore, *Chlorella* is considered an effective food source for the purpose of eliminating dioxins. In this study, we examined the effects of *Chlorella* supplementation on the blood dioxin levels of pregnant Japanese women.

Materials and Methods

Subjects and test diet

Subjects recruited for this study were healthy pregnant women receiving prenatal care at the Shimomura O.B.G.Y. Clinic and the G.Y. & O.B. Miyahara Clinic, Fukuoka, Japan, from August-December 2010

and February-April 2012, respectively. Exclusion criteria included severe medical illness, warfarin use and ongoing *Chlorella* supplementation. Twenty healthy pregnant women (age range, 24-39 years) provided written consent to participate in the study. Of these, 10 subjects (*Chlorella* group) agreed to take *Chlorella* tablets ("Biorinck" Chlorella Industry Co. Ltd., Tokyo, Japan) for approximately 6 months, from gestational week 16-20 until the day of delivery. The daily dose was 6 g of *Chlorella* (30 tablets/day), in portions of 10 tablets after each main meal.

Biorinck tablets have been manufactured since 2008 by the Chlorella Industry Co., Ltd., and are ingested by more than 100,000 people as a health food. The same company prepared the Chlorophyll and *Chlorella* employed in the animal experiments described above [17,18]. Biorinck tablets contain dried *Chlorella* (*Parachlorella bijerinkii* CK-5) powder as the active ingredient. According to our analysis, each tablet is comprised of the following (/100 g): protein, 62 g; lipid, 11 g; dietary fiber, 11 g; chlorophylls, 3.2 g; lutein, 270 mg; β-carotene, 90 mg; zeaxanthin, 30 mg; α-carotene, 7 mg; vitamin B1, 1.8 mg; vitamin B2, 5 mg; vitamin B12, 500 μg; vitamin C, 60 mg; folic acid, 2500 μg; biotin, 300 μg; α- tocopherol, 30 mg; vitamin K1, 3000 μg; iron, 75 mg; potassium, 1000 mg and Magnesium, 350 mg.

No restrictions were imposed on the participants of the control group other than that they were prohibited from taking *Chlorella*. This study was conducted in accordance with the general principles of the Helsinki Declaration, and all procedures involving human subjects were approved by the ethics committee of the Shimomura O.B.G.Y. Clinics (ethics no. 2010001). All participants gave their written informed consent prior to study initiation.

Specimen sampling and dioxins analysis

At the beginning of the study (gestational week 16-20), maternal blood samples (30 ml) were collected from each of the 20 participants, and concentrations of PCDDs and PCDFs were determined and expressed as the original concentrations. On the day of delivery, maternal blood samples (30 ml) were again drawn from the 20 participants and analyzed for PCDDs and PCDFs; these concentrations were expressed as the levels at delivery.

Analyses of PCDDs and PCDFs in the maternal blood were conducted according to the method described in Takasuga et al. [16]. Briefly, maternal blood and breast milk samples were spiked using 13C-labeled internal standards of PCDDs and PCDFs. Dioxin-containing lipid was then separated from maternal blood and breast milk samples by combination with ethanol, saturated sulfuric acid, and n-hexane. The lipid was removed from the dioxin-containing lipid using a multilayer silica-gel column. The multilayer cleanup samples were further subjected to aluminum oxide column chromatography, and fractionated with dichloromethane in n-hexane to the fractions of PCDD/DFs. Identification and quantification of PCDDs and PCDFs were achieved using high-resolution gas chromatography (HRGC) (Hewlett Packard 6890 Series) coupled with high-resolution mass spectrometry (HRMS) (Micromass AutoSpec Ultima). Both DB-5 and DB-17 columns (J & W Scientific) were used to separate PCDD/DF congeners. The selected ion monitoring (SIM) mode was used, and the resolution was kept higher than 10,000 (5% valley). Specific congeners of 7 PCDDs and 10 PCDFs were analyzed.

Data analysis

Toxic equivalent (TEQ) concentrations were calculated for PCDDs and PCDFs using 2005 WHO toxic equivalency factor values [2]. In these calculations, measured values of congeners with concentrations below the detection limit were regarded as zero. These dioxin concentrations were expressed as means and standard deviations. General characteristics of the subjects, except for the sex of the new-born, were also expressed as means and standard deviations. The Wilcoxon signed-rank test was used for comparison of original and at-delivery values, and the Mann-Whitney U test was used for comparisons of data between *Chlorella* and control groups. Sex of new-borns in general characteristics of subjects was analyzed by Fisher's exact test. Differences were considered significant at $p<0.05$. All statistical analyses were performed using Excel Toukai 2012 software for Windows (SSRI, Tokyo, Japan).

Results

Characteristics of participants

Characteristics of mothers and infants are listed in Table1. There were no significant differences in maternal and new-born characteristics between the control and *Chlorella* groups, although new-born weights in the *Chlorella* group tended to be higher than those in the control group. The stools of subjects in the *Chlorella* group displayed green discoloration due to the excretion of chlorophyll in the *Chlorella*. No other adverse reactions were observed. There were no differences found between the control and *Chlorella* groups with regard to the results of normal clinical examinations or interviews at Shimomura O.B.G.Y. Clinic and G.Y. & O.B. Miyahara Clinic during pregnancy (data not shown).

Characteristic	Control group	*Chlorella* group	p value[a]
Maternal			
Age (years)	30.8 ± 5.2	31.9 ± 2.5	0.544
Weight before pregnancy (kg)	51.4 ± 5.9	52.0 ± 8.2	0.97
Height (cm)	158 ± 4	160 ± 8	0.648
Parity (times)	2.1 ± 1.2	2.2 ± 0.9	0.782
New born			
Gestational age (days)	272 ± 15	278 ± 7	0.34

Weight (g)	2977 ± 442	3302 ± 529	0.07
Height (cm)	49.4 ± 2.8	49.7 ± 2.2	0.79
Head circumference (cm)	32.8 ± 1.4	33.3 ± 1.8	0.34
Sex (male/female)	6/4	7/3	1
Figure indicates mean ± SD, except sex. [a]Between control and *Chlorella* groups.			

Table 1: Characteristics of participants.

Dioxins in maternal blood

Concentrations (pg/g lipid and pg-TEQ/g lipid) of PCDDs, PCDFs and PCDD/DFs in the maternal blood of the control and *Chlorella* groups are shown in Table 2.

The principal congeners found in maternal blood were 12378-PeCDD, 123678-HxCDD, and 23478-PeCDF, in both the control and *Chlorella* groups. These three congeners represented more than 80% of the PCDD/DFs TEQ. In the *Chlorella* group, two of these congeners (concentrations of 123678-HxCDD and 23478-PeCDF) were significantly decreased during the supplementation period, from 6.5 ± 4.4 to 3.9 ± 2.3 pg/g lipid ($p<0.05$) and from 4.8 ± 3.2 to 2.9 ± 1.7 pg/g lipid ($p<0.05$), respectively. Thereby, the TEQs of PCDFs and PCDD/DFs significantly decreased in the *Chlorella* group, from 2.20 ± 1.66 to 1.00 ± 0.61 pg-TEQ/g lipid ($p<0.05$) and from 5.48 ± 4.01 to 3.38 ± 1.96 pg-TEQ/g lipid ($p<0.05$), respectively. On the other hand, the TEQs in control group did not change significantly, although there were significant changes in some minor congeners during the supplementation period. Although, the original TEQs of PCDDs, PCDFs and PCDD/DFs in the *Chlorella* group were somewhat higher than those in the control group, all of them at delivery were rather lower than those in the control group, although the difference was not significant. These results suggest that *Chlorella* supplementation may be useful in pregnant women exposed to dioxins.

Congener	TEF	Control group		*Chlorella* group	
		Original	At delivery	Original	At delivery
PCDDs					
2378-TeCDD	1	<LOD[a]	<LOD	0.12 ± 0.37	0.07 ± 0.21
12378-PeCDD	1	2.2 ± 1.4	2.3 ± 1.3	2.3 ± 1.9	1.8 ± 1.1
123478HxCDD	0.1	<LOD	<LOD	<LOD	<LOD
123678HxCDD	0.1	6.5 ± 3.6	5.9 ± 4.0	6.5 ± 4.4	3.9 ± 2.3*
123789HxCDD	0.1	<LOD	0.3 ± 0.8	0.3 ± 1.0	0.1 ± 0.4
1234678-HpCDD	0.01	10.0 ± 4.3	6.0 ± 2.9*	13.0 ± 7.7	8.9 ± 4.8
OCDD	0.0003	142 ± 63	111 ± 53	136 ± 61	120 ± 59
PCDFs					
2378-TeCDF	0.1	< LOD	< LOD	<LOD	< LOD
12378-PeCDF	0.03	<LOD	<LOD	0.1 ± 0.4	<LOD
23478-PeCDF	0.3	4.7 ± 1.6	4.0 ± 2.4	4.8 ± 3.2	2.9 ± 1.7*
123478HxCDF	0.1	1.4 ± 1.5	0.3 ± 0.8	1.7 ± 2.4	0.45 ±0.7
123678HxCDF	0.1	2.0 ± 2.1	0.4 ± 0.1*	2.7 ± 3.1	0.8 ± 1.0
123789HxCDF	0.1	<LOD	<LOD	< LOD	<LOD
234678HxCDF	0.1	1.4 ± 2.5	<LOD	2.7± 3.1	<LOD*
1234678-HpCDF	0.01	2.7 ± 2.3	0.7 ± 1.1*	3.2 ± 3.1	0.8 ± 1.4
1234789-HpCDF	0.01	<LOD	<LOD	<LOD	<LOD
OCDF	0.0003	<LOD	<LOD	<LOD	<LOD

TEQ					
PCDDs		2.98 ± 1.72	2.97 ± 1.6	3.28 ± 2.5	2.38 ± 1.4
PCDFs		1.91 ± 0.86	1.28 ± 0.8	2.20 ± 1.6	1.00 ± 0.6*
PCDD/DFs		0.864.89 ± 2.23	4.25 ± 2.4	5.48 ± 4.0	3.38 ±1.9*
Figure indicates mean ± SD. [a]Values below the detection limit. *p<0.05, between original and at delivery.					

Table 2: Concentrations (pg/g lipid and pg-TEQ/g lipid) of PCDDs, PCDFs and PCDD/DFs in the maternal blood of the control and *Chlorella* groups.

Discussion

This study was the first trial to assess the effects of supplementation with *Chlorella* on the change of blood dioxin levels during the supplementation period in pregnant women. The findings showed the effectiveness of daily intake of *Chlorella* from gestational week 16-20 until the day of delivery for lowering of maternal blood dioxin levels. As mentioned above, adverse health consequences from prenatal, perinatal and postnatal exposure to background levels of dioxins, including PCDDs and PCDFs, have been reported [5-12]. There is a positive relationship between dioxin concentration in the blood and that in cord blood, breast milk, and various tissues, including lung, liver, spleen, pancreas, kidney, mesentery fat, and subcutaneous fat [19-22]. Thus, the present results suggest that *Chlorella* supplementation in pregnant woman might contribute to the healthy development of foetuses and nursing infants by improving maternal dioxin status. This study also suggests that *Chlorella* is safe for use by pregnant women.

In an animal study, Morita et al. [18] showed that administration of *Chlorella* was effective in preventing gastrointestinal absorption of 7 congeners of PCDD and 10 congeners of PCDF, as well as for promoting the excretion of these substances already absorbed into tissues. In this study, we confirmed that *Chlorella* supplementation in pregnant women contributed to a reduction of blood PCDD and PCDF levels. Small amounts of dioxin invade our bodies daily through many kind of food, including fish, shellfish, meat, eggs, milk and dairy products [23]. Dioxins that have accumulated in the body are also secreted with bile into the intestine [24,25]. Rather than being eliminated, however, they can be reabsorbed from mucosal cells lining the gastrointestinal tract. Thus, inhibition of dioxin absorption and reabsorption in the gastrointestinal tract increases its excretion in faeces, resulting in a decrease of the dioxin level in the body.

Three *Chlorella* ingredients appear to be associated with inhibition of absorption and reabsorption in the intestine. The first is the chlorophyll in *Chlorella* cells. Morita et al. [17] confirmed that administration of chlorophyll derived from *Chlorella* at a dose of 2.6-130 mg/kg body weight per day accelerated dioxin fecal excretion and reduced body dioxin level in rats. This was because chlorophyll forms a complex with dioxin congeners that have a planar structure, thereby inhibiting absorption in the digestive tract. In this study, daily intake of chlorophyll was calculated to be 3.7 mg/kg body weight, a dose within the range of the animal study. The second component of *Chlorella* that may be involved in the absorption of dioxins is dietary fiber. Several types of dietary fiber have been reported to bind dioxins and stimulate the excretion of PCDD and PCDF congeners [13,15,26]. After ingestion of *Chlorella*, dietary fibers might inhibit dioxin absorption from the digestive tract, promoting its excretion into faeces. The third component is the complex lipids in *Chlorella* cells, which promoted hepatic bile acid synthesis from endogenous cholesterol and its excretion into faeces in rats, due to prevention of bile acid reabsorption in the intestinal tract [27]. Since dioxins that have accumulated in the body are secreted with bile into the intestine [24,25], the acceleration of bile acid excretion by the complex lipids in *Chlorella* might contribute to the excretion of dioxins in faeces. Intake of *Chlorella* that is abundant in chlorophyll, dietary fiber and complex lipids must therefore contribute to the acceleration of PCDD and PCDF excretion to faeces via prevention of gastrointestinal absorption. It was thought this was why the dioxin level in maternal blood decreased during the *Chlorella* supplementation period.

Infant exposure to dioxins by the breast milk pathway can be significant. For example, an infant who had been breast-fed for one year had an accumulated dioxin dose six times higher than a 1-year-old formula-fed infant [28]. There is a positive relationship between the concentration of dioxin in the blood and in breast milk [19,22]. Thus, by lowering the level of dioxin in maternal blood, it should be possible to decrease the dioxin level in breast milk. Actually, the PCDD/DF levels of colostrum in the *Chlorella* group (4.16 ± 2.09 pg-TEQ/g lipid) were somewhat lower than those in the control group (4.37 ± 1.89 pg-TEQ/g lipid), similar to observations of the blood dioxin levels at delivery. However, the sample size of this study was not large, and *Chlorella* supplementation (yes/no) and other factors (maternal age, parity and BMI before pregnancy) were not significantly associated with the change of blood dioxin levels during experimental period in multiple regression analysis. Future larger and double-blind, placebo-controlled study will be necessary to confirm these findings.

Restrictions on the industrial release of PCDD/DFs into the environment have resulted in an attendant decrease in levels of these compounds in the environment. Dioxin accumulation levels in humans evaluated by concentration in breast milk notably decreased from the 1970s to the 1990s in Japan (survey of the Ministry of the Environment, Japan, 2012) and other industrialized countries [29]. However, in the past decade, there has been little decline of dioxin concentrations in blood and breast milk (survey of the Ministry of the Environment, Japan, 2012). Several large studies are continuing to elucidate the environmental factors that affect children's health and development [30,31]. We must await the results of detailed long-term studies to thoroughly ascertain any causal relationship. Meanwhile, parallel measures should also be pursued to reduce health risks to foetuses and nursing infants posed by environmental contaminants such as dioxins. Reducing maternal dioxin levels and the transfer of dioxins to foetuses and nursing infants represents one of the most realistic ways to accomplish this goal. The present results suggest that *Chlorella* supplementation for pregnant woman may be a simple way

of reducing maternal dioxin levels, and may reduce the transfer of dioxins to foetuses and nursing infants. *Chlorella* tablets is also abundant in nutrients necessary for pregnant women such as folic acid (2500 μg/100 g), vitamin B12 (500 μg/100 g), iron (75 mg/100 g) and carotenoids (lutein, 270 mg/100 g; β-carotene, 90 mg/100 g; zeaxanthin, 30 mg/100 g). We recently reported that maternal supplementation with *Chlorella* improve the carotenoid status of maternal blood and breast milk and thereby probably contributing to healthy development of foetus and newborn [32,33]. This evidence suggests that *Chlorella* may be an excellent food for pregnant women due to both the reduction of dioxin levels and the supplementation of nutrients necessary for pregnant women.

Reducing dioxin levels would also be helpful for Yusho patients, whose dioxin levels continue to be notably higher than that of the general population, even more than 40 years after the Yusho incident [34]. Based on the results of this study, we also strongly recommend that it be considered as a fundamental therapeutic supplementation for patients with Yusho disease, which is PCDF intoxication [35-36]. *Chlorella* is likely to reduce the PCDF body burden of such patients.

Conclusion

Adverse health consequences from pre-, peri- and postnatal exposure to background levels of dioxins have been reported. In order to prevent and/or reduce such health risks, it is important to decrease maternal exposure to dioxins. We investigated the effect of maternal supplementation with *Chlorella* on dioxin levels in the blood during pregnancy. The findings showed the effectiveness of daily intake of *Chlorella* for lowering of maternal blood dioxin levels.

Conflict of Interest

Chlorella Industry Co. Ltd. provided the test supplements used in the study. Junya Nagayama, Takumi Takasuga, Hiroshi Shimomura and Michiyoshi Miyahar have no competing interests.

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