CHLORELLA SUPPLEMENTATION DECREASES THE BLOOD LEVELS OF DIOXINS IN JAPANESE PREGNANT WOMEN

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Introduction

Our environments including food have been polluted with extremely toxic Dioxins which are polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls (DL-PCBs) [1]. Consequently, humans and breast milk also have already been contaminated with these compounds [2,3,4].

Adverse health consequences of Dioxins have been investigated in the fetus and suckling which are considered the most sensitive stages of human life. In fact, we have already observed their unfavorable effects on thyroid hormone and immune response systems in Japanese infants perinatally and lactationally exposed to them [5, 6, 7, 8, 9].

In order to avoid or prevent their adverse health consequences on fetuses and sucklings, accelerative reduction of their contamination levels in mother's body, particularly for highly toxic congeners, seems quite important. In rats, dietary fiber and chlorophyll have been shown to promote the fecal excretion of PCDDs and PCDFs probably due to the inhibition of their absorption and reabsorption in the digestive tract to some extent, and therefore to decrease their levels in rat liver [10, 11, 12].

In this study, we examined whether such kinds of effect were observed by Chlorella supplements, which was rich in chlorophylls and dietary fibers, for PCDDs, PCDFs and DL-PCBs or not in Japanese pregnant women.

Materials and methods

Subjects of this study comprised 20 healthy pregnant women (age range, 24~39 years) who were receiving prenatal care at Shimomura O.B.G.Y. Clinic and G.Y. & O.B. Miyahara Clinic, Fukuoka, Japan and who provided written consent to participate in this study. Of these, 10 subjects (2 primiparae, 8multiparae) agreed to take Chlorella tablets (chlorella group). These subjects were asked to take Chlorella tablets for approximately 6 months, from gestational week 16~20 up until day of delivery. Dose was 6g/day of Chlorella(30 tablets/day), in portions of 10 tablets after each main meal. Biorinck tablets, which hasbeen manufactured since 2008 with Chlorella Industry Co. Ltd, Tokyo, Japan and taken by more than 100,000 people as one of the health foods,containing dried Chlorellapowder as the active ingredient were used. Each tablet comprised the following (g/100g): chlorophyll, 3.2; dietary fiber, 11; protein, 62; lipid, 11. No restrictions were imposed on the remaining participants (5primiparae, 5multiparae) who comprised the control group, with the exception that they were prohibited from taking Chlorellatablets.

This study was conducted in accordance with the general principles of the Helsinki Declaration.

At the beginning of this study, that is, August to December 2010 and February to April 2012, maternal blood samples (30 ml) were taken from each of the 20 participants and blood concentrations of PCDDs, PCDFs and DL-PCBs in both the chlorella and control groups were determined by high resolution GC/MS method [13], which were expressed as the initial concentrations.

In order to examine the effects of Chlorella on the accelerative excretion of PCDDs, PCDFs and DL-PCBs from the maternal body, on the day of delivery, which were between February and April 2011 and between July and September 2012, again maternal blood samples (30 ml) were taken from each of the 20 participants and their blood concentrations were determined by the same analytical method in both the chlorella and control groups, which were expressedas the final concentrations. At the respective same periods of time, breast milk samples (20ml) were also obtained from each of them and the concentrations of PCDDs, PCDFs and DL-PCBs were determined by almost the same analytical method of the blood.

Means and standard deviations (SDs) were calculated for the concentrations of each congener, and for PCDDs, PCDFs, DL-PCBs and Dioxins (PCDDs + PCDFs + DL-PCBs), respectively,in the maternal blood. Toxic equivalent (TEQ) concentrations were calculated using WHO toxic equivalency factor values for each congener [14]. In these calculations, measured values of congeners with concentrations below the detection limit were regarded as half of the detection limit.

Statistical significance of the differences between the initial and final concentrations of each group was evaluated by a two-tailed paired student's *t*-test and in order to prevent the error of second kind, as a rule*p*-value less than 0.10 was considered significant.

Results and discussion

As shown in Fig. 1, initial mean TEQ concentrations of PCDFs in the maternal blood of chlorella and control groups were 2.7 and 2.4pg/g lipid, respectively, and the concentration of chlorella group was slightly higher than that of control group. However, final mean TEQ concentration of PCDFs was a little bit lower than that of control group, that is, 1.5 and 1.8pg/g lipid. Therefore, in chlorella group about 44% reduction was observed, but 25% reduction was seen in control group.

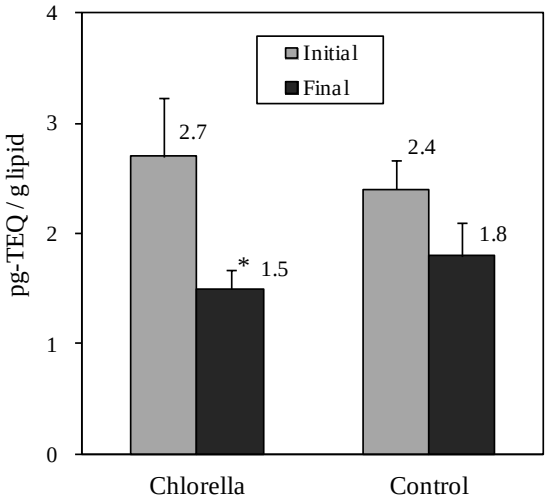

Fig. 1. Mean TEQ concentrations of PCDFs in the maternal blood of chlorella and control groups.
* : p= 0.033

In mean TEQ concentrations of PCDDs in the maternal blood, the initial and final concentrations in chlorella group were, respectively, 4.3 and 3.2pg/g lipid and those in control group 4.1 and 3.8pg/g lipid. Therefore, reduction rate of chlorella group was 26% and that of control group 7%. Consequently, the reduction rate in chlorella group was much greater than that in control group.

In mean TEQ concentrations of DL-PCBs in the maternal blood, the respective initial concentrations of chlorella and control groups were 2.8 and 2.5pg/g lipid and the respective final concentrations of both groups 2.4 and 2.5 pg/g lipid. Hence, the reduction of DL-PCBs was only observed in chlorella group.

As a consequence, initial and final mean TEQ concentrations of Dioxins in the maternal blood of chlorella and control groups, which were indicated in Fig. 2, were 9.8 and 7.1 pg/g lipid, and 9.1 and 8.1pg/g lipid, respectively. Therefore, the reduction rate of chlorella group was 28% and that of control group 11%. Finally, concentrations of Dioxins in the maternal blood of chlorella group was 17% more reduced due to the accelerative excretion of PCDDs, PCDFs and DL-PCBsfrom the maternal body probably by Chlorella Biorinckintake than those of control group.

The primary mechanism by which Chlorellaaccelerates excretion of Dioxins is presumably through the formation of complexes between Dioxins and the chlorophyll in Chlorella [15]. Dioxins are then absorbed by the dietary fiber also contained in Chlorella[16], thereby inhibiting the absorption and reabsorption of Dioxins from the gastrointestinal tract.

We have observed very good correlation between the concentrations of Dioxins in the blood and those in other tissues such as the liver and adipose tissue [17, 18]. Therefore, the body burdens of Dioxins in chlorella and control groups were computed on the assumptions that the mean body weight was 50kg, the body fat was 20% of body weight, that is, 10kg and the body fat was also contaminated with the same concentrations of Dioxins as those in the maternal blood. Then the initial body burdens of Dioxins in chlorella and control groups were, respectively, 97ng and 91ng, and the

respective final body burdens of Dioxins were 71ng and 81ng. In consequence, the net reduction of body burden in chlorella group was 26ng and that in control group 10ng, as shown in Fig. 3. The respective net reduction rates were 27% and 11%. As already mentioned above, body burden of Dioxins in chlorella group was 16% more reduced by Chlorella intake than that in control group.

The concentrations of Dioxins in the blood also correlated quite well with those in the breast milk [19]. Therefore, we observed somewhat lower concentrations of Dioxins (7.6 pg/g lipid) in chlorella group than those (8.0 pg/g lipid) in control group in the breast milk, like the respective final concentrations in the blood showed.

In conclusion, Chlorella supplements (Biorinck tablets) seemed very much effective for reducing Dioxin exposure, and also considered effective for reducing their risks in fetuses and sucklings.

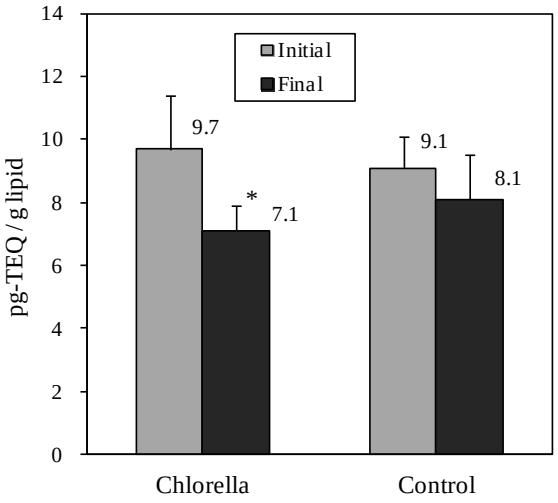

Fig. 2. Mean TEQ concentrations of Dioxins in the maternal blood of chlorella and control groups.
* : $p = 0.113$

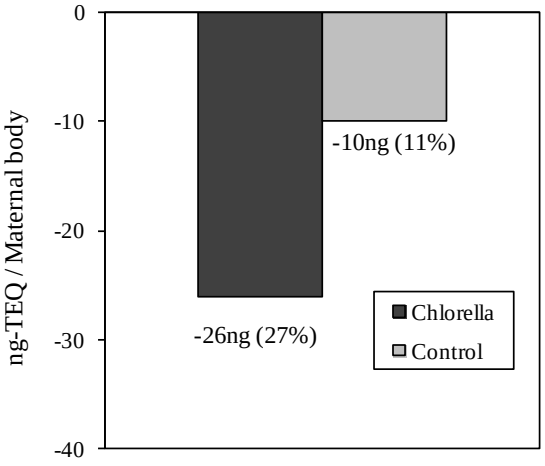

Fig. 3. Accelerative reduction of Dioxins in body burden by Chlorella intake
Figures in parentheses indicate respective reduction rates in chlorella and control groups

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