The Journal of Toxicological Sciences (J. Toxicol. Sci.)
Vol.35, No.1, 101-105, 2010

Letter

The influence of *Parachlorella beyerinckii* CK-5 on the absorption and excretion of methylmercury (MeHg) in mice

Takuya Uchikawa[1], Akira Yasutake[2], Yoshimitsu Kumamoto[1], Isao Maruyama[1], Shoichiro Kumamoto[1] and Yotaro Ando[1]

[1]Department of Research & Development, Chlorella Industry Co., Ltd., 1343 Hisatomi, Chikugo, Fukuoka 833-0056, Japan
[2]Biochemistry Section, National Institute for Minamata Disease, 4058-18 Hama, Minamata, Kumamoto 867-0008, Japan

(Received September 17, 2009; Accepted November 13, 2009)

ABSTRACT — *Chlorella* (*Parachlorella beyerinckii* CK-5), previously identified as *Chlorella vulgaris* CK-5, is a unicellular green algae that has for many years been used as a nutritional supplement. In order to investigate the effects of methylmercury (MeHg) detoxification by *Chlorella*, we examined the absorption and excretion of MeHg in mice. Female C57BL/6N mice were randomly divided into three groups of five, and were housed in metabolism cages. Mice were orally administered MeHg chloride at doses of 5 mg (4 mg Hg)/kg body weight with or without 100 mg/mouse of *P. beyerinckii* powder (BP), and were assigned to either a MeHg group or MeHg + BP group, accordingly. Twenty-four hr after oral administration, feces and urine were collected, and blood, liver, and kidney samples were obtained. Total mercury contents in the samples obtained were determined using an atomic absorption method. The amounts of Hg excreted in feces and urine of the MeHg + BP group were increased nearly 1.9 and 2.2-fold compared with those of the MeHg group. On the other hand, blood and organ Hg levels were not significantly different between two groups. These results suggest that the intake of BP may induce the excretion of Hg both in feces and urine, although it does not affect MeHg absorption from the gastrointestinal tract. The effect of BP on the tissue mercury accumulation may become evident in a long-term experiment.

Key words: *Parachlorella beyerinckii*, MeHg, *Chlorella*, Detoxification, Excretion

INTRODUCTION

Methylmercury (MeHg) is a neurotoxic metal-compound that has been widely utilized for industrial purposes. Recently, individual consumption of seafood has increased worldwide. However, pregnant women are increasingly being cautioned against consuming seafood in many places, including United States and Europe (Mahaffey, 1999; FDA, 2001; European Commission, 2004). These warnings are based on fears regarding the harmful influence of MeHg contained in seafood on the developing embryo.

Chlorella (*Parachlorella beyerinckii* CK-5) is an unicellular green algae approximately 3 to 8 μm in diameter, and it has been eaten as a nutritional food for many years due to its abundance of nutritional components such as proteins, vitamins, minerals and dietary fibers. It is already known that *Chlorella* has the ability to adsorb some metals such as Cd, Zn, Cu, and Pb (Sandau *et al*., 1996). It has therefore been utilized in waste water treatment systems to reduce contaminated heavy metals (Almaguer Cantu *et al*., 2008). In addition, *Chlorella* has been reported to be useful in detoxifying cadmium and dioxins in animal experiments (Nagao *et al*., 1983; Morita *et al*., 2001). However, the effects of *Chlorella* with regard to MeHg have remained unclear. In order to investigate the influence of *Chlorella* on MeHg absorption and excretion in mice, we examined here excretion of Hg as well as the accumulation levels of internal Hg using mice after MeHg administration.

Correspondence: Takuya Uchikawa (E-mail: takuya_uchikawa@chlorella.co.jp)

MATERIALS AND METHODS

Preparation of *Parachlorella beyerinckii* powder (BP)

Parachlorella beyerinckii CK-5, an unicellular green algae approximately 3 to 8 μm in diameter, was used in this study. It was originally identified as *C. vulgaris* based on its morphological characteristics according to the description of Fott and Nov'akov'a (1969), and has been re-identified as *P. beyerinckii* based on both the sequences of the 18S rRNA gene and its morphological characteristics according to the description of Krienitz *et al*. (2004). The algae cells were cultured in an outdoor pool, harvested, and washed with water by a centrifuge separator. The obtained algae slurry was heated at 118°C for 1 min with a plate-heater, and was dried with a spray-drier under a blower temperature of 170°C.

Animals and chemicals

Female C57BL/6N mice (aged 10 weeks) were purchased from Charles River Japan Co., Ltd. (Kanagawa, Japan) and used in this study. MeHg chloride and all chemicals were obtained from Wako Pure Chemicals Ind. (Osaka, Japan). Mice were randomly divided into three groups of five animals and were housed in metabolic cages (one mouse per cage) with a 12-hr light cycle (6:00 to 18:00) at 23 ± 0.5°C and 55 ± 5% relative humidity. Animals were allowed free access to feed (pelleted rodent diet, CE-2, CREA Japan Inc., Tokyo, Japan) and water. MeHg chloride was dissolved in distilled water (184 μg/ml) and administered to the mice at a dose of 5 mg (4 mg Hg)/kg body weight with or without 100 mg/mouse of BP. The mice were assigned accordingly to a MeHg group or MeHg + BP group. In the control group, mice were administered an equal volume of distilled water instead of MeHg. Twenty-four hr after administration, blood was collected from the inferior vena cava under pentobarbital anesthesia; the animals were then perfused with phosphate buffered saline (pH 7.3), and the liver and kidney were removed. The animals were cared for according to the NIH published guideline.

Analysis of total Hg

All samples were degraded by the wet-ashing method (Ministry of Environ, 2004) as a pretreatment, and total Hg levels in all samples were then determined by the reducing-vaporization method using a Mercury Analyzer RA-3320 (Nippon Instruments Corp., Tokyo, Japan).

MeHg adsorption to BP and pepsin non-digestive residue of BP (dBP)

The pepsin digestion of BP was carried out according to the method of AOAC (1990). After the enzymatic reaction, non-digestive residue was collected by centrifugation at 3,000 rpm for 10 min at room temperature, and then freeze-dried. The yield of the freeze-dried preparation was used as dBP. In the experiment of MeHg adsorption *in vitr*o, 100 mg of BP and dBP were shaken in 10 ml of 180 μg/ml MeHg chloride solution (144 μg Hg/ml) at 37°C for 16 hr. After that, BP and dBP were removed from the solution by centrifugation at 3,000 rpm for 15 min at room temperature. Hg concentrations in supernatants were measured, and then the adsorption rates of MeHg to BP and dBP were calculated. The experiments were performed in triplicate.

Statistical analysis

The significance of difference was calculated according to the Student's *t*-test using Microsoft Office Excel 2007 for Windows (Microsoft Co., Ltd., Tokyo, Japan). Each value of $p < 0.05^{*}$ or $p < 0.01^{**}$ was considered statistically significant.

RESULTS AND DISCUSSION

To investigate the influence of *Chlorella* on MeHg excretion and absorption using mice, we divided the animals into three groups, MeHg group, MeHg + BP group, and control group. Each of mice was administered MeHg (5 mg/kg of MeHg chloride), MeHg + BP (100 mg/mouse) or distilled water, respectively. The initial average body weight of the three groups was 18.4 g, and no difference was observed among three groups 24 hr after administration. Additionally, no significant differences were found in feed and water intakes for 24 hr among the groups (data not shown), suggesting that body weight was presumed not to be affected by the administration of MeHg and BP.

The excretions of Hg in feces and urine for 24 hr after MeHg administration are shown in Fig. 1. The amounts of Hg excreted in feces of the MeHg and MeHg + BP groups were 3.12 ± 0.42 μg/mouse (mean ± S.D.) and 5.98 ± 0.52 μg/mouse, respectively. Fecal excretion of Hg in the MeHg + BP group was 1.9-fold higher than that in the MeHg group. In the control group, Hg was not detected in the feces. Moreover, there was no significant difference in the amounts of feces for 24 hr after administration among the three groups (data not shown). MeHg is an easily absorbable toxic substance, and it is known that 95% or more of the MeHg consumed is absorbed from

the gastrointestinal tracts in humans (Canuel *et al*., 2006). Since *Chlorella* is known to have an ability to adsorb several heavy metals (Sandau *et al*., 1996), the dietary components of BP may adsorb a part of MeHg in the gastrointestinal tract. In fact, 80% of lead was adsorbed on dBP in 200 ppm of lead solution (Uchikawa *et al*., 2009). The adsorbed portion of MeHg may appear in the feces without intestinal absorption. MeHg was incubated with dBP *in vitro* to examine its adsorption to dBP. The adsorption rate of MeHg to dBP was found to be as low as 6.6% even after 16-hr incubation (Table 1). Since a time for the interaction between MeHg and dBP in the gastrointestinal tract would be much shorter than 16 hr, an actual adsorption rate occurred there should be much lower than 6.6%. Accordingly, MeHg absorption at the gastrointestinal tract would have occurred mostly at the equal rates in MeHg and MeHg + BP groups.

As alternative factor in the increase in fecal excretion of Hg, we hypothesized that the intake of BP might accelerate the biliary elimination of MeHg. Sano (1982) has reported that BP components such as compound lipid and dietary fiber inhibit the re-absorption of bile acid in the intestinal tract, thus accelerating the secretion of bile in the enterohepatic circulation. Most MeHg has been found to be conjugated with glutathione in liver and blood, with the MeHg-glutathione complex then being secreted from the liver with bile (Ballatori and Clarkson, 1983; Hirayama *et al*., 1987), then small portion of them could be excreted in the feces. Thus, combined effects of dBP adsorption and accelerated biliary elimination might contribute to the increased fecal Hg excretion in the MeHg + BP group observed in the present study.

As to the urinary excretion of Hg, the amounts of excreted Hg in the MeHg and MeHg + BP groups were 0.17 ± 0.07 μg/mouse and 0.38 ± 0.16 μg/mouse, respectively. The levels of urinary-excreted Hg in the MeHg + BP group were 2.2-fold higher than those in the MeHg group, similar to the increase in fecal-excreted Hg in the MeHg + BP group. In the control group, no urinary Hg excretion was detected. Although urinary MeHg excretion was reported to occur as its cysteine-conjugate (Yasutake *et al*., 1989), we considered that the BP-derived met-

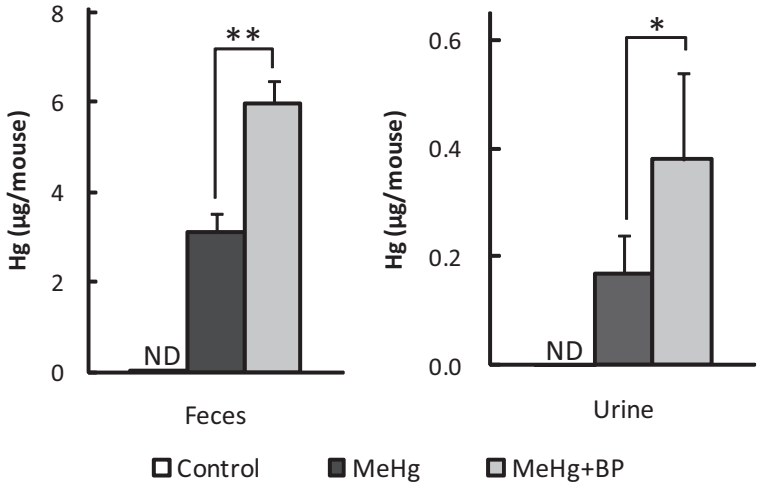

Fig. 1. Excretion of MeHg in feces and urine for 24 hr after the administration of MeHg and BP. Values represent mean ± S.D.. n = 5. ND: Not Detected. Significant differences were shown by * ($p < 0.05$) and ** ($p < 0.01$).

Table 1. The MeHg adsorption toBP and dBP *in vitro*

	BP	dBP
Initial mercury concentrations in flask (μg Hg/ml)	144	144
Mercury concentration in supernatant (μg Hg/ml)	124.0 ± 6.7	134.5 ± 5.1
Adsorption rates of Hg to BP and dBP (%)	13.9 ± 4.7	6.6 ± 3.5

Values represent mean ± S.D.. n = 3.

al-binding factor might contribute to the urinary MeHg excretion. Alternative possibility may be a excretion of inorganic Hg. It has previously been reported that an MT-like metal-binding protein derived from *Chlorella* had a high ability of the adsorption to some metals such as Cd, Zn, and Cu (Yoshida *et al*., 2006; Huang *et al.*, 2008). Nagao *et al*. (1983) reported that co-administration of the *Chlorella*-derived metal-binding protein to rat caused an enhanced urinary excretion of Cd. MeHg is well known to change to inorganic Hg in animal tissue. It may be possible that inorganic Hg thus formed can interact with the *Chlorella*-derived metal-binding protein. Further study using chromatography and/or selective quantification of urinary Hg will provide a clue to clarify a mechanism for BP-induced urinary Hg excretion.

In addition, to investigate the absorption of Hg, the Hg levels of blood, liver, and kidney were also analyzed 24 hr after administration. As shown in Fig. 2A, blood Hg levels of the MeHg + BP group (2.70 ± 0.15 μg/ml) were slightly, but not significantly, lower than those of the MeHg group (2.48 ± 0.19 μg/ml). Hg accumulations in the liver and kidney are shown in Figs. 2B and C. No significant differences were found both in liver and kidney Hg levels between the MeHg and MeHg + BP groups, suggesting that MeHg absorption might have occurred at the similar rates in both groups. That is, BP would have little effect on the absorption of MeHg from the gastrointestinal tract. In the control group, no accumulated Hg was detected in the liver and kidney.

In the present study BP was found to enhance Hg excretion from MeHg-treated mice. The percentages of total excretion for administered dose (73.5 μg Hg/mouse)

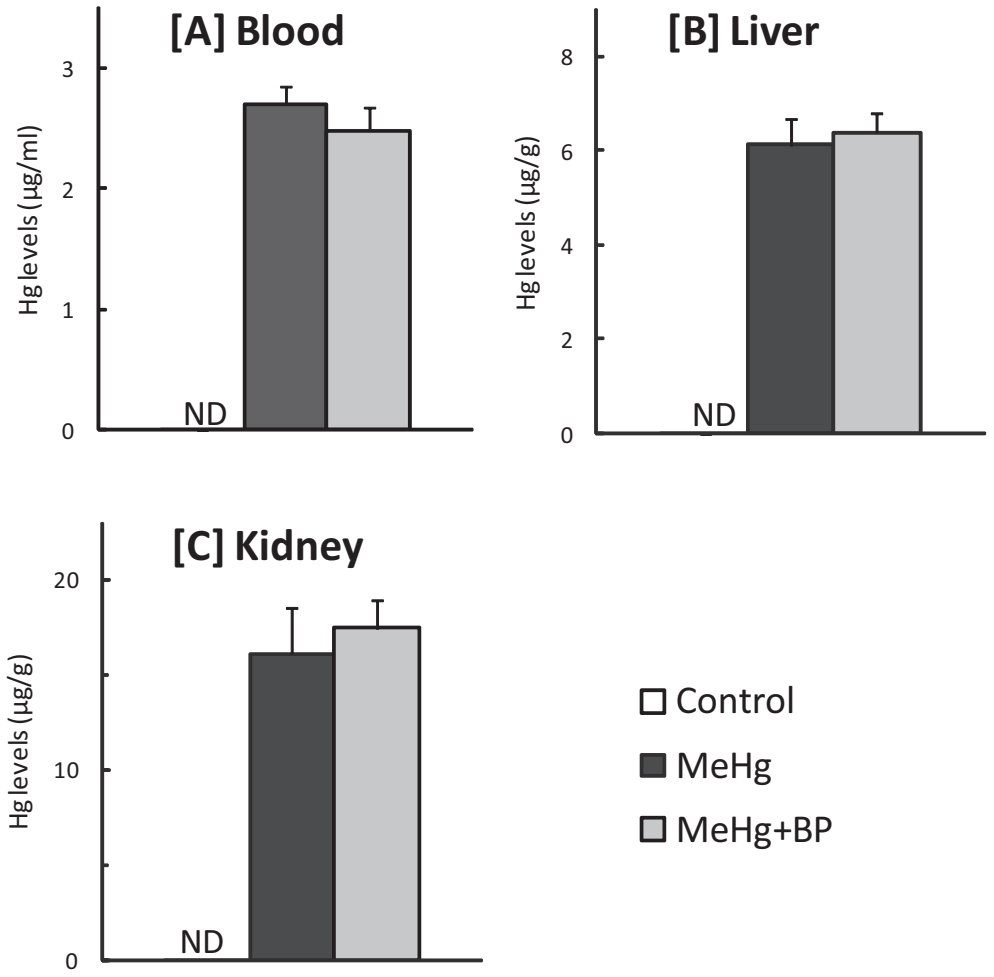

Fig. 2. The mercury levels of blood [A] and the mercury contens of liver [B] and kidney [C] at 24 hr after MeHg administration. Values represent mean ± S.D.. $n = 5$. ND: Not Detected.

were 4.5% and 8.7% in MeHg and MeHg + BP groups, respectively. However, no difference was observed in the tissue Hg accumulation. The BP-induced increased rate in the excretion, 4.2% of the injected amount, might not be sufficient to observe significant reduction in the tissue Hg accumulation. A long term study may be necessary to observe reduced Hg in blood and organs. The present results, however, suggest that *Chlorella* may be a food material that can be successfully used to detoxify MeHg.

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