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Original Article

Intake of wheat bran after administration of methylmercury reduces mercury accumulation in mice

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ABSTRACT — Methylmercury (MeHg) exposure during pregnancy is a concern because of its potential health risks to the fetus. Wheat bran (bran), which is the outer layer of wheat kernel, is used as a functional substance in food for specified health uses in Japan. In the present study, we examined the effect of bran on the accumulation and excretion of Hg in mice to evaluate its potential use for reducing the health risk of MeHg. Female BALB/cByJ mice were administered MeHg chloride (4 mg Hg/kg, p.o.). Immediately after administration, the mice were fed a basal diet supplemented with 0%, 5%, or 15% bran, and urine and feces were collected for 14 days. The bran groups had lower total Hg levels in all tissues including the brain compared with the control group, but the effects were significant only in the blood and brain of mice on the 15% bran diet. Urinary Hg excretion in the bran groups was markedly higher than in the control group, although there was no difference in the excretion between the bran groups. Moreover, fecal Hg excretion in the bran groups was substantially higher than in the control group and was dose-dependent. These results suggest that bran intake after MeHg exposure may enhance Hg excretion both in urine and feces and decrease tissue Hg levels. In conclusion, dietary bran might be useful for reducing Hg burden in humans ingested MeHg in food.

Key words: Wheat bran, Methylmercury, Excretion, Accumulation

INTRODUCTION

Methylmercury (MeHg) is a ubiquitous environmental pollutant and well-known neurotoxicant. Humans are exposed to MeHg mainly through the consumption of fish, shellfish, and sea mammals (National Research Council, 2000). Pregnant women are cautioned against consuming seafood in Canada, UK, USA, Australia, Norway, and Japan because the developing fetal brain is highly susceptible to MeHg (Sakamoto *et al.*, 2002). However, seafood is generally richer in high-quality proteins and omega-3 polyunsaturated fatty acids, such as eicosapentaenoic acid and docosahexaenoic acid, than other foods, and is an important source of beneficial nutrients.

Among the possibilities for reducing exposure, reduced absorption of toxic elements after ingestion has been considered. Some studies have suggested that dietary factors, including fibers and phytochemicals, can impact MeHg bioavailability (Girard *et al.*, 2018; He and Wang, 2011; Kiyozumi *et al.*, 1982; Ou *et al.*, 1999; Rowland *et al.*, 1986; Shim *et al.*, 2009). Indeed, mice fed a 15% or 30% wheat bran (bran) diet from 3 months before MeHg administration to the end of the experiment had decreased Hg concentrations in the blood and brain 2 weeks after administration (Rowland *et al.*, 1986). However, the mechanism of Hg elimination by bran is not well understood.

Bran is the outer layer of wheat kernel and is a food additive that is used to add texture and richness to udon,

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as well as bread, cereal, and other baked goods. Since the late 1990s, bran has also been used as a functional substance in foods for specified health uses in Japan. By the way, bran had the greatest reducing effect on Hg bioaccessibility *in vitro* (the amount of Hg that is released from fish into gastrointestinal tract fluid following simulated digestion and, as a result, is available for absorption by the intestinal mucosa) among dietary fibers (bran, oat bran, and psyllium), suggesting that co-consumption of bran with MeHg may potentially reduce MeHg absorption by the intestine (Shim *et al.*, 2009). Here, we examined the effect of bran intake after MeHg exposure on the accumulation and excretion of Hg in mice which is closer MeHg toxicokinetics to humans (Nielsen and Andersen, 1991).

MATERIALS AND METHODS

Chemicals

MeHg chloride (MeHgCl) was purchased from Nacalai Tesque (Kyoto, Japan). All other reagents and chemicals were of the highest grades available.

Animals

Twelve BALB/cByJ female mice (aged 3 weeks; CLEA Japan, Inc., Tokyo, Japan) were housed in individual plastic cages and maintained on a 12-hr light cycle (07:00 to 19:00) at 25 ± 1°C and at a humidity of 50 ± 5%. The mice were fed a basal diet (AIN-76, CLEA Japan), and food and tap water were provided *ad libitum*. The protocol for animal experiments was approved by the Ethics and Safety Committee on Animals at the National Institute for Minamata Disease. All care and experimental procedures for mice were carried out according to the standards of the Ministry of the Environment, Japan (Notice No. 88 of 2006), and the fundamental guidelines of the Ministry of Education, Culture, Sports, Science and Technology, Japan (Notice No. 71 of 2006). All efforts were made to minimize animal suffering.

MeHg exposure and diets

Five weeks after feeding, mice were administered a single dose of MeHgCl (4 mg Hg/kg body weight, p.o.). Immediately after administration, the diet was switched to the basal diet supplemented with 5% or 15% bran (Table 1). The control group was maintained on the basal diet. Then, mice were housed in metabolic cages (one mouse per cage) for 14 days, and urine and feces were collected every day. Daily food intake was measured for 4 days. Sixteen days after MeHg administration, blood was collected from the heart using a heparinized syringe under isoflurane anesthesia. After perfusion with physiologic (0.9%) saline, the brain, liver, and kidneys were removed for Hg analysis. All samples were stored at −80°C until Hg analysis.

Table 1. Composition of the experimental diets.

Ingredients (g/kg)	Basal diet (AIN-76)	5% Bran diet	15% Bran diet
Sucrose	500	450	350
Bran	0	50	150
Casein	200	200	200
Cornstarch	150	150	150
Corn oil	50	50	50
Cellulose	50	50	50
Mineral mix[#]	35	35	35
Vitamin mix[#]	10	10	10
DL-methionine	3	3	3
Choline bitartrate	2	2	2

[#]Prepared according to AIN-76 formulation.

Hg analysis

Total Hg content in each sample was determined by the oxygen combustion-gold amalgamation method using a mercury analyzer (MA3000; Nippon Instruments Co., Tokyo, Japan).

Statistical analysis

The normality of data distribution was analyzed using Bartlett's test. Unless stated otherwise, differences in data between groups were determined using a one-way ANOVA with the *post hoc* Bonferroni test. $p < 0.05$ was considered statistically significant.

RESULTS

Daily food intake was similar in all three groups (Table 2). Additionally, there was no significant difference in body weights before MeHg administration and at the end of the experiment among the three groups.

Sixteen days after MeHg administration, the bran groups had lower total Hg concentrations in all analyzed tissues compared with the control group (Table 3). In particular, mice fed the 15% bran diet had significantly decreased total Hg concentrations in the blood and brain, which were 73% and 72% that of levels in control mice, respectively.

During the 14 days after MeHg administration, control mice excreted 8.37% and 12.51% of the Hg dose in the urine and feces, respectively (Fig. 1A, C). The sum of fecal and urinary Hg excretions was 20.87 ± 1.23%, 28.60 ± 2.57%, and 34.66 ± 3.61% in the control group,

Table 2. Food intake and body weight of mice fed experimental diets.

	Control (n = 4)	5% Bran (n = 4)	15% Bran (n = 4)
Food intake (g/day)	3.39 ± 0.28	3.27 ± 0.13	3.51 ± 0.20
Body weight			
Before administration (g)	22.55 ± 1.09	21.60 ± 0.80	23.28 ± 2.04
At the end point (g)	24.80 ± 0.93	23.45 ± 0.72	24.63 ± 1.83

Mice were fed either a basal diet, 5% or 15% bran diet after MeHg administration. Food and water were provided *ad libitum* for 16 days. Food intake was measured for 4 days, and the average was calculated. Data represent the mean ± S.D.

Table 3. Total Hg concentrations in tissues at 16 days after MeHg administration in mice.

	Total Hg concentration (μg/g tissue)		
	Control (n = 4)	5% Bran (n = 4)	15% Bran (n = 4)
Blood	3.04 ± 0.18	2.47 ± 0.42	2.22 ± 0.33**
Brain	1.96 ± 0.31	1.71 ± 0.09	1.41 ± 0.12**
Liver	4.93 ± 1.36	4.55 ± 0.62	4.26 ± 0.21
Kidney	11.34 ± 0.76	10.26 ± 1.61	9.51 ± 1.10

Mice were administered a single dose of MeHgCl (4 mg Hg/kg body weight, p.o.). Total Hg contents in the tissue were determined by the oxygen-combustion-gold amalgamation method. Data represent the mean ± S.D. Significantly different from the control group (**$p < 0.01$).

5% and 15% bran groups, respectively, and the sum of each bran group was significantly higher than that in the control group ($p < 0.01$). Additionally, the sum of the excretions was significantly higher in the 15% bran group compared with the 5% bran group ($p < 0.05$).

Urinary Hg excretion in the bran-fed groups was significantly higher than that in the control group on day 3 after MeHg administration (Fig. 1A). Cumulative amounts of Hg in the urine of the 5% and 15% bran groups increased thereafter, and were approximately 1.6- and 1.8-fold higher, respectively, than those of the control group on day 14. Urinary Hg excretion in the first week after MeHg administration was significantly higher in the bran groups than in the control group; however, in the second week, it was significantly higher only in the 15% bran group (Fig. 1B). Additionally, urinary Hg excretion in the second week after administration was significantly higher in the 15% bran group compared with the 5% bran group.

Fecal Hg excretion in the 15% bran group was significantly higher than that in the control group on all days (Fig. 1C). Meanwhile, the 5% bran group had significantly increased fecal Hg excretion from day 10 compared with the control group. Cumulative amounts of Hg in the feces of the 5% and 15% bran groups increased thereafter, and were approximately 1.2- and 1.5-fold higher, respectively, than those of the control group on day 14. Although fecal Hg excretion in the first week after MeHg administration was significantly higher only in the 15% bran group, in the second week both bran groups showed higher Hg excretion compared with the control group (Fig. 1D). Additionally, fecal Hg excretion was significantly higher in the 15% bran group than in the 5% bran group in both the first and second week after administration.

DISCUSSION

We demonstrated that intake of bran after a single oral dose of MeHg enhanced Hg excretion in both urine and feces and decreased Hg accumulation in the tissues of mice. Notably, 15% bran diet-fed mice had significantly lower Hg concentrations in the blood and brain than basal diet-fed mice. The effect of bran on tissue Hg levels in the present study is consistent with the results of an earlier study (Rowland *et al.*, 1986) in which mice were fed bran diets from 3 months before MeHg administration to 2 weeks after administration. This previous study suggested that wheat bran exerts its effects on Hg retention and Hg levels via modifying the metabolic activity of the gut microbiota (Rowland *et al.*, 1986); however, this study did not determine the Hg contents in the urine and feces. In the present study, we did determine the Hg contents in the urine and feces of mice after MeHg administration. Surprisingly, cumulative amounts of Hg in the urine of bran-fed groups were significantly increased compared with the control group. The sum of urinary and

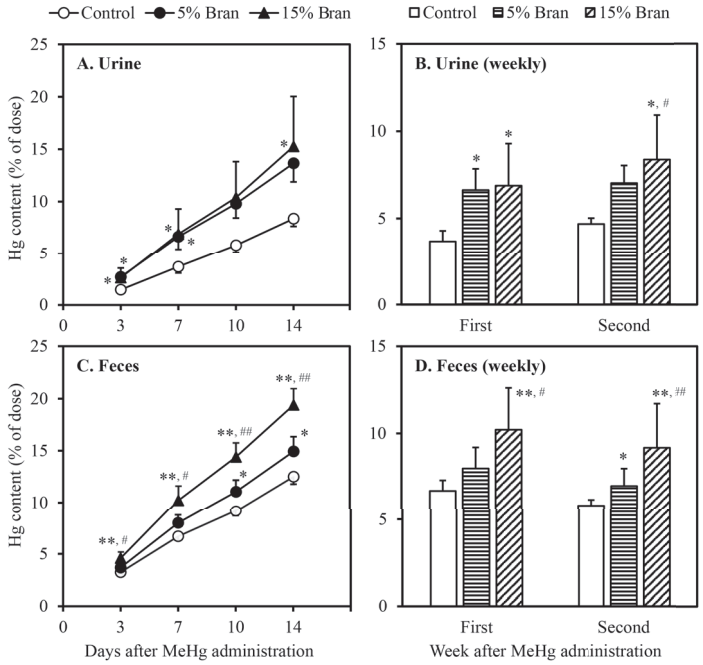

Fig. 1. Cumulative amounts of Hg excretion in urine (A, B) and feces (C, D) for 14 days and weekly after MeHg administration in mice. Data represent the mean ± S.D. (n = 4). Significantly different from the control group (*$p < 0.05$, **$p < 0.01$) and from the 5% bran group ($^{\#}p < 0.05$, $^{\#\#}p < 0.01$) by one-way ANOVA with the *post hoc* Bonferroni test or Kruskal–Wallis test with the *post hoc* Tukey's test.

fecal Hg excretions was significantly higher in the 15% bran group than in the 5% bran group, although urinary Hg excretion was similar between the two groups. These results suggest that fecal Hg excretion contributes largely to the increased Hg excretion following bran intake.

Bran contains dietary fibers (hemicellulose, cellulose, lignin, and fructan), phytic acid, and various nutrients such as proteins, vitamins, minerals, and polyphenols (Katayanagi, 2013; Sugawa-Katayama *et al.*, 2001). Studies on bran components suggest that alkylresorcinols, a type of polyphenol, may play a role in the recycling of oxidized glutathione into its reduced form in murine tissues, including the liver and heart (Agil *et al.*, 2016). Phytic acid has been reported to significantly increase the concentration of acid-soluble sulfhydryl groups in the liver of mice (Singh *et al.*, 1997), while MeHg has a high affinity for sulfhydryl groups (Simpson, 1961), and forms complexes with various thiols such as glutathione, cysteine, and cysteinyl residues of proteins. Additionally, most low molecular weight MeHg metabolites in the kidney and urine are glutathione and cysteine conjugates, respectively (Yasutake *et al.*, 1989). Therefore, although the mechanism of action requires further investigation, it can be speculated that the bran-induced urinary Hg excretion observed in our study was caused by the action of multiple components, rather than a single component, on factors determining the fate of MeHg.

Cumulative amounts of Hg in the feces of the bran groups increased significantly in a dose-dependent manner, and significant differences were shown between the two bran groups. Cumulative amounts of Hg in the feces of the 15% bran group increased significantly from 3 days after MeHg administration. Furthermore, the differences in fecal Hg excretion between the 15% bran group and the control group at 3, 7, 10, and 14 days after MeHg administration was about 2.8−3 times the difference between the 5% bran group and the control group. This difference is similar to the magnification difference between the bran concentrations in the diets, suggesting that bran-induced fecal Hg excretion may occur through the binding or adsorption of MeHg with its components. This hypothesis is supported by an *in vitro* study in which wheat bran decreased Hg bioaccessibility (Shim *et al.*, 2009). Of the several bran components, alkylresorcinols might bind or adsorb MeHg, because polyphenols (catechins, rutin, and caffeic acid) have been reported to decrease Hg bioaccessibility (Girard *et al.*, 2018). Additionally, lignin and hemicellulose may adsorb MeHg, as

these components have been shown to adsorb Hg(II) *in vitro* (Dias *et al.*, 2021; Lv *et al.*, 2012). Therefore, our current results and previous reports suggest that certain components of bran can bind with or adsorb Hg(II) and MeHg, reduce their absorption in the intestine, which may lead to increased fecal Hg excretion. However, further studies are needed on the binding or adsorption of bran components with MeHg and the chemical forms of Hg in feces.

Dietary fibers cannot be degraded by digestive enzymes in the small intestine and are fermented by intestinal bacteria in the large intestine (Nakamura *et al.*, 2014). After that, they are metabolized to short-chain fatty acids, such as acetate, propionate, and butyrate (Topping and Clifton., 2001), as well as gases. As mentioned in the previous paragraph, earlier studies suggest that wheat bran exerts its effects on Hg retention and Hg levels via modifying the metabolic activity of the gut microbiota (Rowland *et al.*, 1986), although the effect of bran on the gut microbiota has not been investigated. The demethylation of MeHg by the gut microbiota, which converts MeHg to inorganic Hg, increases fecal Hg excretion and decreases tissue Hg concentrations (Nakamura *et al.*, 1977; Seko *et al.*, 1982) because inorganic Hg is poorly absorbed from the intestine compared with MeHg. Thus, demethylation by the gut microbiota is considered another mechanism contributing to bran-induced fecal Hg excretion. In the previous study, mice were fed bran diets from 3 months before MeHg administration to 2 weeks after administration, whereas the mice in the current study were fed the diets immediately after MeHg administration. Another study reported that mice fed an 11.7% bran diet for 4 weeks had significantly increased total short-chain fatty acid and butyrate concentrations in the cecum compared with the control group (Jiminez *et al.*, 2016). Compared with the other two studies, the feed duration in the present study is short. Furthermore, as mentioned above, the cumulative amounts of Hg in the feces of the 15% bran group were increased significantly from 3 days after MeHg administration. Thus, early increases in fecal Hg excretion at shorter feeding periods imply that intestinal bacteria are unlikely to be involved in bran-induced fecal Hg excretion. However, further studies are needed on the chemical form of Hg in feces and the gut microbiota to prove this hypothesis.

To our knowledge, this is the first report to demonstrate the effect of bran intake after MeHg exposure on the accumulation and excretion of Hg in mice. In conclusion, bran intake after MeHg administration decreased tissue Hg concentrations, including those in the brain. Therefore, bran may reduce the neurotoxic effects of MeHg. Although the mechanisms of bran-induced Hg excretion are poorly understood, this study showed that the effect of bran on Hg accumulation is owing to enhance Hg excretion into both urine and feces, and that fecal Hg excretion contributes largely to its effect. Moreover, the results suggest that other mechanisms of bran-induced fecal Hg excretion may exist beside the demethylation of MeHg by the gut microbiota. Further studies are needed to clarify the mechanisms of bran-enhanced urinary and fecal Hg excretion.

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Conflict of interest---- The authors declare that there is no conflict of interest.

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