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Using nanoselenium to combat Minamata disease in rats: the regulation of gut microbes†

Yang Liu ,‡§[ab] Wei Zhang,‡[cbd] Jiating Zhao, [bd] Xiaoying Lin,[eb] Liming Wang, [bd] Liwei Cui,[bd] Junfang Zhang,*[f] Bai Li[b] and Yu-Feng Li *[bd]

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Methylmercury (MeHg) can magnify through food chains and cause potent neurological problems like Minamata disease. Gut microbes have been found to demethylate MeHg while MeHg can destroy the diversity of gut microbes. Our recent study found that selenite (Se^{4+}) could promote the demethylation of MeHg and regulate the diversity of gut microbes in MeHg-poisoned rats (Minamata disease). Elemental selenium at nano-size (nanoSe) was less toxic than Se^{4+} with comparable bioavailability. In this study, nanoSe was studied on its demethylation of MeHg and regulation of gut microbes in rats with Minamata disease using Se^{4+} as a positive control. Rats with Minamata disease were treated with nanoSe or Se^{4+} every other day for 90 days. Fecal samples were collected on days 8, 30, 60 and 90, and concentrations of Se, Hg and MeHg were measured. The diversity and abundance of gut microbes were determined using 16S rRNA gene profiling. It was found that both nanoSe and Se^{4+} enhanced the demethylation of MeHg, especially before day 30. The percentage of MeHg (of total mercury) in the MeHg-poisoned group was in the range of 81–105% while it was 23–79% in the nanoSe group and 65–84% in the Se^{4+} group, suggesting the higher demethylation rate of nanoSe than that of Se^{4+}. Both nanoSe and Se^{4+} regulated the diversity and abundance of gut microbes at both the phylum and genus rank. Therefore, considering its low toxicity, nanoSe is more desirable in combating Minamata disease. Besides, this study confirmed the new role of Se against MeHg poisoning, *i.e.* the regulation of gut microbes.

Environmental significance

Methylmercury (MeHg) in the environment can magnify through food chains and cause potent neurological problems to human beings. Gut microbes have been found to demethylate MeHg while MeHg can destroy the diversity of gut microbes and decrease their capability to transform MeHg. Considering the low toxicity of nano-selenium, we used nano-selenium to treat MeHg-poisoned rats while using sodium selenite as a positive control. It was found that the therapeutic effect of nano-selenium was better than that of sodium selenite, and nano-selenium promoted much more demethylation of methylmercury than sodium selenite did. This study also confirmed the new role of Se against the toxicity of MeHg, *i.e.* the regulation of gut microbes.

[a] *Department of Preclinical Medicine and Forensic, Baotou Medical College, Inner Mongolia University of Science & Technology, Baotou 014010, Inner Mongolia, China*
[b] *CAS Key Laboratory for Biological Effects of Nanomaterials and Nanosafety, CAS-HKU Joint Laboratory of Metallomics on Health and Environment, Beijing Metallomics Facility, National Consortium for Excellence in Metallomics, Institute of High Energy Physics, Chinese Academy of Sciences, Beijing 100049, China. E-mail: liyf@ihep.ac.cn; Tel: +86 10 88233908*
[c] *State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang 550081, Guizhou, China*
[d] *University of the Chinese Academy of Sciences, Beijing 100049, China*
[e] *Jilin Medical University, Jilin 132013, China*
[f] *Guizhou Institute of Environmental Science and Designing, Guiyang 550081, Guizhou, China. E-mail: 7385969@qq.com*

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‡ These authors contributed equally to this work.

§ Present address: School of Medicine, Southern University of Science and Technology, Shenzhen 518055, Guangdong, China.

Introduction

Methylmercury (MeHg) is one of the most toxic mercury (Hg) species, and can magnify through food chains and lead to potent neurological problems like Minamata disease.[1,2] Approximately 95% of MeHg ingested can be absorbed by the gastrointestinal tract while other forms of Hg like inorganic and elemental Hg are much less absorbed.[3]

Gut microbes live in the digestive tracts of humans and other animals, and play important roles in immunity, cognitive development, and metabolic processing. They also act as an important mediator on the toxicity of environmental pollutants like Hg. Studies have found that MeHg can be demethylated by gut microbes.[4–6] For example, it was found that people receiving antibiotics had lower MeHg elimination rates than untreated ones, highlighting

the importance of gut microbes in the demethylation of MeHg.[5] On the other hand, repeated dietary MeHg exposure for 30 days was found to alter the gut microbiome in fish.[7] Our study found that even acute exposure to MeHg could largely perturb the diversity of gut microbes.[8] Therefore, the regulation of gut microbes may help the host to mediate the toxicity of MeHg.

Selenium (Se) is an essential element in human beings and animals, which exists as selenite, selenate, elemental Se, *etc.*[9,10] It has been found to play protective roles against the toxicity of Hg when co-administered. For example, selenite (Se^{4+}) could dramatically decrease the acute toxicity of Hg in rats and many other animals as well as plants.[11–15] Elemental selenium (Se^0) at nano-size (nanoSe) has been studied for the treatment of Huntington's disease.[16] The toxicity of nanoSe (LD_{50}, 113 mg per kg bw) is lower than that of other forms of Se[17,18] and can be efficiently absorbed.[19–21]

The use of Se as an antidote against MeHg poisoning is less studied. Se supplementation using Se-enriched yeast in fish consumption population found reduced Hg levels in pubic hair.[22] Se^{4+} promoted weight gain, decreased hepatic damage, increased serum Hg levels and promoted Hg to bind with selenoprotein P in MeHg-poisoned rats, confirming the detoxifying role of Se against MeHg poisoning.[23,24]

The detoxification of Hg poisoning by Se was generally ascribed to the following reasons: firstly, Se can bind more readily to Hg through its highly reactive selenol group, and secondly, Se increases the levels of enzymes and proteins like GPx and selenoprotein P, which help compromise the reactive oxygen species induced by Hg *in vivo*.[25] Our previous study found that Se^{4+} could partially restore the gut microbes in MeHg-poisoned rats, suggesting a new role of Se in detoxifying MeHg poisoning.[26]

In this study, nanoSe was studied in rats with MeHg poisoning (Minamata disease) considering its low toxicity and comparable bioavailability. Besides, the effects on regulation of gut microbes between nanoSe and Se^{4+} were studied. To the best of our knowledge, this is the first study on nanoSe in the demethylation of MeHg and regulation of gut microbes in rats with Minamata disease.

Experimental section

Reagents

All reagents were of analytical reagent grade. Nitric acid and sodium selenite were obtained from Beihua (China), while MeHg was from Alfa Aesar. NanoSe was synthesized and characterized according to Gao *et al.*[27] Briefly, 0.5 mmol sodium selenite was dissolved in 97 mL of distilled water contained in a glass beaker. Then, 2 mmol of mercaptoethanol followed by 400 mg of bovine serum albumin was added to the solution and dissolved. Finally, 3 mL of 0.9 mmol L^{-1} aqueous NaOH was introduced. The solution (which was stirred magnetically) was kept at ~2–8 °C for 72 h to ensure the sufficient reaction. The final nanoSe solution was bright red.

Rat model with Minamata disease and Se treatment

Twenty male SD rats (8 weeks old) were purchased from Beijing Vitalriver Experimental Animal Technology Co., Ltd. The animals were housed in stainless-steel cages in a ventilated room. The room temperature was maintained at 20 ± 2 °C, and the relative humidity at 60 ± 10% with a 12 h light/dark cycle. Distilled water and sterilized food for rats were available *ad libitum*. The animals were acclimated to this environment for 5 days before dosing. All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Institute of High Energy Physics, Chinese Academy of Sciences and approved by the Animal Ethics Committee of Institute of High Energy Physics, Chinese Academy of Sciences.

Five rats were randomly selected as the control group, which were given normal saline during the whole experiment. The other 15 rats were orally gavaged with MeHg (4 mg per kg bw) diluted in normal saline every other day. After 4 weeks (28 days) of gavage, behavioural symptoms of MeHg intoxication were shown in rats, such as decreased mobility and hind-leg ataxia, which is the typical symptom of Minamata disease.[26,23] From the 29th day on (considered as day 0 of Se treatment in this study), the 15 MeHg-poisoned rats were further randomly divided into the MeHg-poisoned group, the Se^{4+} group and the nanoSe group, respectively. The Se^{4+} group and nanoSe group were given Se^{4+} (2.74 mg per kg bw) and nanoSe (1.25 mg kg^{-1}, both are equimolar Se to Hg in 4 mg per kg bw MeHg), respectively, while the MeHg-poisoned group was orally gavaged with normal saline every other day for another 90 days, and then all the rats were sacrificed. Before each gavage, the rats were weighed and recorded. Fecal samples were collected with metabolic cages on day 8, day 30, day 60 and day 90 after Se treatment, which were stored at −20 °C before analysis.

Analysis of Se and total Hg (THg)

Hg and Se in serum, red blood cells, feces and some organs (brain, liver and kidney) in the rats were determined. About 0.1 g samples (100 μL of serum or red blood cells, respectively) were digested in concentrated nitric acid overnight. Then, the solutions were heated at 120 °C to remove the remaining nitric acid until the solutions were colourless and clear. Finally, the remaining solutions were diluted to 4 mL with 2% nitric acid and 0.1% β-mercaptoethanol. The concentrations of Se and THg were analyzed using a Thermo X7 ICP-MS. Reference material dogfish muscle DORM-2 (Canada) was used for quality control, with the recovery in the range of 85–120%.

Analysis of MeHg in feces

For MeHg, fecal samples (~0.6 g) were leached in 1.5 mL 1 M copper sulfate, 7.5 mL 25% nitric acid, and 10 mL dichloromethane (CH_2Cl_2) and MeHg was back-extracted into DDI-H_2O.[28] MeHg extracts were analyzed following EPA

method 1630 using gas chromatography (GC)-CVAFS (Brooks Rand Model III). Reference material dogfish muscle DORM-2 (Canada) was used for quality control with the recovery falling in the range of 85–120%.

Chemical forms of Se in the small intestine

The mid-ileum of the small intestine was embedded in an embedding agent (Sakura Tissue-Tek OCT, Japan), frozen at −80 °C, and then cut into 30 μm slices with a freezing microtome (Reichert-Jung, Germany). The slices were put on a Mylar membrane (polycarbonate membrane), and were stored at −20 °C before analysis. Se K-edge XANES was performed at the BL15U1 beamline at the Shanghai Synchrotron Radiation Facility (SSRF). Na_2SeO_3, Na_2SeO_4 (Alfa Aesar), SeCys (Sigma), SeMet (Sigma) and SeMeCys (Sigma) were used as reference materials. The XANES spectra were analyzed using WinXAS. The least squares method was used for linear fitting of the XANES spectra of the samples to obtain the corresponding proportion of Se in the samples.[29,30]

16S rRNA sequencing of gut microbes in feces

Microbial genomic DNA was extracted from faecal samples collected on day 90 using a QIAamp Fast DNA Stool Mini Kit [Qiagen]. The V3–V4 hypervariable regions of 16S rRNA were amplified by PCR. The primer sequence was as follows: F: CCTACGGGNBGCASCAG; R: GACTACNVGGGTATCTAATCC. The PCR conditions were as follows: initial denaturation at 95 °C for 5 min, 95 °C denaturation for 30 s, 50 °C annealing for 30 s and 72 °C extension for 60 s, repeated for 25 cycles; final extension at 72 °C for 10 min. The PCR products were purified using a Qiagen quick Gel Extraction Kit (Qiagen, cat# 28706). PCR production from each sample was used to construct a sequencing library by using an Illumina TruSeq DNA Sample Preparation Kit. For each sample, barcoded V3–V4 PCR amplicons were sequenced on an Illumina Miseq platform.

The trimmed and assembled sequences from each sample were aligned to the RDP 16S rRNA training set 10 using the best hit classification option to classify the taxonomy abundance in QIIME.[31] Bacterial operational taxonomic units (OTUs) were generated using the cluster function in QIIME. The mean and standard deviation (SD) were calculated for each parameter. Results were expressed as mean ± SD.

Statistical analysis

Microbial diversity analyses[32–35] were performed by clustering sequence tags into groups of defined sequence variation as previously described.[36] All analyses were conducted using the R programming language. Multigroup comparisons of variables were carried out by a multiple comparison test method (ANOVA *t*-test) using SAS 6.12 (SAS Institute, Inc., Cary, NC). Statistical significance was set at $p < 0.05$.

Results

Characterization of nanoSe

NanoSe is approximately spherical with a size of 39.2 ± 19.2 nm.[37] Through DLS analysis, the hydrodynamic size of nanoSe in water is 47.6 ± 1.7 nm.[37] In aqueous solution, the zeta potential is −24.7 ± 2.2 mV, and the surface charge of nanoSe is negative, indicating that these nanoparticles have good dispersibility in the solution.

Changes in animal body weight, total mercury and total selenium content

During the set up of the Minamata disease model, the weight of each experimental group decreased significantly from the 16th day on (Fig. S1†). After selenium treatment, the weight gain of rats in the nanoSe group was significantly higher than that of the other groups (Fig. S2†). The total selenium and total mercury contents in the rat serum, red blood cells, liver, kidney and brain were not significantly different between the nanoSe group and the Se^{4+} group (Fig. S3–S12†).

Concentration of Se, THg and MeHg in feces

The Se concentration in feces from different groups is shown in Fig. 1. It can be seen that the Se level in the nanoSe group is significantly higher than that in the Se^{4+} group on the same day, suggesting that more Se was excreted through feces in the nanoSe group. The level of Se in both Se treatment groups was higher than that in the MeHg-poisoned group, suggesting that excessive Se was not retained in the body.

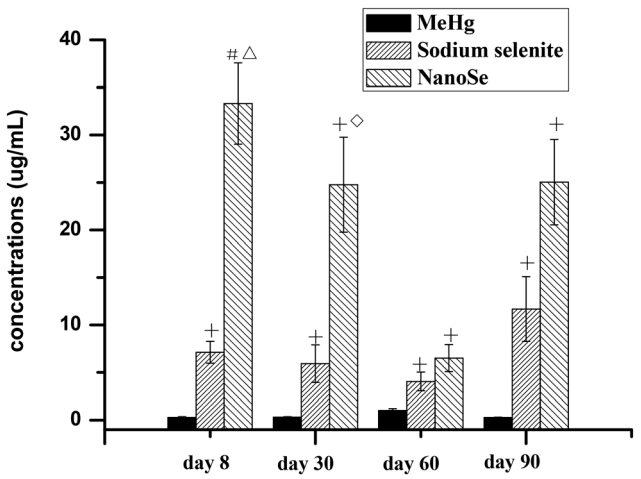

Fig. 1 Selenium concentration in feces of different groups on different days. MeHg: the MeHg-poisoned group, sodium selenite: the MeHg-poisoned with sodium selenite treatment group and nanoSe: the MeHg-poisoned with nanoSe treatment group. + significant difference to the MeHg-poisoned group ($p < 0.05$), # significant difference to the MeHg-poisoned group ($p < 0.01$), ◇ significant difference to the sodium selenite group ($p < 0.05$) and △ significant difference to the sodium selenite group ($p < 0.01$).

Table 1 Total mercury, MeHg and percentage of MeHg (of total mercury) in feces of different groups on different days

	MeHg-Poisoned group (μg g^{-1})				Sodium selenite group (μg g^{-1})				NanoSe group (μg g^{-1})			
Parameter	Day 8	Day 30	Day 60	Day 90	Day 8	Day 30	Day 60	Day 90	Day 8	Day 30	Day 60	Day 90
THg	22.78 ± 7.81	4.5 ± 0.97	1.43 ± 0.55	3.77 ± 1.37	16.36 ± 5.48	10.36 ± 3.09[a]	1.89 ± 0.81	1.16 ± 0.44[a]	21.14 ± 4.13	8.38 ± 1.72[a]	1.06 ± 0.35	1.69 ± 0.37[a]
MeHg	20.44 ± 4.25	4.74 ± 1.50	1.26 ± 0.06	3.07 ± 0.46	11.66 ± 1.85	6.76 ± 0.17	1.60 ± 0.02	0.81 ± 0.08[a]	10.22 ± 0.60	5.97 ± 0.76	0.84 ± 0.14	0.39 ± 0.02[a]
% MeHg (of THg)	89	105	88	81	71	65	84	69	48	71	79	23

[a] Represents significant difference from the MeHg-poisoned group ($p < 0.05$).

The concentrations of THg and MeHg in feces in each group on different days are shown in Table 1. The THg and MeHg levels in the control group were negligible and therefore are not listed in Table 1. For the MeHg-poisoned group, the levels of both THg and MeHg decreased with time, from 22.78 ± 7.81 and 20.44 ± 4.25 μg g^{-1}, respectively, on day 8, to 3.77 ± 1.37 and 3.07 ± 0.46 μg g^{-1}, respectively, on day 90. For both Se treatment groups, the levels of THg and MeHg also decreased with time. The concentrations of THg and MeHg were lower in both Se treatment groups than those in the MeHg-poisoned group on days 8, 60, and 90. However, on day 30, significantly more THg and MeHg were excreted in both Se treatment groups than those in the MeHg-poisoned group, suggesting that day 30 may be an important time point for Se to promote the excretion of MeHg.

Table 1 also shows that the percentage of MeHg to THg in the MeHg-poisoned group on different days was in the range of 81–105% while it was 23–79% in the nanoSe group and 65–84% in the Se^{4+} group on different days, suggesting the higher demethylation rate by nanoSe than that by Se^{4+}. Over 80% of the Hg was MeHg in the MeHg-poisoned group while it was reduced to 71% by selenite and 48% by nanoSe on day 8. This is more evident on day 90, where it was 81% in the MeHg-poisoned group and 69% in the Se^{4+} group while it was 23% in the nanoSe group. Less MeHg was found in the Se treatment groups than that in the MeHg-poisoned group on the same day, suggesting that the Se treatment promoted the demethylation of MeHg.

The chemical forms of Se in the small intestine wall

As shown in Fig. S13,† the chemical form of Se in the duodenum wall was studied using μ-XANES. In the nanoSe group, Se mainly existed in the form of SeCys and Se^0, accounting for 58% and 42%, respectively, by least-squares fitting. In the Se^{4+} group, Se mainly appeared in the form of SeMet and Se^{6+}, the proportions of which were 32% and 68%, respectively.

Profiles of gut microbes in feces

Fig. 2 shows the profiles of gut microbes in feces on day 90 at the rank of phylum. 16S rRNA data were assigned to 13 phyla, among which *Firmicutes*, *Bacteroidetes* and *Tenericutes* represented about 98% of gut microbes. Compared with the control group (1.2% ± 0.2%), the abundance of *Cyanobacteria* was significantly lower in the experimental groups. The abundance of *Bacteroidetes* also decreased in the MeHg-poisoned group, but the abundance of *Firmicutes* increased in the MeHg-poisoned group. It is worth noting that the abundance of *Firmicutes* and *Bacteroidetes* in the nanoSe group is the closest to that in the control group. The abundance of *Tenericutes* did not differ much between the control group and the MeHg-poisoned group. When comparing the other groups the abundance of TM7 increased significantly in the nanoSe group.

Fig. 3 shows the profiles of gut microbes in feces on day 90 at the rank of genus. It can be seen that the abundance of *Ruminococcus*, *Anaeroplasma*, *Lactobacillus* and *Clostridium* increased while the abundance of *Oscillospira*, *Paraprevotella*, *Bacteroides*, and *Roseburia* decreased in the MeHg-poisoned group compared with the control group.

At the rank of genus, 15 of 113 genera had significant differences in the different groups ($p < 0.05$). Table 2 shows that some of the bacteria (*Flexispira*, *Providencia* and *Treponema*) disappeared after MeHg poisoning, indicating that these bacteria are more sensitive to MeHg. The abundance of *Prevotella*, *Akkermansia*, *etc.* decreased in the MeHg-poisoned group, indicating that MeHg caused damage to these bacteria. After Se treatment, the abundance of

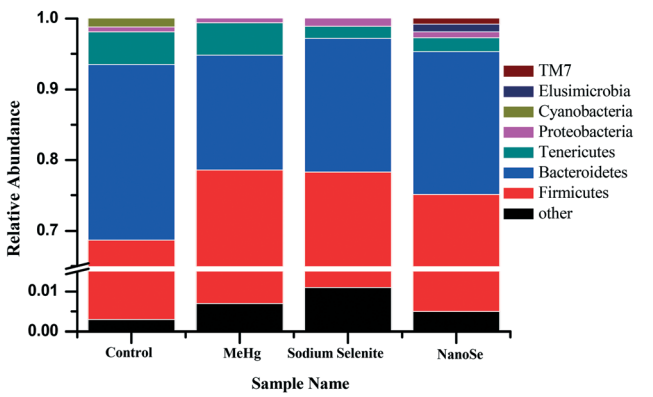

Fig. 2 Profiles of gut microbes in feces at the rank of phylum on day 90. Control: The control group, MeHg: the MeHg-poisoned group, sodium selenite: the MeHg-poisoned with sodium selenite treatment group and nanoSe: the MeHg-poisoned with nanoSe treatment group.

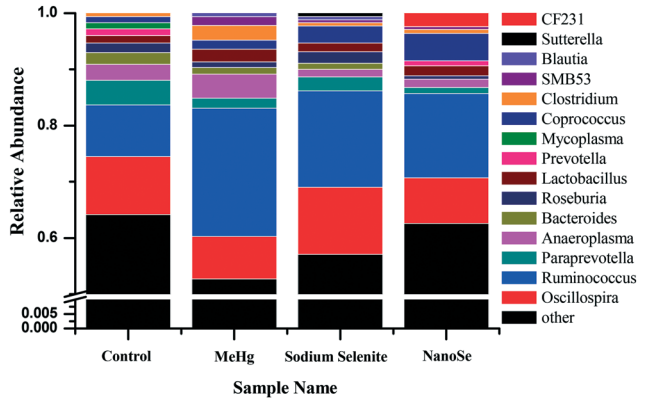

Fig. 3 Profiles of gut microbes in feces at the rank of genus on day 90. Control: The control group, MeHg: the MeHg-poisoned group, sodium selenite: the MeHg-poisoned with sodium selenite treatment group and nanoSe: the MeHg-poisoned with nanoSe treatment group.

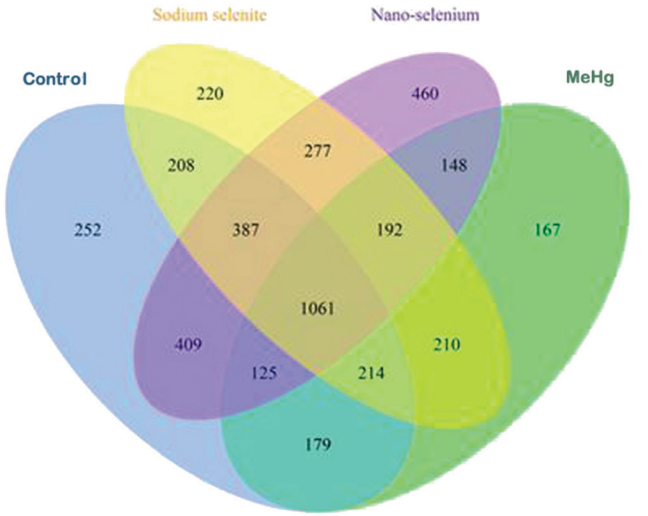

Fig. 4 Numbers of OTUs shared among the different groups in rat feces on day 90. The numbers in the shaded overlapping zones indicate how many OTUs of the total OTUs were shared among different groups. Control: The control group, MeHg: the MeHg-poisoned group, sodium selenite: the MeHg-poisoned with sodium selenite treatment group and nanoSe: the MeHg-poisoned with nanoSe treatment group.

Prevotella, *Treponema*, *Rothia*, CF231 and *Coprococcus* was close to or even greater than that in the control group. Moreover, the nanoSe treatment brought significantly higher species abundance for the following bacteria: *Flexispira*, *Paraprevotella*, *Coprococcus*, *Dorea*, *and Treponema*.

Fig. 4 shows the numbers of OTUs in the different groups. Clustered at the 97% similarity level, each group contains the following number of OTUs: 3430 in the control group, 2296 in the MeHg-poisoned group, 2769 in the Se^{4+} group and 3059 in the nanoSe group, respectively. There were 1061 shared OTUs in the four groups. The Se treatment groups contain more OTUs than the MeHg-poisoned group, suggesting the recovery of microbial species after the Se treatment. Besides, more OTUs were found in the nanoSe group than those in the Se^{4+} group. The shared number of OTUs between the MeHg-poisoned group, Se^{4+} group and nanoSe group, and the control group is 179, 208 and 409, respectively, indicating that although both Se treatments improved the diversity of gut microbes, nanoSe is more efficient in the improvement.

Discussion

Effects of nanoSe treatment on fecal excretion of THg and MeHg

From Table 1, the total mercury levels of the two Se treatment groups were higher than that in the MeHg-poisoned group on day 30, suggesting that this may be a critical time point for the promotion of Hg excretion by Se. On the other hand, the lowered fecal Hg levels in the MeHg-poisoned group may

Table 2 Comparison of the abundance between the MeHg-poisoned group and other groups at the rank of genus

Genus	Control (mean ± SD) %	MeHg (mean ± SD) %	Sodium selenite (mean ± SD) %	NanoSe (mean ± SD) %
Prevotella	1.16 ± 0.11[a]	0.36 ± 0.13	0.49 ± 0.22	0.94 ± 0.21
Alistipes	0.01 ± 0.001[a]	0.004 ± 0	0.0001 ± 0.0001	0.002 ± 0.002
Butyricimonas	0.23 ± 0.01[a]	0.09 ± 0.02	0.08 ± 0.01	0.02 ± 0.01
Mucispirillum	0.01 ± 0.002[a]	0.001 ± 0	0.0007 ± 0.0007	0.009 ± 0.009
Rc4-4	0.73 ± 0.09[a]	0.28 ± 0.05	0.22 ± 0.12	0.08 ± 0.03
Flexispira	0.002 ± 0[a]	0	0	0.18 ± 0.04
Providencia	0.002 ± 0[a]	0	0	0
Rothia	0.02 ± 0.002	0.008 ± 0.006	0.10 ± 0.01[a]	0.03 ± 0.02
Paraprevotella	4.43 ± 1.87	1.84 ± 0.14	0.01 ± 0[a]	1.05 ± 0.007[a]
Aggregatibacter	0.14 ± 0.09	0.03 ± 0.003	0.05 ± 0.03	0.002 ± 0[a]
CF231	0.01 ± 0.002	0.006 ± 0.001	0.01 ± 0[a]	2.37 ± 0.71
Coprococcus	1.14 ± 0.33	1.58 ± 0.34	3.04 ± 2.15	4.91 ± 0.37[a]
Dorea	0.10 ± 0.04	0.14 ± 0.01	0.06 ± 0.02	0.23 ± 0.002[a]
Treponema	0.001 ± 0	0	0	0.25 ± 0.003[a]
Akkermansia	0.004 ± 0.001	0.002 ± 0	0.43 ± 0.42	0[a]

[a] Represents significant difference from the MeHg-poisoned group ($p < 0.05$). Control: the control group, MeHg: the MeHg-poisoned group, sodium selenite: the MeHg-poisoned with sodium selenite treatment group and nanoSe: the MeHg-poisoned with nanoSe treatment group.

be ascribed to its retention in the body since it was found that the blood Hg content in the MeHg-poisoned group was much higher than that in the Se treatment group.[38]

Our results found that most of the Hg in feces existed in the form of MeHg, and the nanoSe group had a significant decrease in MeHg content (23%) on day 90. Studies have found that in MeHg-poisoned rats, more than 50% of Hg in feces was in the form of inorganic mercury (IHg).[6] Earlier studies have also proved that the intestine plays an important role in the demethylation of MeHg.[39,40] The demethylation ability was significantly reduced in the MeHg-poisoned rats while nanoSe promoted the demethylation of MeHg as found in this study.

Se has long been found to have a protective effect against MeHg toxicity when co-administered[41,42] and has been proposed to play an important role in MeHg demethylation in avian livers, where both contaminants have been shown to co-accumulate.[43,44] It can be seen from Fig. 1 that after Se treatment, the excretion of Se also increased, and this is consistent with previous reports.[45] The Se^0 found in the small intestinal wall (Fig. S13†) in the nanoSe group suggested that nanoSe was directly absorbed by the intestinal wall while Se^{4+} was oxidized to Se^{6+} in the Se^{4+} group. The nanoSe group excreted high levels of Se, indicating that excessive nanoSe was not stored in the body and would not cause additional metabolic burden to the body.

Effects of Se treatment on the diversity and abundance of gut microbes

The diversity of bacteria was different between the MeHg-poisoned rats and the control group. As can be seen in Fig. 4, the MeHg-poisoned rats contain the smallest number of OTUs, reflecting the strong toxicity of MeHg to gut microbes, while OTUs in the nanoSe group were more close to those in the blank group with 1982 shared OTUs, suggesting that the diversity of gut microbes is restored.

The abundance of gut microbes at the ranks of phylum (Fig. 2) and genus (Fig. 3) also shows the significant difference among the four groups. *Firmicutes* and *Bacteroidetes* are the main gut microbes at the phylum level. Many reports indicate that about 90% of the human gut microbiota is composed of them.[46,47] In general, the ratio of *Firmicutes* and *Bacteroidetes* in the intestine is significantly correlated with the overall health of the human GIT.[48] Exposure to MeHg increased the ratio of *Firmicutes* and *Bacteroidetes* (F/B ratio, Fig. 2), indicating that the balance of intestinal microbes was disrupted. Both nanoSe and selenite treatments reduced the F/B ratio to the level of the control group, confirming the partial restoration of the intestinal microbial balance of the intestinal flora. Gut microbes in humans and rats are similar at the phylum level.[49] This may suggest that, at least at the phylum level, our results may be extrapolated to humans.

When considering the difference at the rank of genus, it can be seen from Fig. 3 and Table 2 that the nanoSe treatment could efficiently reverse the abundance of intestinal *Ruminococcus*, *Anaeroplasma*, *Lactobacillus*, *Clostridium* and *Roseburia* towards the levels in the blank group, suggesting the restoration of the balance of the gut microbes in the MeHg-poisoned rats. Although there is no direct evidence for demethylation by these bacteria, the regulated abundance of gut microbes like *Lactobacillus* and *Clostridium* may suggest the improvement of intestinal health.

The possible mechanism of Se against the toxicity of MeHg by regulating intestinal microbes

Se could be a scavenger of inorganic Hg resulting from the demethylation of MeHg[50] or may facilitate the radical attack on MeHg.[51] However, little has been addressed on the possible role of Se in the demethylation of MeHg through gut microbes. Studies have shown that nanoSe is not easily absorbed in the intestine.[52] Therefore, nanoSe may reduce the toxicity of MeHg by changing the intestinal flora and intestinal environment. Sulfur and iron-reducing bacteria have been reported to be related to Hg methylation and demethylation,[53,54] however, there was no significant difference of these bacteria found in both nanoSe and Se^{4+} groups in this study. Therefore, gut microbes should demethylate MeHg using a different metabolic pathway (*i.e.*, oxidative demethylation),[55,56] which deserves further study.

From Table 2, it can be seen that most of the differentiated bacteria between the experimental groups and the control group are related to inflammation and intestinal function. *Alistipes* can slow down intestinal inflammation, thereby reducing the occurrence of inflammatory bowel disease.[57] *Butyricimonas* can convert glucose into butyric acid and isobutyric acid,[58] and can also generate other types of short-chain fatty acids such as acetic acid, propionic acid and succinic acid. These short-chain fatty acids are an important source of energy for intestinal mucosal cells, helping build and repair the intestinal mucosal barrier, resist oxidative stress, and play an important role in colon function and health.[59] The abundance of the above two bacteria in the control group was significantly higher than that in the other three experimental groups. *Rothia* is one of the common bacteria related to the production of butyric acid, which affects colon movement and has immune maintenance and anti-inflammatory effects.[60] The significantly increased *Rothia* in the Se^{4+} group may contribute to the improvement of intestinal health.

The abundance of *Paraprevotella* in both the nanoSe and Se^{4+} groups reduced significantly, while it decreased more in the Se^{4+} group. *Paraprevotella* is a Gram-negative anaerobic bacteria producing succinic acid and acetic acid, in which succinic acid can produce butyrate through symbiosis to exert anti-inflammatory effects.[61] These metabolites can also protect the intestinal mucosal barrier by affecting the intestinal pH, electrical impedance, *etc.*[62] The decreased *Paraprevotella* in both the nanoSe and Se^{4+} groups may suggest that the damage

to the mucosal barrier by MeHg could not be fully recovered.[63,64] The abundance of *Coprococcus* and *Dorea* increased significantly in the nanoSe group compared to that in the MeHg-poisoned rats while no significant difference was found in the Se^{4+} group. *Coprococcus* has anti-inflammatory effects and *Dorea* belongs to butyric acid-producing bacteria, which can maintain intestinal health. Therefore, the Se treatment especially nanoSe contributed to maintaining the balance of intestinal function by regulation of the gut microbes. This may be one of the underlying mechanisms of Se against the toxicity of MeHg.

Conclusion

In all, our results show that Se promoted the demethylation of MeHg, especially nanoSe. MeHg poisoning interfered with the number of intestinal microbes and increased the F/B ratio. After Se treatment, the diversity and abundance of intestinal microorganisms were restored, and nanoSe was more prominent than selenite in the regulation of gut microbes. These findings are meaningful, indicating that Se can effectively reduce the level of more toxic forms of Hg (*i.e.* MeHg), especially nanoSe. Besides, Se can help restore the diversity of intestinal microbes, thereby improving the health status of MeHg poisoned rats. Therefore, considering its low toxicity, nanoSe is more desirable in fighting against MeHg poisoning, *i.e.* Minamata disease.

Conflicts of interest

No potential conflict of interest was reported by the authors.

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