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Metagenomic profiles and antibiotic resistance genes in gut microbiota of mice exposed to arsenic and iron

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HIGHLIGHTS

- Changes of mice gut microbiota under As and/or Fe exposure were analyzed.
- Co-exposure of As and Fe mitigated effects on gut microbial community in mice.
- Exposure of As and/or Fe changed types and abundance of ARGs.
- Changes of gut microbiota influenced host metabolic profiles.
- Gut microbiota should be considered during risk assessment of As and/or Fe.

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ABSTRACT

Iron (Fe) has been widely applied to treat arsenic (As)-contaminated water, and Fe could influence bioavailability and toxicity of As. However, little is known about the impact of As and/or Fe on gut microbiota, which plays important roles in host health. In this study, high-throughput sequencing and quantitative real time PCR were applied to analyze the impact of As and Fe on mouse gut microbiota. Co-exposure of As and Fe mitigated effects on microbial community to a certain extent. Correlation analysis showed the shifts in gut microbiota caused by As and/or Fe exposure might be important reason of changes in metabolic profiles of mouse. For antibiotic resistance genes (ARGs), co-exposure of As and Fe increased types and abundance of ARGs. But for high abundance ARGs, such as *tetQ*, *tetO* and *tetM*, co-exposure of As and Fe mitigated effects on their abundances compared to exposure to As and Fe alone. No obvious relationship between ARGs and mobile genetic elements were found. The changes in ARGs caused by metal exposure might be due to the alteration of gut microbial diversity. Our results show that changes of gut microbial community caused by As and/or Fe can influence host metabolisms and abundances of ARGs in gut, indicating that changes of gut microbiota should be considered during the risk assessment of As and/or Fe.

1. Introduction

Gut harbors diverse microbes that play a key role in well-being of their host. The gut microbiota acts in a concerted manner to achieve metabolic communication with the host, and many different bacterial genera and species are involved in metabolite production (Wikoff et al., 2009; Mestdagh et al., 2012; Martinez et al., 2013). Changes in gut microbiota are linked with inflammatory and metabolic disorders (Nicholson et al., 2012). Many researches have showed that metal exposure could change the gut microbiota (Dostal et al., 2012). On the other hand, the gut microbiota could change transportation and metabolism of metals (Wiele et al., 2010). Thus, it is necessary to identify impacts of metal on the gut microbiota under oral metal exposure. Arsenic (As) as ubiquitous metalloid has been paid much attention due to its high toxicity. Consumption of drinking water is the main source of As exposure. Iron (Fe) coagulation/flocculation has been widely applied in the actual treatment of As-contaminated water due to its low cost and high efficiency (Mohan and Pittman, 2007). Our previous study demonstrated that combined exposure of As and Fe in mouse could significantly reduce hepatic toxicity of As (Liu et al., 2013). In addition, some altered host-gut co-metabolites in serum and urine were identified, indicating the possible changes of gut microbiota. Thus, it is necessary to explore the impacts of As and/or Fe on gut microbiota to better understand their combined effects.

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Gut microbiota is an important antibiotic resistance genes (ARGs) reservoir, playing important roles in host health. Recently, it has been suggested that environmental pollution could affect abundance of resistance traits. In fact, there are various known mechanisms by which the resistance traits may be retained or propagated in the presence of metals (Baker-Austin et al., 2006; Stepanauskas et al., 2006). Bacterial resistance mechanisms exist to mitigate toxicological effects of excessive bioavailable metals as part of their stress response strategy. Defense-associated metals are often closely associated with those responsible for antibiotic resistance on mobile genetic elements (MGEs) (Beaber et al., 2004). These genes can encode for generic detoxifying mechanisms (e.g. efflux pumps), which non-specifically reduce intracellular concentrations of both metals and antibiotics (cross resistance) (Berg et al., 2010; Knapp et al., 2011). Thus, constant exposure to metals can increase ARGs' frequency in the gene pool in environmental or gut bacteria. There are many reports on correlation between tolerance to metals (including As and Fe) and antibiotic resistance in environment (Tuckfield and McArthur, 2008; Kaur et al., 2011; Ji et al., 2012). However, effects of As and/or Fe on the ARGs in gut microbiota are still unknown.

In the present study, we exposed pure water, As alone, Fe alone and As + Fe to male mice for 90 d, respectively. After exposure, gut microbiota were analyzed by high-throughput sequencing. Relationships between gut microbiota and mouse metabolic profiles were characterized by correlation analysis. The ARGs in gut microbiota were determined based on high throughput sequencing and verified by quantitative real-time PCR (qRT-PCR). This study firstly provides the effects of As and/or Fe exposure on gut microbiota and ARGs. Combined with results on metabolic profiles of mouse serum and urine, this study might be very useful for understanding of toxicological effects and mechanism of actions of As and/or Fe exposure.

2. Materials and methods

2.1. Animal treatment

Five-week-old male mice (*Mus musculus*, ICR) were purchased from the experimental animal center of Academy of Military Medical Science of China. Forty mice (about 18 g) were randomly assigned to four groups (ten mice in one group). The mice in four groups were exposed to pure water, 3 mg L^{-1} As, 5 mg L^{-1} Fe and 3 mg $^{-1}$ As + 5 mg L^{-1} Fe under ambient conditions (25 ± 3 °C, 50 ± 5% relative humidity, and a 12/12 h light/dark cycle) for 90 d, respectively. The concentrations selected for As and Fe were based on our preliminary work (Liu et al., 2013). Arsenic oxide was obtained from NSI Solution Inc. Ferric chloride was obtained from Sigma Chemical Co. All experimental processes were in accordance with NIH Guide for the Care and Use of Laboratory animals. And the protocol was approved by the Committee on the Ethics of Animal Experiments of the Nanjing Military General Hospital.

2.2. Histopathological analysis

On day 90, mice were anesthetized with diethyl ether to minimize suffering. Parts of intestine were dissected and fixed in 10% formalin solution. After 24–28 h the samples were dehydrated in a grade alcohol series and embedded in paraffin wax. Sections of 4–5 μm thickness were stained with hematoxylin–eosin (H&E) for pathological studies.

2.3. DNA extraction

Fecal samples were collected from each mouse on day 90 to identify the effects of long-term metal exposure on gut microbiota. Approximately 200 mg of feces was applied for total genomic DNA extraction in duplicate using FastDNA Soil Kit (MP Biomedicals, USA). Concentration and quality of the extracted DNA were determined using Nanodrop (ND-1000, NanoDrop Technologies, USA). DNA from the feces was pooled by treatment group were sequenced by pyrosequencing and Illumina sequencing (Looft et al., 2012).

2.4. Pyrosequencing

Feces genomic DNA was amplified with a set of primers targeting the hypervariable V3–V4 region of 16S rRNA gene. PCRs were conducted in a reaction system (50 μL) containing 1 × Amplification Buffer (Invitrogen, USA), 0.4 mM dNTP, 2 mM $MgSO_4$, 0.4 μM each fusion primer, 1 μL template DNA and 2 U DNA Polymerase (Invitrogen, USA). Nucleotide "barcode" was permuted for each sample to separate corresponding reads from data pool generated in a pyrosequencing run. The protocols for PCR amplification were initial denaturation at 94 °C for 3 min, followed by 30 cycles of 94 °C for 30 s, annealing at 62 °C for 30 s and extension at 70 °C for 45 s, with a final elongation step at 70 °C for 7 min. In order to minimize the impact of potential early-round PCR errors, amplicon libraries were prepared by a cocktail of three independent PCR products for each sample. After purification using quick-spin PCR Product Purification Kit (iNtRON Biotechnology), the PCR products were quantified using Nanodrop. Then they were sent out for pyrosequencing on the Roche 454 FLX Titanium platform (Roche, Nutley, NJ). The fragment size for each library is about 300 bp. The obtained data are publicly available at European Nucleotide Archive database (Accession PRJEB3374).

Following pyrosequencing, pipeline initial process tool in Ribosomal Database Project's (RDP) was applied to remove sequences containing more than one ambiguous base 'N' or/and shorter than 150 bp, and check completeness of the barcodes and the adaptor. After denoising, filtering out chimeras, and removing archaeal sequences, the library size of each sample was normalized to the same sequences (the smallest sequences among four samples) to make the samples at the same sequencing depth (Ye and Zhang, 2011). Then, all effective sequences were assigned to taxonomic ranks with MEGAN program using the Lowest Common Ancestor (LCA) algorithm (Huson et al., 2007). Default parameters (absolute cutoff: BLAST bit score 35, and the relative cutoff: 10% of the top hits) were applied.

2.5. Illumina sequencing

Mouse fecal genomic DNA was further sequenced using Illumina Hiseq 2000. About 10 μg DNA samples were applied to construct a library consisting of 180 bp DNA fragment sequences according to manufacturer's instructions. Then, the sequencing strategy was paired end sequencing, 101 bp reads and 8 bp index sequence. More than 1 Gb of sequences was generated for each DNA sample. The raw reads containing three or more 'N' or contaminated with adaptors were removed to obtain clean reads (91.79–98.27% of raw reads). The obtained data are publicly available at European Nucleotide Archive database (Accession PRJEB3374).

The clean reads from Illumina sequencing were submitted to MG-RAST (Meta Genome Rapid Annotation using Subsystem Technology, v3.2) deposited in the Argonne National Library (http://metagenomics.nmpdr.org) under ID numbers of 4491170.3 (Control), 4491169.3 (As alone), 4491168.3 (Fe alone) and 4491167.3 (As + Fe). The nucleotide data were annotated by Clusters of Orthologous Groups (COGs) to identify their function (Koenig et al., 2011; Yu and Zhang, 2012). The maximum *e*-value cutoff,