Thallium(I) exposure perturbs the gut microbiota and metabolic profile as well as the regional immune function of C57BL/6 J mice

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Abstract
Intestinal microbes regulate the development of diseases induced by environmental exposure. Thallium (Tl) is a highly toxic heavy metal, and its toxicity is rarely discussed in relation to gut microbes. Herein, we showed that Tl(I) exposure (10 ppm for 2 weeks) affected the alpha diversity of bacteria in the ileum, colon, and feces, but had little effect on the beta diversity of bacteria through 16S rRNA sequencing. LEfSe analysis revealed that Tl(I) exposure changed the abundance of intestinal microbiota along the digestive tract. Cecum metabolomic detection and analysis showed that Tl(I) exposure altered the abundance and composition of metabolites. In addition, the Kyoto Encyclopaedia of Genes and Genomes (KEGG) enrichment analysis revealed that Tl(I) exposure impaired amino acid, lipid, purine metabolism, and G protein-coupled receptor signalling pathways. A consistency test revealed a strong correlation, and a Pearson’s correlation analysis showed an extensive interaction, between microorganisms and metabolites. Analysis of the intestinal immunity revealed that Tl(I) exposure suppressed the immune responses, which also had regional differences. These results identify the perturbation of the intestinal microenvironment by Tl exposure and provide a new explanation for Tl toxicity.

Keywords Thallium pollution · Intestinal microbes · Metabolome · Immune response

Introduction
Thallium (Tl) is a trace metal with higher toxicity than that of other common heavy metals such as mercury (Hg), lead, cadmium (Cd), and copper, which are widely used in rat poison, pesticides, semiconductors, and electronic equipment (Peter and Viraraghavan 2005; Lennartson 2015; Wu et al. 2019; Hsu et al. 2021). Tl is considered a priority pollutant by the Environmental Protection Agency of the USA (Cvjetko et al. 2010). In recent years, human activities and the rapid development of the high-tech industry have led to a rapid increase in the discharge of Tl to the environment (Campanella et al. 2016; Hsu et al. 2021). Humans are easily exposed to Tl by consuming contaminated food and water or inhaling contaminated air (Liu et al. 2017). The maximum contaminant level for Tl in drinking water is 2 ppb; this concentration will not affect the human health (Cvjetko et al. 2010). The maximum admissible concentration for Tl is 100 ppb (Karbowska 2016). The toxicity induced by Tl exposure is multifaceted, and a part of its toxicity mechanism has been studied. Tl has two oxidation states, Tl(I) and Tl(III). Tl(I) and potassium have similar ionic radii and charges. It can compete with potassium, thereby modifying the enzymatic activation of the Na+/K+ ATPases, pyruvate kinase, and other proteins dependent on K+ (Rodríguez-Mercado and Altamirano-Lozano 2013; Osorio-Rico et al. 2017). Tl exposure can impair mitochondrial respiratory complexes (I, II, and IV), leading to increased production of reactive oxygen species. Tl can affect oxidative phosphorylation, ATP synthesis, mitochondrial membrane potential, and outer membrane integrity, ultimately leading to the release of cytochrome C into the cytoplasm, and inducing cell apoptosis (Eskandari et al. 2015). Tl can also interact with...
sulphydryl groups located on enzymes, such as glutathione and DL-dithiothreitol, to affect their function (Rodriguez-Mercedo and Altamirano-Lozano 2013). Currently, research on Tl toxicity is mainly focused on the study of cells in vitro. The human intestinal tract is populated with as many as 100 trillion microbes, with conferred several essential functions, such as the digestion of food, the synthesis of essential vitamins, the strengthening of the intestinal barrier, maintaining epithelial homeostasis, and the stimulation and regulation of the immune system, revealing the profound role of intestinal microbes in the lifespan (Ley et al. 2006; Lu et al. 2014; Heintz-Buschart and Wilmes 2018). The composition/development of the human gut microbiota may affect the risk factors associated with adult health under environmental exposure. For example, smoking reduces the diversity of the gut microbiota and increases the abundance of Firmicutes and Actinobacteria. It decreases Bacteroidetes and Proteobacteria abundance, changes the epithelial mucin composition of the mucus layer, and increases inflammatory responses to elevate the risk of colorectal cancer (Biedermann et al. 2014; Song and Chan 2019). In addition, emerging evidence supports that environmental exposure is a crucial determinant of gut microbial composition and function. Their alterations can cause changes in host gene expression, metabolic regulation, and local and systemic immune responses, thereby affecting the development of diseases. For instance, the abundance of gut microbes in pregnant women exposed to heavy metals can change and potentially lead to glucose metabolism disorders, which may contribute to the development of gestational diabetes mellitus (GDM) (Zhang et al. 2021). Heavy metal exposure (such as Cd and arsenic) induces changes in the abundance of intestinal microbes, which disturb bile acid and amino acid metabolic pathways that may induce type 2 diabetes (T2DM) (Li et al. 2019). Long-term Cd exposure reduces the abundance of short-chain fatty acid (SCFA)-producing bacteria, which in turn affects the incidence of intestinal inflammation (He et al. 2020). Exposure to other heavy metals, such as Hg, can induce intestinal microbiota dysbiosis and metabolic disorder, which in turn exacerbate intestinal cell apoptosis in mice (Zhao et al. 2020). As a heavy metal, Tl can induce many diseases and even death. However, the toxic effects of Tl exposure on the intestinal microenvironment are obscure.

The goal of the present study was to examine the effects of Tl(I) exposure on gut microbes along the digestive tract and explore the characteristics of metabolome changes and immune responses. The changes in the abundance of gut microbes following Tl(I) exposure along the digestive tract were first shown using 16S rRNA gene sequencing. Using ultra-high-performance liquid chromatography coupled with quadrupole time-of-flight tandem mass spectrometry (UHPLC-QTOF-MS/MS) analysis, we reported that the cecum content metabolome was greatly disturbed, and many metabolic pathways were affected. Gene expression analysis confirmed that the intestinal immune response was suppressed. These findings indicate that the diseases induced by Tl exposure may be related to immunosuppression caused by disorders of intestinal microbes and their metabolic profiles.

Materials and methods

Chemicals and animal exposure strategy

Thallium(I) nitrate (TINO₃), with a purity of 99.9%, was purchased from Sigma-Aldrich (Shanghai, China) and diluted in deionized water to the required concentration. Male mice were selected as the object to preclude gender effects in this experiment. Seven-week-old specific pathogen-free (SPF) C57BL/6 J mice were purchased from Ensiweier Biotechnology Co., Ltd. (Chongqing, China). A total of 24 mice were housed in plastic cages under a standard 12-h light/dark cycle at a temperature of 22 ± 1 °C and 50–60% humidity, with free access to standardised pellet food. The growth maintenance food (SPF grade) used for the experiment was purchased from Xietong Pharmaceutical Bio-engineering Co., Ltd (Jiangsu, China), which was prepared according to GB 14,924.1, GB 13,078, GB 14,924.2, and GB 14,924.3. After 1 week of acclimatisation, the animals were randomly divided into two groups and six cages (4 mice/cage): the control group (n = 12) and the 10 ppm TINO₃ exposure group (n = 12). Mice in the experimental group were exposed to 10 ppm TINO₃ based on an established dose-equivalent equation that adjusts for surface area differences between mice and humans (Reagan-Shaw et al. 2008; Perry et al. 2013). For mice, drinking water containing TINO₃ at 10 mg·L⁻¹ is equivalent to a 60 kg man drinking 3 L·d⁻¹ of water contaminated with TINO₃ at 1.2 mg·L⁻¹. This exposure concentration has been reported in the blood of patients with Tl poisoning (Heath et al. 1983; Di Candia et al. 2020). The food and water of both groups were changed every 3 days, food and water intake were measured, and the animals were weighed once every 3 days. After 2 weeks of continuous exposure, all mice were sacrificed after being anaesthetised with ether on the last day of exposure. The ileum, cecum, colon, and their contents and feces were collected.

DNA extraction, 16S rRNA gene sequencing, and analysis

The ileum, cecum, colon contents, and fecal DNA were used for bacterial population analysis. Bacterial DNA was extracted using the QIAamp Fast DNA stool Mini Kit (Qiaegen, China), and the concentration and purity were determined using NanoDrop 2000 (Thermo, USA). The 16S rRNA gene V3–V4 hypervariable region was amplified with 50 ng DNA, 0.3 μL of 10 μM of each primer, 5 μL KOD FX Neo Buffer, 2 μL
dNTP (2 mmol each), 0.2 μL KOD FX Neo (Takara, China), and an appropriate amount of sterilised water (up to 10 μL), using universal polymerase chain reaction (PCR) primers 338F/806R (Chen et al. 2019), according to the following PCR program: 5 min at 95 °C, 25 cycles of 30 s at 95 °C, 30 s at 50 °C, and 40 s at 72 °C, and then 7 min at 72 °C. In the second PCR (Solexa PCR), the sequencing primers and adaptors were attached to the amplicon library under the following PCR conditions: 30 s at 98 °C, 10 cycles of 10 s at 98 °C, 30 s at 65 °C, and 30 s at 72 °C, followed by 5 min at 72 °C. The amplicon products were identified, quantified, and purified using 1.8% agarose gel electrophoresis, the DNA purification kit (Omega, Bio-Tek), and the Monarch DNA gel extraction kit (NEB, USA). Sequencing libraries were constructed and paired-end sequencing was performed on an Illumina NovaSeq 6000 platform according to standard protocols. Paired-end reads were merged using Flash (v. 1.2.7) (Yang et al. 2020a), and tags with more than six mismatches were discarded. The merged tags with an average quality score of less than 20 in a 50 bp sliding window were determined using Trimmomatic (v. 0.33) (Sheng et al. 2019), and those shorter than 350 bp were removed. Chimeras were removed, and the denoised sequences were clustered into operational taxonomic units (OTUs) with a 97% sequence homology. For the statistical analysis, alpha diversity between the groups was drawn through the open-source statistical program ‘R’ for the statistical analysis of microbiota data. Beta diversity was ordinated using principal coordinates analysis (PCoA) based on Bray–Curtis distance and drawn using the R-packages ‘vegan’ and ‘ggplot2’. Samples were hierarchical-clustered with the unweighted pair group method with arithmetic mean (UPGMA) method and using different beta-diversity metrics and visualised using the R packages ‘vegan’, ‘cluster’, and ‘factoextra’. Linear discriminant analysis (LDA) and linear discriminant analysis effect size (LEfSe) were performed for the identification of differentially abundant features between groups (Segata et al. 2011).

**Non-target metabolome analyses**

The metabolite detection method was based on our previous research (Li et al. 2021a). In brief, the metabolites of the cecum contents were extracted using an extraction solution (methanol–acetonitrile–water volume ratio, 2:2:1) supplemented with an internal standard solution, and UHPLC-QTOF-MS/MS (Waters, USA) was used for the analysis. The capillary voltages of the positive and negative ion modes were 2000 V and –1500 V, respectively. Raw data were collected using MassLynx (v. 4.2) and processed using Progenesis QI software. The collected data were normalised to the peak area before statistical analysis. For the statistical analysis, principal component analysis (PCA) was used to identify differences between groups, and the significance was estimated using the PERMANOVA test (also known as the Adonis test). Orthogonal partial least-squares discriminant analysis (OPLS-DA) was used to observe the separation between the different groups and to observe the similarities within a group. Volcano map analysis was used to identify and screen different metabolites based on fold change, P-value, and variable importance in the projection (VIP, obtained from the OPLS-DA analysis). The differential metabolites obtained in the positive and negative ion modes were subjected to KEGG annotation (http://www.kegg.jp/). The KEGG pathway enrichment analysis was performed using MetaboAnalyst (v. 5.0) (Pang et al. 2021) and MBRole (v. 2.0) (López-Ibáñez et al. 2016), and visualised using a bubble chart. Network analysis between differential metabolites and KEGG pathway enrichment results was performed using Cytoscape (v. 3.8.2).

**Microbiota and metabolome interactions analysis**

The method of microbiota and metabolome correlation analysis is based on our previous research (Li et al. 2021a). The correlation matrix between the gut bacterial species and metabolites was generated using Pearson’s correlation coefficient (r). The network was very robust if the absolute value of r was > 0.8 and P < 0.05. The concordance between metabolite and microbiota data was performed using the R-package ‘vegan’. The networks were visualised using the Gephi interactive platform (v. 0.9.2). Nodes represented the individual microbial genus or metabolite, and the edges represented linear correlations between the nodes in the integrative network. The role of an individual node was determined according to the topological features of degree and closeness centrality. The nodes with high values of degree (> 60) and closeness centrality (> 0.3) in the network were determined as ‘Hub nodes’ (Xiong et al. 2021).

**Real-time quantitative PCR (RT-qPCR)**

Total RNA was extracted from the ileum, cecum, and colon tissues using the TRIzol reagent (Takara). The purity and concentration of RNA were determined using NanoDrop 2000 (Thermo). cDNA was prepared using the Prime Script RT reagent kit (Takara). The total volume of the RT-qPCR reaction system was 10 μL, including 100 ng total RNA, 0.3 μL of 10 μM of each primer, 5 μL SYBR green II (Takara), and an appropriate amount of sterilised water. PCR amplification was conducted using a Bio-Rad CFX96 Real-Time PCR (USA) system under the following conditions: 95 °C for 5 min; 40 cycles of 95 °C for 30 s, and 56 °C annealing temperature for 30 s; melt curve analysis was carried out by increasing the temperature from 65 to 95 °C in 0.5 °C increments for 0.05 s each. All operations were performed according to the manufacturer’s instructions. The
primers used to amplify specific gene fragments are listed in Table S1. The relative fold change (FC) value was calculated using the Pfaffl method (Pfaffl 2001) with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the reference gene.

Statistical analysis

Body weight, feed and water consumption, and gene expression data are shown as the mean ± SEM. Before statistical testing, the Shapiro–Wilk normality test was performed. Differences in feed and water consumption, alpha diversity, and gene expression between the groups were evaluated using the Student’s t-test. The PERMANOVA test was used to determine whether beta diversity and metabolites were significantly different between the control and treatment groups. LEfSe analyses were used to identify the biomarker taxa for each group (Wilcoxon P-value <0.05, LDA > 2). The correlation matrix between the gut microflora and metabolites was generated using Pearson’s correlation coefficient. The KEGG enrichment analysis of differential metabolites was performed using a hypergeometric test. The concordance between the microbial community and metabolites was determined using the Procrustes test. Statistical significance was set at P < 0.05. All statistical analyses were performed using GraphPad Prism (v. 9.0.0) or R software (v. 4.0.5).

Results

TI(I) exposure had no significant effect on the animal condition

The body weight of the mice was recorded on the first day of exposure, and every 3 days thereafter, with a 2-day interval in the last recordings. In addition, we recorded the average food intake and water consumption of each mouse. The exposure and control groups showed no significant differences in body weight, food intake, and water consumption (Fig. S1a–c).

TI(I) exposure significantly altered the gut microbial diversity

To investigate the changes in gut microbial community triggered by TI(I) exposure, we analysed the ACE, Chao1, Simpson, and Shannon indices in four different sampling sites (ileum, cecum, colon, and feces) of mice. The results showed that TI(I) exposure reduced the ACE and Chao1 indices in the ileum, colon, and feces, but had no significant effect on the ACE and Chao1 indices in the cecum (Fig. 1a and b). The Simpson and Shannon indices showed no significant differences in the cecum, colon, and feces but were significantly lower in the ileum of the exposure group than in that of the control group (Fig. 1c and d). Thus, the bacterial population was significantly decreased in the ileum, colon, and feces, but only the evenness decreased in the ileum under TI(I) exposure.

To further explore the differences in the gut microbial community, we conducted beta diversity analysis based on the Bray–Curtis distance metric and visualised them using PCoA and UPGMA tree diagrams. We first analysed the significance of gut microbial differences along the digestive tract in the control and exposure groups. As shown in Fig. 2a and c, the beta diversity of the gut microbiota showed a significant difference along the digestive tract in both control (P = 0.006) and exposure (P = 0.016) groups according to the Adonis test, indicating that the microbiota changed significantly along the digestive tract. The pairwise analysis did not find significant differences between any two sampling sites in the control and exposure groups (Table S2). In addition, cluster analysis based on the UPGMA method showed that OTUs of the ileum were grouped together and separated from all other samples in the control and exposure groups, respectively (Fig. 2b and d). It suggests that the ileum microbial diversity is different from the microbial diversity of the cecum, colon, and feces. Furthermore, we analysed the influence of TI(I) exposure on gut microbial beta diversity at the four sampling sites between the two groups. Under TI exposure, the gut microbial beta diversity did not show significant differences in the ileum (P = 0.1) (Fig. 2e), cecum (P = 0.2) (Fig. 2g), colon (P = 0.2) (Fig. 2i), and feces (P = 0.5) (Fig. 2k). However, cluster analysis results showed that microbial diversity was different between the two groups (Fig. 2f, h, j, and l).

TI(I) exposure induced significant changes in the gut microbiota

To identify specific microorganisms in four different sampling sites under TI(I) exposure, we conducted LEfSe analysis at the genus level. Figure 3 shows the overall structural changes (Fig. 3a and b) and detailed differential bacterial genera (Fig. 3c–f) along the digestive tract. TI(I) exposure changed the enrichment of specific microorganisms along the digestive tract compared with that of the control group (Fig. 3a and b). In the ileum, the abundance of Devosia, Bradyrhizobium, Sphingomonas, Sporobacter, Citrobacter, Prevotella, Kineothrix, Tyzzerella, Anaerotruncus, Alistipes, Dielma, and Pseudoflavonifractor decreased significantly, whereas the abundance of Faecalibaculum, Staphylococcus, Oscillatoria, Citrobacter, and Hungatella increased in the exposure group (Fig. 3c). In the cecum, the abundance of Sporobacter, Streptococcus, Harryflintia, Faecalibacterium, Candidatus_Koribacter, Devosia, Roseburia, and Sphingomonas was significantly reduced, whereas the abundance of Alloprevotella was significantly increased in the exposure group.
In the colon, Tl(I) exposure remarkably reduced the abundance of *Sphingomonas*, *Citrobacter*, *Faecalibacterium*, *Sporobacter*, *Herbinix*, *Lachnolaelae*, *Fusobacterium*, *Bradyrhizobium*, *Devosia*, *Candidatus Koribacter*, *Anaerotruncus*, *Streptococcus*, and *Roseburia*, but increased *Bifidobacterium* and *Escherichia* abundances (Fig. 3d). In the feces, Tl(I) exposure notably reduced the abundance of *Sphingomonas*, *Devosia*, *Paraeggerthella*, *Sporobacter*, *Candidatus Koribacter*, *Anaeroplasma*, *Intestinimonas*, and *Lactobacillus*, but significantly increased the abundance of *Escherichia* and *Robinsoniella* (Fig. 3e). These observations demonstrate that microbial abundance was disturbed along the intestinal tract under Tl(I) exposure.

**Tl(I) exposure induced global changes in the gut metabolome**

Relevant evidence shows that disturbance of gut microbes is often accompanied by changes in metabolites. The metabolomic profiles of the cecum contents were generated using UHPLC-QTOF-MS/MS, including two detection modes of positive and negative ions to determine whether Tl(I) exposure affects the gut metabolome. Multivariate differences between the exposure and control groups were assessed using PERMANOVA and PCA. The results showed that the metabolites detected in the positive and negative ion modes were significantly different between the exposure and control groups ($P = 0.002$ for positive ion mode, $P = 0.004$ for negative ion mode) (Fig. 4a and d). OPLS-DA analysis showed that the components of metabolites detected under positive and negative ion conditions in the exposure group were quite distinct from those in the control (Fig. 4b and e). A total of 807 metabolites were detected in 12 samples, including 239 downregulated and 58 upregulated metabolites in the positive ion mode (Fig. 4c). In the negative ion mode, 1156 metabolites were detected, including 134 upregulated metabolites and 138 downregulated metabolites (Fig. 4f). Furthermore, we performed KEGG annotations on the differential metabolites detected in the positive and negative ion modes. The results showed that 141 and 145 different metabolites were annotated in the positive and negative ion modes, respectively (Fig. 4g). After merging all the annotated metabolites and eliminating duplicates, we performed a KEGG pathway enrichment analysis as
Twenty-six KEGG pathways were significantly enriched, including 10 pathways with \( P < 0.05 \) and false discovery rate (FDR) < 0.05 (mainly including amino acids, lipids, and purine metabolism), and 16 pathways with \( P < 0.05 \) and FDR > 0.05. The KEGG molecular interaction analysis showed that the GPCRs signalling pathway and lipid-related molecules were significantly changed under Tl(I) exposure (Fig. 4i). To explore the role of differential metabolites between KEGG pathways, we conducted a network analysis between KEGG pathways and differential metabolites. The results showed that, except for pyrimidine metabolism, steroid biosynthesis, and riboflavin metabolism, the other metabolic pathways contained at least one differential metabolite involved in another metabolic pathway (Fig. 4j).

**A strong interaction was found between metabolites and gut microbes**

Related studies have reported that disorders of the intestinal metabolome are closely related to microbes under environmental exposure (Lu et al. 2014; Li et al. 2019, 2021a). To identify whether metabolites and gut microbes are related and their mode of action under Tl(I) exposure, we first performed a concordance analysis between metabolite and microbiota data by Procrustes correlation. The results shown in Fig. 4h. Twenty-six KEGG pathways were significantly enriched, including 10 pathways with \( P < 0.05 \) and false discovery rate (FDR) < 0.05 (mainly including amino acids, lipids, and purine metabolism), and 16 pathways with \( P < 0.05 \) and FDR > 0.05. The KEGG molecular interaction analysis showed that the GPCRs signalling pathway and lipid-related molecules were significantly changed under Tl(I) exposure (Fig. 4i). To explore the role of differential metabolites between KEGG pathways, we conducted a network analysis between KEGG pathways and differential metabolites. The results showed that, except for pyrimidine metabolism, steroid biosynthesis, and riboflavin metabolism, the other metabolic pathways contained at least one differential metabolite involved in another metabolic pathway (Fig. 4j).
Fig. 3  Significantly differentially abundant genera between exposure and control groups. 

a and b LEfSe analysis at the genus level among four different sampling sites (including ileum, cecum, colon, and feces) of mice in control and exposure groups. c–f LEfSe analysis at the genus level for four different sampling sites between control and exposure groups (blue, taxa enriched in control mice; orange, taxa enriched in exposed mice)
were significant (protest, correlation = 0.85 and $P = 0.02$ for positive ion mode, correlation $= 0.82$ and $P = 0.03$ for negative ion mode) (Fig. 5a and b), suggesting that a fraction of gut content metabolites are substrates or products of the microbiota. We then analysed the co-occurrence profiles of bacteria and metabolites (positive or negative correlations). The results showed that differential metabolites and intestinal flora were mainly positively correlated (positive correlation accounted for 59.38%, and negative correlation accounted for 40.62% for positive ion mode; positive correlation accounted for 56.4% and negative correlation accounted for 43.6% for negative ion mode) (Fig. 5c and d). The network complexity was 27.918 and 25.134 in the positive and negative ion modes, respectively (Fig. 5c and d). In addition, the number of hub nodes under positive ion mode was 38, whereas the number of hub nodes under negative ion mode was 34 (Fig. 5e and f). Detailed information on the hub nodes is presented in Table S3.

**Tl(I) exposure impaired regional intestinal immunity**

To identify whether Tl(I) exposure affects intestinal immunity, RT-qPCR analysis was performed on ileum, cecum, and colon tissues using a three-step process. First, we selected GAPDH as a reference gene because its expression is the most stable among the four candidate reference genes ($\beta$-actin, GAPDH, 18S rRNA, and HPRT) according to the evaluation results of the NormFinder software (Table S4). Second, we calculated the amplification efficiency of all genes to be detected using established standard curves. Third, the relative expression levels of genes were calculated using the Pfaffl method. The results showed that the expression of IL-17 ($P = 0.004$) and RORγ ($P < 0.001$) significantly decreased in the ileum (Fig. 6a). In the cecum, IL-10 ($P = 0.003$), IL-17 ($P = 0.04$), IL-1β ($P = 0.009$), RORγ ($P = 0.01$), and TNF-α ($P = 0.02$) expression decreased significantly (Fig. 6b). In the colon, the abundance of none of the detected genes changed significantly, but the expression of IL-17 showed a downward trend ($P = 0.07$) (Fig. 6c). Collectively, these data demonstrate that intestinal immunity showed regional differences.

**Discussion**

The present study showed that Tl(I) exposure changed the gut microbial community at the abundance level, disturbed metabolic profiles at the functional level, and altered gut immune status at the molecular level. Data revealed that Tl exposure had a significant impact on the intestinal microenvironment, which may provide mechanistic insights regarding Tl exposure-induced disease.

Gastrointestinal symptoms and signs, such as nausea, vomiting, poor appetite, and anorexia, occur after Tl poisoning (Tsai et al. 2006; Yumoto et al. 2017; Di Candia et al. 2020). A study of children with Tl intoxication (urinary Tl levels of 13.4–60.1 μg/L) shows that Tl exposure slightly affects their appetite (Duan et al. 2020). In this study, we found that Tl exposure for 2 weeks did not significantly affect food or water intake in mice. This may be due to differences in exposure levels, including exposure time, frequency, and/or dose (Li et al. 2021b).

The gut microbiota plays an important role in regulating the critical balance between health and disease throughout the human life cycle (Zhuang et al. 2019). Different types of environmental exposure have different effects on microbial diversity. Rubidium was positively associated with alpha diversity indices (Shannon and Simpson), whereas mercury and vanadium showed negative associations (Zhang et al. 2021). Cd exposure increased the alpha diversity of gut microbes, whereas arsenic (As) exposure had no significant effect (Li et al. 2019, 2021a). These results show that different exposure characteristics have different effects on the intestinal microbial community. In this study, Tl exposure reduced the alpha diversity of gut bacteria but did not significantly influence the beta diversity. These results contrast with previous studies in the zebrafish (Wang et al. 2021). Possible reasons include exposure dose and species differences.

Diseases induced by environmental exposure may be regulated by gut microbes. As and Cd exposures induce significant changes in gut microbiota and metabolome by affecting bile acids and amino acids, which may be related to T2DM (Li et al. 2019). Rubidium and antimony exposures were negatively correlated with the risk of GDM by changing the abundance levels of the co-abundance group enriched for Lachnospiraceae, Coriobacteriales, and Ruminococcaceae (Zhang et al. 2021). Tl exposure induces intestinal abnormalities in zebrafish, changes the structure of gut microbiota, and increases the level of pathogenic intestinal bacteria such as Mycobacterium, which may induce zebrafish death (Wang et al. 2021). These results indicate
that environmental exposure affects intestinal microbes and the occurrence and development of diseases. In this study, TI exposure affected gut microbiota abundance along the digestive tract and altered the composition and abundance of metabolites; these gut microenvironmental changes can reflect the toxicity profile to some extent before TI induces significant pathological symptoms.

Changes in the diversity of intestinal microbes can cause changes in metabolites. Both As and Cd exposures show that disturbance of intestinal microbes is closely related to the perturbation of metabolites (Lu et al. 2014; Li et al. 2019, 2021a). We found that the number of microorganisms related to differential metabolites was far greater than that of the screened differential microorganisms (Figs. 5e and f and 3d). This indicates that there are significant interactions among gut microbes. The disturbance of the metabolome may be the result of the joint action of microorganisms, rather than being mediated by different microorganisms independently.

Fatty acids are essential components of all biological membrane lipids and are important substrates for energy metabolism; they play an important role in regulating various biological functions, such as cell division and inflammation (Guillou et al. 2008, 2010). The disorder of fatty acid biosynthesis induced by TI exposure implies a change in energy absorption and energy metabolism in the intestine. Unsaturated fatty acids, such as polyunsaturated fatty acids (PUFAs), have important physiological functions, such as regulating cell membrane permeability (Yang et al. 2020b). Under normal conditions, PUFAs are frequently oxidised by lipooxygenase, but they are immediately reduced by glutathione peroxidase 4 (GPX4) and its cofactor glutathione (GSH) (Lee et al. 2020). GPX4 activity is inhibited or GSH level is depleted, which can induce the accumulation of lipid peroxides in the cells and eventually lead to cell death (Seiler et al. 2008; Dixon et al. 2012; Stockwell et al. 2017). In vitro studies have found that TI exposure can reduce the content of GSH and GPX enzyme activity in rat brain cytosolic fractions, which implies the depletion of GPX and GSH under Tl exposure (Hanzel et al. 2005). GSH, which is synthesised by glutamate, cysteine, and glycine, plays a key role in maintaining the cellular thiol redox state and protecting cells from oxidative stress (Denke and Fanburg 1989). In the current study, KEGG pathway enrichment results showed a disorder in the biosynthesis of unsaturated fatty acids and glutamate metabolism, which may reveal another mechanism by which TI exposure induces changes in the intestinal microenvironment. Namely, TI exposure depletes intestinal GPX and GSH, which leads to disturbance of unsaturated fatty acid synthesis, especially of PUFAs, inducing an imbalance in intestinal cell homeostasis.

Intestinal microbes and their metabolites, and intestinal immunity are closely related. Segmented filamentous bacteria can induce the activation of CCR6+ ILC3s distributed in isolated lymphoid follicles and crypts to promote IL-22 secretion (Sano et al. 2015). In addition, the capsular polysaccharide A contained in Bacteroides fragilis upregulates IL-10 levels to activate FoxP3+CD4+ regulatory T cells (Tregs) (Chang et al. 2017). Furthermore, intestinal metabolites, which include products of both host and microbial metabolism, can also affect intestinal immunity. For example, intestinal microorganisms digest dietary fibre into SCFA (such as butyric acid, propionic acid, and acetic acid), which is identified by GPCRs located in intestinal epithelial cells to regulate the production of cytokines, including TNF-α, IL-6, CXC chemokine ligand 1 (CXCL1), and CXCL10 (Kim et al. 2013; Zhou et al. 2020). In addition, naive T cells that reside in the intestine can differentiate into T helper type 1 (Th1) cells, Th2, Th17, and Treg cells, which promote the secretion of IFN-γ, IL-4, IL-17, and IL-10, respectively (Zhou et al. 2020). Changes in the levels of these cytokines indicate immune disorders. In this study, TI exposure disrupted gut microbial communities, which in turn affected fatty acid and protein metabolism. The disturbance of these two metabolic pathways may be the direct cause of TI-induced intestinal immunosuppression. The KEGG enrichment analysis showed that TI exposure significantly affects fatty acid biosynthesis, which may perturb the levels of SCFA and mediate disturbances in the intestinal immune response. Metabotropic glutamate receptors (mGlurRs) are dimeric class C GPCRs; they include mGlur4, mGlur6, mGlur7, and mGlur8, which modulate neuronal excitability and synaptic plasticity throughout the central nervous system (Niswender and Conn 2010). In addition, mGlurRs are widely expressed in immune cells and regulate immune functions (Xue and Field 2011). Disturbance of intestinal glutamate metabolism induces changes in the mGluR signalling pathway in immune cells, which may be the cause of intestinal immune cell dysfunction. Moreover, TI exposure may directly affect intestinal immune cells and induce immunosuppression. Distinct immune responses along the intestinal tract were uncovered. This is not surprising, because different intestinal tissues have structural and functional differences. For example, in
the study of long-term As exposure, the abundance of As resistance genes showed differences in the cecum and colon (Li et al. 2021a). Furthermore, the transcript level of IL-17 was significantly decreased in the ileum and cecum, while its decrease was not significant in the colon, suggesting that TI exposure may influence the body's defense against extracellular pathogens and autoimmune diseases. The transcription factor RORγ that regulates IL-17 expression was significantly inhibited, suggesting that TI exposure inhibits transcription factor activation, thus affecting immune responses, and may be another pathway of intestinal immunosuppression. Further research is needed on the mechanisms of interaction between intestinal microbes, metabolites, and the intestinal immune response.

Conclusions

In this research, we presented a longitudinal study on the changes in gut microbiota along the intestinal tract following water TI(I) exposure. We demonstrated that TI(I) exposure altered microbial abundance at genus levels at the four sampling sites (ileum, cecum, colon, and feces). The gut microbiota disturbance was associated with substantial metabolic functional changes, characterised by significantly affected amino acid, lipid, purine metabolism, and GPCRs signalling pathways. Perturbation of metabolic pathways may be related to intestinal immunosuppression induced by TI(I) exposure. The perturbations of the gut microbiota and its metabolic profile, and intestinal immunosuppression, may serve as a new pathway to explain how TI exposure leads to or exacerbates human disease.

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Author contribution XYZ and XHZ conceived and designed the project, and modified the grammar and increased the readability of the manuscript. DL wrote the paper with input from the authors. HY and ZQL modified the grammar and corrected the table.

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Data availability Raw sequences of 16S rRNA were deposited in NCBI Sequence Read Archive database (accession number PRJNA777011). Raw metabolomics data will be made available on Dryad, http://datadryad.org/.
Declarations

Ethics approval and consent to participate The handling of animals was performed in accordance with the protocols approved by the Animal Protection Committee of China West Normal University and Sichuan Agricultural University.

Consent for publication The authors declare that they consent for the publication of this study.

Conflict of interest The authors declare no competing interests.

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