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Polystyrene microplastics induce gut microbiota dysbiosis and hepatic lipid metabolism disorder in mice

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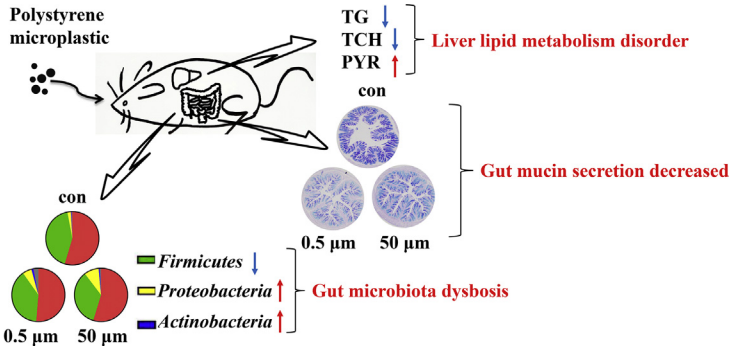
HIGHLIGHTS

- Polystyrene microplastic decreased the secretion of mucin in gut of mice.
- Polystyrene microplastic induced gut microbiota dysbiosis in mice.
- Polystyrene microplastic induced hepatic lipid metabolism disorder in mice.

GRAPHICAL ABSTRACT



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ABSTRACT

Microplastic (MP) has become a concerning global environmental problem. It is toxic to aquatic organisms and can spread through the food chain to ultimately pose a threat to humans. In the environment, MP can interact with microbes and act as a microbial habitat. However, effects of polystyrene MP on the gut microbiota in mammals remain unclear. Here, male mice were exposed to two different sizes of polystyrene MP for 5 weeks to explore its effect. We observed that oral exposure to 1000 μg/L of 0.5 and 50 μm polystyrene MP decreased the body, liver and lipid weights in mice. Mucus secretion in the gut decreased in both sizes of polystyrene MP-treated groups. Regarding the gut microbiota, at the phylum level, polystyrene MP exposure decreased the relative abundances of *Firmicutes* and α-*Proteobacteria* in the feces. Furthermore, high throughput sequencing of the V3-V4 region of the 16S rRNA gene revealed significant changes in the richness and diversity of the gut microbiota in the cecums of polystyrene MP-treated mice. At the genus level, a total of 6 and 8 types of bacteria changed in the 0.5 and 50 μm polystyrene MP-treated groups, respectively. Furthermore, an operational taxonomic unit (OTU) analysis identified that 310 and 160 gut microbes were changed in the 0.5 and 50 μm polystyrene MP-treated groups, respectively. In addition, the hepatic triglyceride (TG) and total cholesterol (TCH) levels decreased in both 1000 μg/L 0.5 and 50 μm polystyrene MP-treated groups. Correspondingly, the relative mRNA levels of some key genes related to lipogenesis and TG synthesis decreased in the liver and epididymal fat. These results indicated that polystyrene MP could modify the gut microbiota composition and induce hepatic lipid disorder in mice; while the mouse is a common mammal model, consequently, the health risks of MP to animals should not be ignored.

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1. Introduction

Recently, the production of plastics and their products has increased due to their pervasive use in industry, agriculture and daily life (Browne et al., 2011; Vianello et al., 2013). Plastic has excellent physical and chemical properties; the global production and use of plastic have risen sharply, the world's plastic production approached 300 million tons in 2013 (Plastics Europe, 2015), and it is estimated that by 2050, the number of plastic products on earth will increase by 33 billion tons (Rochman et al., 2013; Phillips and Bonner, 2015). In the ocean, the plastic accounts for about 60% to 80% of marine litter, and even reaches 90% to 95% in some areas, indicating that it has become the main component of marine litter (Moore, 2008). Microplastic (MP) is a kind of plastic particles which <5 mm in diameter (Moore, 2008). In the environment, the plastics can be further broken down into small pieces through UV radiation, biological degradation, light degradation and embrittlement (Andrady, 2011; Cózar et al., 2014). Plastic will breakdown into particles even smaller than a millimeter in diameter and cause long-term physical and chemical effects (Thompson et al., 2004; Yamashita et al., 2011). And MP can also come directly from industrial products, such as plastic beads added to toothpaste, cosmetics and personal care products (Cole et al., 2015).

As a new type of environmental pollutant, MP has recently received more and more global attention. Since 2011, the United Nations Environment Program (UNEP) has continued to focus on plastic waste in the ocean, especially MP pollution. In addition, the United States has also forbidden the use of plastic particles in cosmetics and personal care products as of 1 July 2017 (Smith, 2014; Perkins, 2014), and the Canada, the Netherlands and other countries also issued statements to phase out the use of microbeads (Eriksen, 2014; Anderson et al., 2016).

Because the size of MP is similar to that of the food of many aquatic organisms, MP is often eaten by mistake (Steer et al., 2017). Previous studies indicated that the gastrointestinal tracts of 16 species of birds had plastic fragments (Zhao et al., 2016). This MP will flow from the lower nutrient levels to the higher nutrient levels in the food chain; eventually, MP can cause risks to the human health (Miranda and de Carvalho-Souza, 2016; Setälä et al., 2014). In addition, when it is ingested, MP especially the micro and nanoscale can also enter the circulatory system of marine organisms through a variety of ways (Avio et al., 2015; Grigorakis et al., 2017). The toxic cellular effects of MP have also been reported in blue mussels and mesenchymal stem cells (Jiang et al., 2011; Von et al., 2016). Nonetheless, there are some studies showing that MP have no adverse effects on several toxic biomarkers in aquatic animals (Kaposi et al., 2014; Cauwenberghe et al., 2015). whereas some studies had shown that MP could be toxic to aquatic organisms, not only cause physical injury in fish (Jovanović, 2017; Pedà et al., 2016) but also lead to the internal damages such as block the digestive tract, reduce growth rates, block enzyme production, induce oxidative stress and even affect reproduction (Wright et al., 2013; Jeong et al., 2016; Sussarellu et al., 2016; Rodriguez-Seijo et al., 2017).

More and more studies have focused on the effects of MP on marine organisms, and there are some reports showing that MP can enter the terrestrial food chain (Huerta et al., 2017). And the interaction between MP and microbes can't be ignored either. A previous study indicated that marine MP can interact with microorganisms (Harrison et al., 2011). Mccormick et al. (2014) demonstrated that MP in rivers was a distinct microbial habitat and may be a novel vector for the downstream transport of unique bacterial assemblages. Hence, we hypothesized that MP might change or interact with the microbes in the gut of mice. Although one study indicated that MP can enter tissues and accumulate in mice (Deng et al., 2017), there are few studies on the effects of polystyrene MP on the gut microbiota in mice. According to previous studies, some environmental chemicals, including antibiotics, pesticides and several heavy metals, can effectively induce gut microbiota dysbiosis, change the mucus layer and even result in lipid metabolism disorder in different experimental models, including mice (Lu et al., 2014; Jin et al., 2016a, b, 2017b; Wu et al., 2018). In our study, we exposed male ICR mice to two different sizes of polystyrene MP; our data showed that the polystyrene MP could induce gut microbiota dysbiosis and hepatic lipid metabolism disorder in these mice. The results obtained in this study provide new insights into the potential health risks caused by MP in mammals.

2. Materials and methods

2.1. Chemicals

Polystyrene MP was purchased from Microspheres-Nanospheres (New York, USA). The particle sizes were 0.5 and 50 μm, the item catalog numbers were 100,161-10 and 100,259-10, and the corresponding product IDs were C-PS-0.5 and C-PS-50.0, respectively. The MP come wet in solution and the initial concentration is 25 mg/mL. The medium of delivered PS is deionized water. Both of the polystyrene MP particles were used as received. The morphology of the different sizes of polystyrene MP in water by scanning electron microscopy as our previous study (Jin et al., 2018b).

2.2. Animals and experimental scheme

Five-week-old ICR (Institute of Cancer Research) mice ($n = 40$) were purchased from the China National Laboratory Animal Research Center (Shanghai, China). All mice were housed in separate cages in an animal room for a week to adapt to the environment. After one week, they were weighed and divided randomly into five groups, and the body weights of each group were almost the same. During the whole experiment, only one mice was put in per cage (size: 285 × 178 × 150 cm). All the mice were maintained on a twelve hour light and twelve hour dark cycle each day, and water (Reverses Osmosis pure water) and basic diet (Proteng Biotechnology co. LTD, Shanghai, China) were always available.

The four groups (eight in each group) were exposed to 0.5 and 50 μm polystyrene MP at concentrations of 100 and 1000 μg/L (about 1.456×10^{10} particles/L for 0.5 μm and 1.456×10^{4} particles/L for 50 μm), respectively. Polystyrene MP are diluted in RO water for direct drinking with continuous exposure for 5 weeks. The control group drank normal water without polystyrene MP. During the exposure, we replaced the padding in the cages and then collected enough fresh feces immediately in each group on the third and seventh days and then once a week; the feces were stored at −80 °C until disposal. During the exposure, the body weights of each mouse were measured after 8 h fasting at the same point as described above.

All the mice were fasted for 8 h, anesthetized with ether and sacrificed after five weeks of exposure. Blood sera samples were collected quickly with venous blood and stored at −40 °C until further measurement. Livers, epididymal fat, colons and cecum contents were collected quickly and flash-frozen in liquid nitrogen; the samples were stored at −80 °C until further use. All experiments were performed in accordance with the Guiding Principles for the Use of Animals of Zhejiang University of Technology, and all efforts were made to minimize animal suffering.

2.3. Histopathological analysis

The colon was cut into small pieces and fixed immediately in 10% (vol/vol) formaldehyde solution. Subsequently, the fixed gut tissues were dehydrated in a rising series of ethanol, hyalinized in xylene, and embedded in paraffin wax at 56 °C. Then, three middle guts samples from each group were cut into 5 μm-thick sections. After that, at least 4 sections were stained with alcian blue-periodic acid Schiff (AB-PAS) solution (Jin et al., 2018b). The mucus coverage ratio from 6 sections in each group was calculated by the pixels in the mucus area to the