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Impacts of polystyrene microplastic on the gut barrier, microbiota and metabolism of mice

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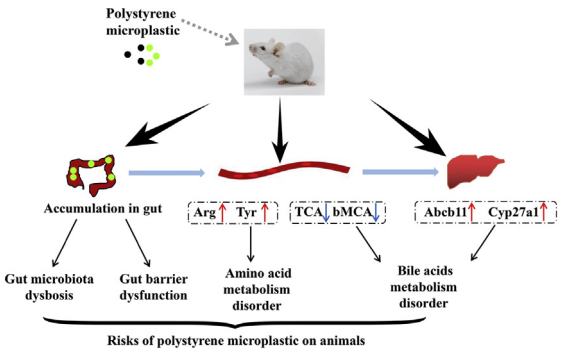
HIGHLIGHTS

- 5 μm polystyrene microplastic could be accumulated in the gut of mice.
- 5 μm polystyrene microplastic induced intestinal barrier dysfunction in mice.
- 5 μm polystyrene microplastic induced gut microbiota dysbiosis in mice.
- 5 μm polystyrene microplastic induced bile acids metabolism disorder in mice.

GRAPHICAL ABSTRACT



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ABSTRACT

Microplastics (MPs), which are new environmental pollutants with a diameter of <5 mm, have received wide attention in recent years. However, there are still very limited data regarding the risks of MPs to animals, especially higher mammals. In this study, we exposed male mice to 5 μm pristine and fluorescent polystyrene MP for six weeks. The results showed that the polystyrene MP was observed in the guts of mice and could reduce the intestinal mucus secretion and cause damage the intestinal barrier function. In addition, high-throughput sequencing of the V3-V4 region of the 16S rRNA gene was used to explore the change of the gut microbiota composition in the cecal content. At the phylum level, the content of *Actinobacteria* decreased significantly in the polystyrene MP-treated group. The PD whole-tree indexes of the alpha diversity and principal component analysis (PCA) of the beta diversity indicated that the diversity of gut microbiota was altered after polystyrene MP exposure. At the genus level, a total of 15 types of bacteria changed significantly after exposure to polystyrene MP. Furthermore, the predicted KEGG (Kyoto Encyclopedia of Genes and Genomes) metabolic pathway differences indicated that the main metabolic pathways of the functional genes in the microbial community were significantly influenced by the polystyrene MP. In addition, indexes of amino acid metabolism and bile acid metabolism in the serum were analyzed after polystyrene MP exposure. These results indicated that polystyrene MP caused metabolic disorders. In conclusion, the polystyrene MP induced gut microbiota dysbiosis, intestinal barrier dysfunction and metabolic disorders in mice. This study provided more data on the toxicity of MPs in a terrestrial organism to aid in the assessment of the health risks of polystyrene MP to animals.

1. Introduction

In the last century, plastics began to appear. These materials have been mass-produced since the 1950s and have subsequently increased

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year by year (Jambeck et al., 2015). Microplastics (MPs), a new type of environmental pollutant that consists of plastic particles <5 mm in diameter, were created due to mass production and large use of plastic in the world (Thompson et al., 2004; Barnes et al., 2009). The scientific community began to study marine plastic pollution in the 1970s (Carpenter et al., 1972; Wong et al., 1974). However, scientists began to pay full attention to MP pollutants in the present century, especially in the most recent decade. For example, Thompson et al. (2004) first proposed the term microplastics. Recently, at the second United Nations conference on the environment in 2015, MP pollution was listed as the second most important scientific problem in the field of environmental and ecological science, which included global threats such as climate change, ocean acid and ozone depletion (Amaral-Zettler et al., 2016).

MPs are widely distributed in seawater, inland lakes and even in polar regions (Barnes et al., 2009; Obbard et al., 2014; Eerkes-Medrano et al., 2015), and they are easily ingested and further accumulated by various organisms due to their small sizes and low rate of degradation (Ivar Do Sul and Costa, 2014). Today, many studies have shown that various organisms, especially those in the marine environment, can ingest MPs. For example, in Shanghai, China, the researchers found that 16 of 17 (94.1%) specimens ingested a total of 364 microscopic anthropogenic items, an average of 10.6 ± 6.4 items per bird (Zhao et al., 2016). And MPs were detected in the fish of the English Channel, the level of MPs in the body is approximately 1.90 ± 0.10 items per fish (Lusher et al., 2013). Studies have mainly evaluated the toxicity of MP on indicators such as feeding rate, growth rate, oxidative damage, quantity of egg-laying, and enzyme activities (Browne et al., 2008; von Moos et al., 2012; Jeong et al., 2016; Sussarellu et al., 2016). Some studies have shown that MPs have no negative effects in some organisms such as the freshwater invertebrate *Gammarus pulex* and the terrestrial isopod *Porcellio scaber* (Weber et al., 2018; Jemec Kokalj et al., 2018). However, a recent review indicated that the extent of MP pollution in the terrestrial environment remained a fundamental gap in our knowledge and should be a future research priority (Horton et al., 2017). Increasing numbers of studies have shown that MPs are an emerging threat to terrestrial ecosystems, and a more balanced discussion on human exposure to MPs is needed (de Souza Machado et al., 2018; Rist et al., 2018). Compared to the effects of MPs on aquatic organisms, the effects of MPs on terrestrial systems have received far less scientific attention (de Souza Machado et al., 2018). The fact is that MPs contamination on terrestrial systems might be 4- to 23-fold greater than in the ocean (Horton et al., 2017), and more significantly, MPs may affect human health through the food chain as a result of the human consumption of MPs via bivalves, chicken gizzards, and even in sea salt and tap water (Van Cauwenberghe and Janssen, 2014; Huerta et al., 2017; Rist et al., 2018).

The intestinal mucosa is the line of defense against the intestinal infection of an animal body (Martínez et al., 2012). Generally, the intestinal mucosal barrier of an animal can effectively prevent the intestinal endogenous and exogenous antigens from being transferred through the intestinal tract to the systemic circulation, thereby ensuring the health of the animals (Kong et al., 2017). Intestinal epithelial cells, the main functional cells in the intestinal tract, are an important element of the intestinal mucosal mechanical, immune and chemical barriers (Sanz and De Palma, 2009). If the intestinal epithelial cells mutate, decrease or are destroyed, the permeability of epithelium will increase, and bacteria, endotoxin and macromolecules can enter the systemic milieu and thus adversely affect the host's health (Chen et al., 2015). However, a number of previous studies have shown that gut microbiota can induce the proliferation of intestinal epithelial cells, strengthen the close connection of the intestinal mucosal epithelium, reduce the damage to the intestinal mucosa by pathogenic bacteria, and maintain the function of intestinal barrier (de Kivit et al., 2014). Thus, the gut microbiota has an essential role in modulating host metabolism and in the development of some metabolic diseases in host (Ley et al., 2005; Allahham et al., 2012). Increasing evidence has supported the concept that the gut microbiota is a toxicological target for different kinds of environmental pollutants (Jin et al., 2017; Xia et al., 2018a). More importantly, some studies have also shown that MPs can also interact with microorganisms and even suggested that MPs can serve as a distinct microbial habitat (Harrison et al., 2011; McCormick et al., 2014). Our previous research has recently shown that the 0.5 and 50 μm polystyrene MP could induce gut microbiota dysbiosis both in zebrafish and mice (Jin et al., 2018a; Lu et al., 2018). In this study, we exposed male ICR mice to 5 μm polystyrene MP. Our results indicated that the 5 μm polystyrene MP could accumulate in the gut and induce gut barrier dysfunction, microbiota dysbiosis and metabolic disorders in male mice. The results obtained in this study provide some new insights into the potential risks of MP exposure to terrestrial ecosystems and provide some basic data on the possible impacts of MPs to human health.

2. Materials and methods

2.1. Chemicals

The 5 μm pristine and fluorescent polystyrene microplastic (MP) suspensions were purchased from Microspheres-Nanospheres (New York, USA). The delivery medium for the two types of MP was deionized water. All the stock solutions were treated with ultrasound for 30 min before dilution. Both of the polystyrene MP particles were used as received.

2.2. Animals and experimental scheme

Five-week-old ICR (Institute of Cancer Research) mice were purchased from the China National Laboratory Animal Research Center (Shanghai, China). All mice were housed in independent cages (size: 285 × 178 × 150 cm) in an animal room with a cycle of twelve hours of light and twelve hours of dark. After a week of accommodation, they were weighed and randomly divided into three groups. Two groups (eight in each group) were exposed to 5 μm polystyrene MP at the concentrations of 100 (approximately 1.456×10^6 particles/L) and 1000 μg/L (approximately 1.456×10^7 particles/L) for the toxicological experiment. The polystyrene MPs were diluted in RO drinking water, and the animals were continuously exposed for six weeks. The control group ($n = 8$) drank normal water without polystyrene MP. In addition, for the histopathological accumulation experiment, two groups were exposed with or without 5 μm fluorescent polystyrene MP at a concentration of 1000 μg/L ($n = 5$). During the whole experiment, the water (Reverse Osmosis pure water) and basic diet (Proteng Biotechnology Co. LTD, Shanghai, China) were always available.

At the end of the experiment, all the mice were fasted for 8 h, anesthetized with ether and sacrificed. Blood sera samples were collected quickly with venous blood and stored at −40 °C until further measurement. Tissue such as liver, colon, ileum 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. Detection of MP in the gut

To examine the existence of polystyrene MP in the gut of mice, after exposed to 5 μm fluorescent polystyrene MP for 6 weeks, the colon was cut into small pieces and fixed with 10% (vol/vol) formaldehyde. Then embedded in paraffin wax, cut into 5 μm-thick sections and unstained. The fluorescently labeled polystyrene MP in the gut of the mice were observed with a high resolution confocal microscope (Olympus FV31-HSU-P, Olympus Corporation, Tokyo, Japan).