The impacts of microplastics on intestinal structure and mucus secretion

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Abstract: Microplastics accumulate in large quantities throughout the ecosystems. Also, microplastics are harmful to the organisms. This paper mainly discusses the effects of microplastics on intestinal structure and mucus secretion. It has been found experimentally that exposure to microplastics and nanoplastics impairing mucus secretion and, in addition, altering the intestinal structure and affecting the formation of intestinal flora.

1. Introduction

In 1868, humans nitrated natural cellulose and used camphor as a plasticizer to make the world's first plastic variety, called celluloid, which began the history of using plastic. Due to their stable properties, plastics are increasingly produced around the world [1]. However, the durability and recyclability of plastics are limited [2], which leads to the continuous accumulation of plastics in the environment. Over time, the plastic breaks down into smaller pieces of various sizes and shapes. Plastic fragments will be restricted as microplastics (MPs) when they are less than 5 mm in diameter [3]; Plastic particles smaller than 1μm in size are defined as nanoplastics (NPs). The term "microplastics" was introduced in 2004 by Professor Richard Thompson, a Marine biologist at Plymouth University.

Concentrations of MPs in oceans and rivers around the world have been reported to reach thousands of particles per cubic metre of water. There were reports, for example, of around 100,000 plastic particles per cubic metre of seawater in the port area near a polyethylene plant in Sweden. More recently, Sutton et al. found that in San Francisco Bay, this number reaches about 700,000 particles per square kilometer [4]. Microplastics (MPs) and nanoplastics (NPs) are mainly absorbed by aquatic organisms through ingestion. When ingested NMPs reach the gut, they appear to migrate more easily to other tissues and become smaller in particle diameter, damaging biological tissue and inhibiting colony growth. The gastrointestinal system is a very composite and highly generalized system in which the body digests and absorbs nutrients [5]. It not only ensures the assimilation of nutrients but also has a barrier function [6]. Change in gut microbiota abundance and mucus changes and even damage immune cells. But studies have shown that the intestinal barrier can be disrupted by the entry of NMPS, which may cause changes in gut microbiota abundance and the amount of mucus, and even damage the immune cells, leading to a leaky bowel and other more serious physical problems.

Most of the waste can only be disposed of through incineration and landfills, and more than 20 million tons of plastic end up in the ocean without treatment. Because plastics are hard to break down, they persist in the food chain as microplastics, eventually making their way into our bodies and intestines through water and food, which is the main source of microplastics in our bodies. MPs and NPs exposure can alter the composition of the intestinal tract, leading to disruption of the intestinal microenvironment. Some experiments showed that polystyrene MP can induce intestinal microflora dysregulation in zebrafish and mice [7], 5μm polystyrene MP can also accumulate in the intestinal tract of male mice, causing intestinal barrier dysfunction, intestinal microflora imbalance and

metabolic disorders. It can also show intestinal damage (e.g., increased mucus ratio) and further alter the gut flora.

2. Intestinal Epithelial Structure and Function

2.1 Intestinal epithelial cells

The intestinal epithelium is constructed, in a cellular level, by different types of epithelial cells. These cells are highly specialized and organized into a monolayer, forming structures called crypts and villi, which not only adapt to the function of nutrient absorption, but also constitute a barrier between the intestinal lumen and the internal environment. Known epithelial cells include enterocytes which are the main component of the intestinal villi and capable of nutrient absorption, goblet cells which produce mucus forming a gel-like layer above the intestinal epithelium, enteroendocrine cells which play an important role in cell signaling and regulating of intestinal functions, paneth cells which secrete antimicrobial proteins, M cells which connect the epithelium with the mucosal immune system, and other cells with relatively low proportions such as cup cells and tuft cells [8].

Epithelial cells are interconnected by junctional complexes composed of serious proteins called tight junctions. These junctions were proved to be associated with intestinal permeability regulation, and therefore maintain the integrity of the epithelial cell layer [9]. The connected epithelial cells along with their secretory proteins provide both physical and chemical protection for the internal environment from the microorganisms and toxic xenobiotics in the intestinal lumen. Furthermore, they regulate and coordinate with the internal immune system, therefore build a connection between physical, chemical, microbiological and immune barriers of the intestine, which are essential to the maintenance of intestinal homeostasis.

2.2 Mucus layer

The mucus layer, flowing on the top of and around the epithelium, is the first defense of invasions of microorganisms, because of its direct contact with the intestinal microbiota. The mucus mainly consists of mucins, with MUC2 as the most abundant component. Other derived products such as TFF3 and RELMβ, also secreted by goblet cells, reinforce the structure of MUC2 and adjust the amount of MUC2 secreted [10]. The mucus layer is divided into two parts according to the thickness of the mucus colon, physical properties and chemical composition. The inner layer is firmer and it works as a filter to prevent large bacteria from getting in, hence the inner layer is said to be devoid of bacteria, meaning that any damage to the mucus layer could leave epithelium cell unprotected from bacteria and lead to serious infections [11]. In contrast to the inner one, the looser and more expanded outer layer is served as a habitats and nutrient source for microbiota. The microorganisms can feed on carbohydrates such as mucin monosaccharides and are hard to wash away when they have embedded in the mucus layer [12].

There are several important biological molecules in the mucus, involving antibacterial substances such as bile acids, which further strengthen the antibacterial properties of the mucus layer [8]. In conclusion, a fully functioning mucus layer with the right amount of mucus secretion is crucial to the prevention of infections and the maintenance of intestinal homeostasis, as it not only separates the intestinal epithelium from the external environment but also supports and strengthens another intestinal barrier such as the microbiota.

3. Effects of Microplastics on the Structure of Intestinal Epithelium

3.1 Histological damages related to the amount of accumulation

Many studies using animal models have shown that MPs do accumulate in the gastrointestinal tract after exposure to a certain concentration of MPs. Not surprisingly, the vast majority of the experiments showed that the number of accumulation increases as the concentration of exposure increases. For example, in the gut of guppy, the MP concentration after exposing to $1g/m^3$ of MPs is significantly

higher than that after exposing to 0.1 g/m^3 MPs [13]. Another experiment also found a higher frequency of the occurrence of MPs in the intestine of the intertidal fish Girella laevifrons, feeding with higher MP concentration diets [14]. Moreover, a study using goldfish discovered that the shape and size of MPs also had effects on the final amount of accumulation. In the experiment, MPs were made into fragments, fibre and pellets, and were then exposed to goldfish. However, only fibre was recovered in the gills and gastrointestinal tract of the goldfish [15]. These observations are important because the amount of MP accumulation in the gastrointestinal system is one of the decisive factors of the toxicity of MPs, the larger amount of accumulation, the severer damage that the MPs might cause.

The histological damages are notable in many studies. Significant histological changes were observed in the gut of adult zebrafish after exposure to both low and high levels of MPs, which showed a decreased thickness of the bowel wall as well as villi and epithelial damage respectively [16]. Similar results were found also using zebrafish but exposed to MPs made of different chemicals (PA, PE, PP, PVC, PS) and within a certain concentration range (0.001–10.0 g/m^3). Cracking of villi and splitting of enterocytes were observed almost in every chemical group, with ignorable differences. The experiment on European sea bass showed the impacts of the amount of exposure. The anterior intestine morphological feature of the sea bass has no obvious alteration after feeding with 0.1 g PVC-MPs/kg comparing to the controlled group, whereas massive signs of injuries were shown in the 0.5 g PVC-MPs/kg group, including increased villus thickness and dissociation of the mucosal epithelium from the lamina propria [17].

3.2 Oxidative stress is the main mechanism

The main mechanism of microplastic toxicology is considered to be oxidative stress, namely, toxic effects induced by the production of reactive oxygen species in the course of metabolism of MPs. Products related to gene expression and intestinal permeability are common indicators of the level of oxidative stress. In Caenorhabditis elegans of nematode, marked gst-4 gene was used as a reflection of oxidative damage, a significant increase of gst-4 gene expression was shown after treatment with 5.0×10^{-3} g m^{-2} of different kinds of MPs. A decrease in intestinal calcium level was also observed [18]. ROS production and lipofuscin accumulation in C. elegans, which are related to oxidative stress, were also analyzed. Significant increases in ROS production and lipofuscin accumulation were observed when exposing to 0.001-0.1 g/m^3 of PS-MPs and 0.01–0.1 g/m^3 of PS-MPs respectively [19]. Similar results were achieved using zebrafish. The CAT and SOD activities in dissected gut tissue were largely increased after exposure to both low and high levels of MPs. The intestinal permeability was evaluated using the levels of DAO and D-lactate in the zebrafish gut, after exposure to MPs. As a result, the level of DAO was significantly decreased while the level of D-Lactate was significantly increased. All these results of in vivo studies would act as strong evidence for the severe oxidative stress induced by MPs.

3.3 Exposure to MPs cause inflammation

The overall histopathological adverse effect of MPs that often occur along with the broken or damaged epithelial structure and oxidative stress is inflammation. For example, when MPs were accumulating in the gut of zebrafish, epithelial tissue damage, oxidative stress and inflammation were all observed [16]. Some signs are usually seen when intestinal inflammation occurs, which might be useful as the parameters for evaluation of the intestinal inflammation, such as the increased phagocytic activity of immune cells [20], leukocyte infiltration as well as Crypt cell loss and Villi cell loss [14].

The occurrence of inflammation also indicates that MPs have disrupted the immune system to a certain extent. In the earthworm Eisenia Andrei, dysfunction of the immune system including inflammation and fibrosis were proved to be associated with MPs [21]. Moreover, at a cellular level, significant reductions in some total counts of hemocytes and phagocytosis were observed in the adult blood clams which were exposed to MPs [22]. Chemicals that are carried by MPs may also be responsible for inducing an inflammatory and immune response. Relevant evidence includes that endotoxin contaminated in nanomaterials could activate the inflammasome and result in immune response [23]. However, the toxicity of materials carried by MPs is not fully understood. Another experiment using mice as a model found that the intestine of mice exposed to high-concentration MPs

showed obvious inflammation and higher TLR4, AP-1, and IRF5 expression [24], which are related to immune proteins so that are signs of inflammation. The results achieved by mammals are of more significance because they provide hints and hypothesis of how MPs would interact with a human.

4. Effects of Microplastics on Intestinal Mucus Secretion

The mucus layer, as an important barrier of the intestine, could also be impacted when interacting with MPs. Changes in the number of secretory cells and the amount of mucus were all observed in many experiments. Also, factors like the concentration and types of MPs are again affecting the toxicity. For example, the study examining European sea bass using distinct types of MPs including PE, PVC showed that when exposing to 0.1g/kg of MPs, there was no significant difference in different types of MPs. However, after exposure to 0.5g/kg of MPs, the PVC group showed an increase in the number of goblet cells; On the other hand, those fed by PE-MPs had a significant decrease in the number of goblet cells [17]. Alteration in some goblet cells would probably induce a change in the amount of mucus. The study on mice showed evidence of that. The secretion of mucus was significantly decreased after exposure to 0.1 and 1 g/m^3 PE MP. Moreover, the transcription levels of genes related to the secretion of mucin including Muc1, Muc2, Muc3, Klf4, Meprin-βand Retnlb tended to decrease, though the extents are different [25]. These experimental results might reveal an explanation in genetic level for the change in the amount of secretion of mucus, which could be crucial for further studies of the effects of MPs on the mucus layer.

As mentioned, the mucus layer is the nutrient source of microorganisms and gives a place for attachment. The damages to microbiota were also observed on adult zebrafish after exposure to MPs. The composition of gut microbiota at the phylum level changed significantly after exposure to 10^{-3} g/m^3 0.5mm and 50mm PE-MPs for 14 days, which means the proportions of different species of microorganisms in the gut have altered and were not balanced. Not only numbers the type (genus) of the bacteria has also changed, including ones that could lead to inflammation [7]. But it is still not clear whether these change in microbiota has a direct relationship with the alteration in the amount of mucus. In addition, a decrease in the number of antibacterial molecules such as bile acids was also observed in the same experiment [25], which indicates a further decline in the antibacterial capability of the mucus layer.

5. Conclusion

This paper has introduced the composition of intestinal epithelial cells and added that they are related to the regulation of intestinal permeability, identified the importance of the mucus layer as a line of defense - the first line of defense and explained the importance of protection of the mucus layer. The harm of MPs entering the human intestine includes that exposure to MPs will cause inflammation; It can also affect the content of antibacterial substances such as bile acids, destroy the intestinal structure and even affect the formation of intestinal microbiota.

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