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Intestinal permeability and gene expression after polyethylene and polyamide microplastic ingestion in Wistar rats

Benuarda Toto [a,*], Alice Refosco [a], Maria O'Keeffe [a], Øyvind Halås Barkhald [c], Aurora Brønstad [e], Gülen Arslan Lied [a], Fekadu Yadetie [c], Anders Goksøyr [c], Tanja Kögel [c,d], Jutta Dierkes [a,b]

[a] Department of Clinical Medicine, Centre for Nutrition, University of Bergen, Bergen, Norway
[b] Department of Medical Biochemistry and Pharmacology, Haukeland University Hospital, Bergen, Norway
[c] Department of Biological Sciences, University of Bergen, Bergen, Norway
[d] Institute of Marine Research, Bergen, Norway
[e] Department of Clinical Medicine, Laboratory Animal Facility, University of Bergen, Norway

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ABSTRACT

Microplastic particles are ubiquitous in the environment. However, little is known about their uptake and effects in humans or mammalian model organisms. Here, we studied the effects of pristine polyamide (15–20 μm) and polyethylene (40–48 μm) particles after oral ingestion in rats. The animals received feed containing microplastic particles (0.1% polyamide or polyethylene, or a mixture of both polymers) or a control diet without microplastic particles, for 5 weeks. The permeability of the duodenum was investigated in an Ussing chamber, whereas gene expression and concentration of tight junction proteins were measured in gut tissue and plasma. Microplastic particles were quantified by pyrolysis-gas chromatography/mass spectrometry in rats' feces. Rats fed with microplastic particles had higher duodenal permeability. Expression of gene coding for the tight junction protein occludin (OCLN) was higher in PE treated animals compared to control or the PA group. No changes in the expression of the gene coding for zonula occludens protein 1 were detected. Occludin protein concentrations were below the limit of detection of the applied method in both gut and plasma. Zonula occludens protein 1 concentrations in the gut were significantly higher in groups exposed to PA and PE as compared to control, while zonula occludens protein 1 concentrations in plasma did not show significant changes. These results demonstrated that short-term exposure to a dose of 0.1% (w/w) microplastic particles in feed had limited effects on duodenal permeability, expression of pro-inflammatory protein genes and tight junction protein genes in the duodenum.

1. Introduction

Plastic production has increased constantly over the past 70 years, and plastic is nowadays widely used in everyday life, for such different purposes as food packaging, building and construction, clothing, or fishing gear. Plastic breakdown in the environment leads to increasingly smaller fragments, called microplastic and nanoplastic. Microplastic particles (MP) are defined as plastic particles with a size of less than 5 mm. They are found ubiquitously in the environment and there is increasing concern about their effects on living organisms, including humans (Vethaak and Legler, 2021).

MP that are manufactured to be smaller than 5 mm are defined as primary MP (Gesamp, 2016), while secondary MP are formed when larger plastic particles break down into MP as a result of weathering conditions such as ultraviolet radiation, wind, waves, abrasion, mechanical and physical forces, and other factors (Andrady, 2011; Gewert et al., 2015). Expanding expertise shows that MP cause adverse effects on terrestrial and aquatic life (de Souza Machado et al., 2018).

MP are found in the gut and in the edible parts of fish and in other seafood (AMAP, 2021; Kögel et al., 2020). However, MP are not limited to the marine environment, and it has been estimated that the burden of MP in terrestrial systems may be even higher compared to marine systems, while data on the potential health risks in terrestrial animals and humans is scarce (Horton et al., 2017).

Exposure to MP in humans can occur through inhalation, dermal contact or oral intake. Ingestion through the diet is considered to be one

* Corresponding author.
E-mail address: Benuarda.Toto@uib.no (B. Toto).

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of the most important routes for humans (Galloway, 2015). A study that investigated the presence of MP in human feces from eight volunteers, detected MP in all samples measured (Schwabl et al., 2019). Furthermore, a preliminary study in young male students showed that MP were present in 23 of the 24 (95.8%) fecal samples (Zhang et al., 2021). An important question for human health is if MP leave the intestinal tract into body fluids, tissues and organs to a significant portion, and if that causes negative effects at current or expected environmental, occupational or food/food packaging exposure levels. However, the fate of MP in the human gastrointestinal tract is largely unknown. While the intestinal epithelial layer is an effective physical barrier against the external environment, controlled transport across this barrier can occur via transcellular or paracellular pathways (Tsukita et al., 2001). Paracellular pathways involve tight junctions (TJ), which are selectively permeable barriers consisting of tight junction proteins that are crucial for the integrity of the barrier function. Among others, occludin, and zonula occludens protein 1 are important TJ proteins. These TJ complexes are modulated by external stimuli and intracellular signaling transduction systems including cytokines, small GTPases, and post translational modifications (Buckley and Turner, 2018).

It can be hypothesized that MP can cross the intestinal endothelial layer by transcellular or paracellular pathways. Another hypothesis is that the contact of endothelial cells with MP would cause low grade inflammation in the gut, potentially affecting the integrity and function of the gut mucosa and of the TJ proteins. Both mechanisms may play a role.

Inflammation in the colon was previously demonstrated in mice after exposure to polyethylene (PE) MP (1–10 μm; (Sun et al., 2021) and both in the colon and duodenum after exposure to high concentrations of PE MP (10–150 μm; (Li et al., 2020). It can be hypothesized that defects in the mucosal barrier may lead to increased intestinal permeability and intestinal inflammation (Michielan and D'Incà, 2015). In the present study we tested therefore the effect of orally ingested PE and polyamide (PA) MP in Wistar rats on duodenal permeability in an Ussing chamber, the duodenal expression of TJ proteins and pro-inflammatory proteins, and their concentration in gut tissue and plasma. The expression of the pro-inflammatory markers TNF-α (tumor necrosis factor-alpha), IL-10 (interleukin 10), SOCS3 (suppressor of cytokine signaling 3), as well as the expression of F4/80, a murine macrophage infiltration marker, were used to assess the inflammatory condition of the intestine.

2. Methods

2.1. Rat feeding study

Wistar rats (*Rattus norvegicus*), 12 female and 12 male (Janvier Labs, Le Genest-Saint-Isle, France), aged 8 weeks, were divided into 4 groups with six individuals (n = 6), assigned into pairs in cages according to baseline weight, and acclimated for 1 week. Tap water without additional treatment was provided *ad libitum.* The animals were housed in a temperature- and humidity-controlled room (22 °C, 55 ± 10% relative humidity) on a 12:12 h light:dark schedule. All inlet air to animal rooms and IVC cages were hepa-filtered. The sleeping hut inside the cages was made of porcelain and the feed was offered in porcelain plates with metallic spoons. The common, red-tinted plastic tubes normally used as enrichment in rats cages were replaced by ceramic items where rats can hide and better maintain the temperature in their preferred zone. The bedding material made of aspen wood and gnawing sticks were produced for animal studies supplied by Scanbur. Weight gain and well-being were assessed weekly.

After acclimatization, they were fed with either control feed (standard low-fat powdered feed, RM1 (E) SQC (Scanbur, Witham, England)), or feed containing 0.1% w/w MP of either PE or PA or a 1:1 w/w mixture of both polymers. MP were mixed and homogenized into the feed and thus administered continuously throughout the experiment. The detailed preparation of the homogenized feed is shown in the supplementary information. The following MP polymers were used: polyamide-nylon (PA6) in powder, hereafter abbreviated PA, with a particle size of 5–50 μm (mean 15–20 μm), 1.13 g/ml density, Goodfellow Cambridge Ltd. (Huntingdon, England); polyethylene ultra-high molecular weight (UHMWPE) in powder, hereafter abbreviated PE, particle size of 40–48 μm, 0.94 g/ml density, Sigma-Aldrich (St. Louis, MO, USA). Animals were fed for 5 consecutive weeks with their respective diet based on a normal daily intake of 5 g per 100 g of bodyweight (Harkness et al., 2010). Assuming that the entire amount of 25 g of food given daily is ingested by the rats, this corresponds to the maximum MP daily consumption of 25 mg/day. The similarity in weights between the rats housed in the same cage was beneficial when planning their daily intake based on the expectations that the two rats of each cage would consume a similar amount of food.

After 5 weeks of feeding, the animals were euthanised using carbon dioxide. Blood samples to provide plasma for analysis were obtained by cardiac puncture within 2 min into EDTA tubes and centrifuged at 1800 g at 4.4 °C for 10 min. After euthanising the animal, the duodenum was removed immediately and prepared for insertion into an Ussing chamber. Feces were obtained directly from the colon to avoid possible contamination. They were wrapped into aluminum foil and stored at − 80 °C to be used for MP analysis.

2.2. Particle characterization

Particle shape, sizes and chemical identity were analyzed by ATR-FTIR spectroscopy, laser light scattering and light microscopy. A Nicolet™ Summit PRO FTIR spectrometer equipped with an Everest™ ATR accessory monolithic ATR diamond crystal was used to collect the spectra from 4000 cm^{-1} to 400 cm^{-1}. For each sample 16 co-added scans with spectral resolution of 4 cm^{-1} were collected and 16 pre-recorded background scans were used for correction. Spectra were processed in the OMNIC Paradigm Desktop software (Thermo Scientific™) and compared to spectra of known compounds and polymers in commercial libraries (Thermo Scientific™) and in-house libraries.

The particle size distribution was quantified using a Mastersizer 3000 laser diffraction instrument with a HydroLV dispersion unit (Malvern Instruments Ltd., Worcestershire, UK). The samples were added directly to the dispersion unit until the obscuration reached 5–15%, as suggested by the manufacturer for fine samples. To facilitate the dispersion of the particles, 0.05 ml of dishwashing detergent liquid (Zalo; Orkla home and personal care products, Norway) was added. The refraction index was set to 1.50 and the absorption index to 0.01. All samples were measured at a 2500 rpm stirring speed.

For microscopy images, PA and PE particles were suspended in a solution of water and Zalo to avoid agglomeration of the particles. One drop of the solution was placed on a microscope slide and dried at room temperature. A Leica DM 2500 LED optical microscope (Leica Microsystems, Wetzlar, Germany) was used to image the particles at 4x and 10x for PE and PA, respectively.

2.3. Permeability studies of duodenum in the Ussing chamber

Permeability was measured in an Ussing chamber using the Fluorescein isothiocyanate (FITC) method (Vidyasagar and MacGregor, 2016). Duodenal segments were stripped off the external muscle layers, leaving the submucosal plexus and mucosa intact. The muscle-stripped tissues were mounted in the Ussing chamber and 2 ml of the dextran solution was added to the mucosal chambers, whilst pure PBS was added to the serosal chambers. Carbogen (95% O_2, 5% CO_2) was added to the chambers through injection ports at low pressure.

Fluorescein-Dextran (molecular weight 3000 g/mol, Thermo Fisher, Waltham, MA, USA) was dissolved in PBS buffer solution (EMD Millipore Corp, Burlington, MA, USA) with a final concentration of approximately 8.25 μmol/L (mucosal chamber, at the start of the experiment). Mucosal-to-serosal fluxes of (FITC)-dextran were monitored every 15

min by measuring the fluorescence on both sites of the chamber. Fluorescence was measured at excitation wavelength 491 nm and emission wavelength 521 nm (SPEXTRAmax Gemini EM, Molecular Devices, San Jose, CA). Data were processed using Softmax pro 7.1 software (Molecular Devices, San Jose, CA).

The fluorescence ratio was calculated by dividing the fluorescence (arbitrary units) at the serosal side by the fluorescence of the apical side, in order to correct for differences in starting fluorescence. Two duodenal segments of each rat were run in parallel.

2.4. Gene expression of tight junction proteins occludin and zonula occludens protein 1 and inflammatory proteins in duodenum (TNF-α, IL-10, Socs3, F4/80)

After the animals were euthanized, a part of the duodenum was immediately removed and frozen at - 80 °C for RNA extraction. RNA was isolated using TRI-Reagent (Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer's instructions. Briefly, 30 mg of frozen tissue were homogenized in 1 ml of TRI reagent and incubated for 5 min at room temperature. Then 0.2 ml of chloroform was added to the samples before incubating the tubes for another 15 min. By centrifuging the samples at 12,000 g for 15 min at 4 °C, the mixture was separated into three different phases: a lower organic phase containing protein, an interphase containing DNA and an upper aqueous phase containing RNA. Afterwards, 0.4 ml of the aqueous phase were added to fresh tubes, mixed with 0.4 ml isopropanol and incubated for 10 min at room temperature before centrifuging again at 12,000 g for 10 min at 4 °C. The supernatant was carefully removed, and the remaining RNA pellet was washed with 1 ml 75% ethanol and centrifuged at 7500 g for 5 min at room temperature. Then, the ethanol was removed, and the remaining RNA pellet was allowed to dry for 30 min. The pellet was dissolved in 150 μL RNAse-free water by incubating at 60 °C for 30 min. The concentration and purity of the RNA was measured using a nanodrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and the integrity was visualized by a Doc EZ imager (Bio-Rad) after agarose-gel electrophoresis. The samples were stored at - 80 °C until further use.

2.5. Quantitative polymerase chain reaction

Reverse transcription (RT) for cDNA synthesis was performed for each sample using 1 μg total RNA in 20 μL reactions using the iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA), according to manufacturer's protocol. Briefly, RT reaction mix (containing 1 μg total RNA, dNTPs, random hexamer and oligo (dT) primers, and 1 μL of the reverse transcriptase in reverse transcriptase buffer) was pre-incubated at 25 °C for 5 min, 46 °C for 30 min and at 95 °C for 1 min. After completion, the RT reaction mix was then diluted 1:10 and used as template in quantitative polymerase chain reaction (qPCR). Each of the PCR reaction mix (20 μL total volume) contained 5 μL of the diluted cDNA template, 0.5 μM of each of the forward and reverse primers and 10 μL of 2x LightCycler® 480 SYBR Green I Master mix (Roche Diagnostics, Mannheim, Germany). qPCR was performed on Bio-Rad CFX96 real-time PCR detection system (Bio-Rad, Hercules, CA, USA) with the following reaction conditions: an initial denaturation of 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 10 s, annealing at 55 °C for 20 s, and elongation at 72 °C for 30 s. For each primer pair, two negative controls (with no reverse transcriptase enzyme and no template) were included in the PCR set-up. Amplification efficiency was determined from a standard curve constructed using serial dilutions of cDNA prepared from pooled RNA samples. Melting curve analysis was performed to check specificity of primers. Triplicate reactions were performed for each sample of the PCR assays. As a reference for normalization, the *Rattus norvegicus* actin, beta (Actb) gene (accession: NM_031144) was used. Analysis of relative gene expression was performed using the ΔΔCt method (Schmittgen and Livak, 2008). The expression of genes coding for the tight junction proteins occludin and zonula occludens protein 1, as well as genes coding for the pro-inflammatory proteins (TNF-α, IL-10, SOCS3, F4/80) was performed using specific primers from other studies on rat intestine (Gil-Cardoso et al., 2017), or primers designed using Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) (Table S2 in the supplementary information).

2.6. Protein concentration in duodenum and plasma

The concentration of occludin and zonula occludens protein 1 was measured in gut tissue and plasma, and the concentration of C-reactive protein (CRP) was measured in plasma by specific ELISAs (occludin: Rat Occludin (OCLN) ELISA kit (MyBioSource, San Diego, USA); ZO-1: Rat tight Junction Protein 1 (ZO1) ELISA kit (MyBioSource, San Diego, USA); CRP: Rat C-Reactive Protein ELISA kit (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA).

Protein was extracted from approx. 50 mg frozen duodenum homogenates. Briefly, the duodenum section was rinsed from blood and fecal matter with ice-cold PBS (0.02 mol/L, pH 7.0–7.2). The rinsed gut samples were added to 1 ml of PBS and 5 mm stainless beads (Qiagen, Germany) and then homogenized with a TissueLyser II Qiagen (Qiagen, Germany) for 6 min. Following homogenization, the samples were removed and placed in 15 ml tubes and then subjected to ultrasonication, followed by centrifugation for 15 min at 1500 g at 4 °C. Finally, the supernatant was removed, separated into multiple tubes and stored at − 80 °C. All procedures were performed on ice.

2.7. Analysis of MP in feces

To avoid any contamination fecal matter was directly extracted from the colon after euthanisation of the rat and stored in aluminum foil at -80 °C until analysis. Feces samples were subjected to a three-step digestion protocol: i) oxidative-acidic digestion (H_2O_2 and HNO_3 mixture) ii) alkaline digestion (KOH), and iii) enzymatic digestion with ViscozymeL® (Sigma-Aldrich St. Louis, MO, USA), which will be published separately. MP particles were then measured by py-GC/MS (pyrolysis-gas chromatography mass spectrometry) in the microplastic laboratory at the Institute of Marine Research, Bergen.

2.8. Ethics

The animal study was approved by the Norwegian Food Safety Authority after application through FOTS (Forsøksdyrforvatningen tilsyns- og søknadssystem), with FOTS application ID: 20467.

2.9. Statistics

The study used a 2 × 2 factorial design, testing two different types of MP. As this was a pilot study, there was uncertainty to calculate the number of animals needed. The final calculation was based on differences in the fluorescence ratio (thus gut permeability), and 6 animals per group were sufficient to demonstrate a difference in fluorescence ratio of 0.0015 at a baseline standard deviation of 0.0009 and assuming a power of 80% and a significance level of 0.05. Calculations were made with the program clincalc.com.

Results are expressed either as mean with standard error of the mean, or as median with range. We compared results from the groups that received MP to the control group by non-parametric tests (Wilcoxon signed rank test for unrelated samples). P-values lower than 0.05 were regarded as significant.

In addition to testing the individual time points from the permeability studies, the area under the curve (AUC) was calculated for each of the gut samples according to the trapezial rule (12 segments per group, GraphPad Prism 6.0) and compared the AUC to the control group.

3. Results

3.1. PA and PE characterization

ATR-FTIR analysis of PA and PE used in the study revealed a 93.59% and 98.78% match to PA and high density PE of in-house libraries. The characterization and the microscopy images of PA and PE particles are reported in the supplementary material (Fig. S1 in the supplementary information). The particle size distribution obtained by the Mastersizer is expressed in percentile values (D10, D50 and D90), that indicate the size in μm below which 10%, 50% or 90% of all particles are found (Table 1). The D10, D50, and D90 values correspond to the mean of 5 measurements for each sample and are expressed in volume density of the sample.

3.2. Weight and well-being of the rats

At the beginning of the study, the average weight of male rats was 429 ± 38 g and of female rats 235 ± 19 g. Animals gained weight without differences among the groups, and their weight was 502 ± 28 g in male rats and 266 ± 16 g in female rats when euthanized after 5 weeks (Fig. S2 in the supplementary information). Animals showed no sign of discomfort or pain during the study.

3.3. Ussing Chamber results

When the permeability of the duodenum was measured, setups from control animals and from rats fed with PA + PE mixture showed the lowest increase of fluorescence at the serosal side, while setups from rats fed with either PA or PE had stronger increase of fluorescence, indicating higher permeability. Fig. 1 shows the time course of fluorescence ratio (serosal side corrected for the apical side).

However, when the fluorescence ratio of the individual time points from 0 to 150 min from groups that received MP were compared with the control, there was no significant difference at any time point and group comparison, with all p-values > 0.10, with the exception of the PE group ($p = 0.07$ at 90 min) (Fig. 1).

The AUC was lowest for the control group, followed by the mixed group and the PA and PE group. However, there were no significant differences among the AUC calculations (Fig. 2).

3.4. Biochemical results

3.4.1. Quantitative PCR – increased occludin expression

Results from the qPCR of genes encoding tight junction proteins and inflammatory proteins are presented in Fig. 3. The gene coding for occludin showed significantly higher expression in the groups receiving polyethylene MP (PE and PA + PE), compared to the control. No significant changes were observed for the expression of the gene coding for ZO-1 or inflammatory proteins.

3.4.2. Protein concentration in gut and plasma

The concentrations of the proteins occludin, zonula occludens proteins 1 and CRP were measured in EDTA plasma samples obtained from cardiac puncture and in supernatants of duodenum homogenates by specific ELISAs. For occludin, the signals were extremely low, and the sensitivity of the ELISA did not allow the calculation of either plasma or gut concentrations. For zonula occludens protein 1, the concentrations were much higher in the supernatants of the gut homogenates than in plasma in the MP fed groups, and there were significantly higher concentrations in gut homogenates from the groups exposed to PA and PE, as compared to the control. The group exposed to PA + PE was not significantly different from the control. No significant differences were detected in plasma (Fig. 4).

Feeding with MP did not affect CRP concentrations in rat plasma (Fig. 5).

Table 1
Summary table of the particle size distribution shown in volume %.

Polymer	D10 (μm)	D50 (μm)	D90 (μm)
PA	12.9	20.8	32.9
PE	27.4	44.1	68.1

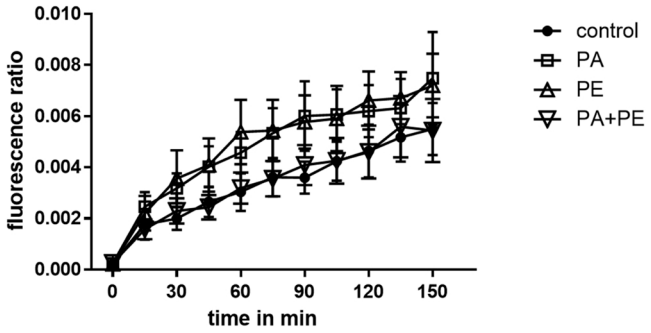

Fig. 1. Fluorescence ratio over time for serosal side chamber representing transport over duodenal segments (n = 2 from each animal, total 24 animals). Fluorescence ratio measured every 15 min over a period of 150 min. Results are presented as mean ± SEM. Abbreviations: PA: polyamide, PE: polyethylene, PA+PE: mixed.

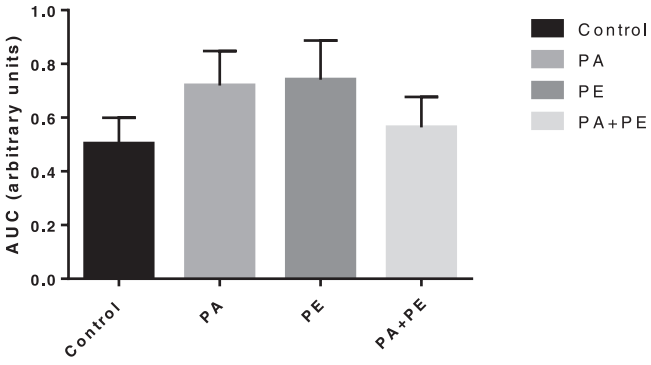

Fig. 2. Mean AUC from the fluorescence ratio 0–150 min for each feeding group (each group n = 12 duodenal segments from 6 animals). Each group receiving MP was compared to the control group (PA $p = 0.30$, PE $p = 0.18$, PA+PE $p = 0.68$). Results are presented as mean ± SEM. Abbreviations: PA: polyamide, PE: polyethylene, PA+PE: Mixed.

3.5. Analysis of MP in feces

Analyses of feces directly obtained from the gut of 14 rats (2 from control and 4 rats from each MP group) showed MP levels below the limit of detection in control rats, and quantifiable amounts of PA particles in the PA-fed rats (0.76 ± 0.17 mg/g feces, mean ± SEM), of PE particles in the PE fed rats (0.66 ± 0.06 mg/g feces) and both PA and PE in the rats fed with PA and PE (0.33 ± 0.09 mg/g PA, 0.42 ± 0.01 mg/g PE;Toto et.al,in preparation).

4. Discussion

To our knowledge, this is the first reported study where gut permeability was tested in rats by applying an Ussing chamber after exposure of MP. The aim of the study was to investigate the effect of a short-term, relatively high dose oral feeding with PA and/or PE MP, on gut permeability, expression and concentration of tight junction proteins and pro-inflammatory proteins. The main result of the study is that PA and PE MP have been tolerated well by the rats for 5 weeks. We did not

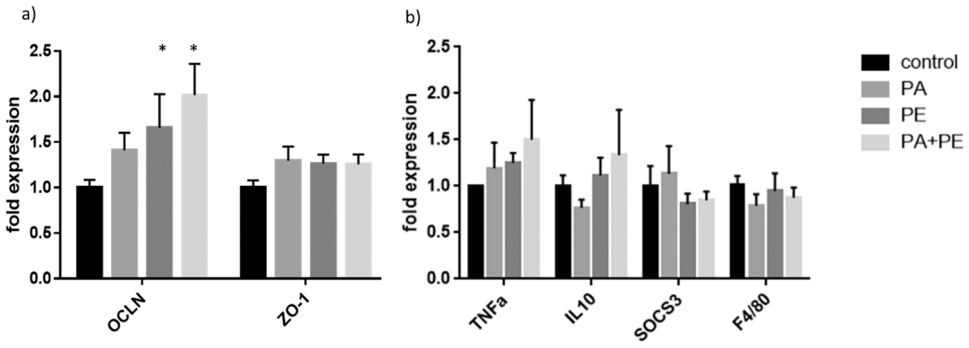

Fig. 3. Expression of genes coding for (a) tight junction proteins occludin and zonula occludens protein 1 and (b) inflammatory proteins TNF-α, IL-10 and SOCS3 in rat duodenum in control animals (n = 6), and animals fed with similar feed containing PA (n = 6), PE (n = 6) and PA+PE (n = 6), measured by qPCR, normalized for the house-keeping gene Actb, and expressed relative to the control group. Results are presented as mean ± SEM. Differences at $p < 0.05$ are indicated by asterisks.

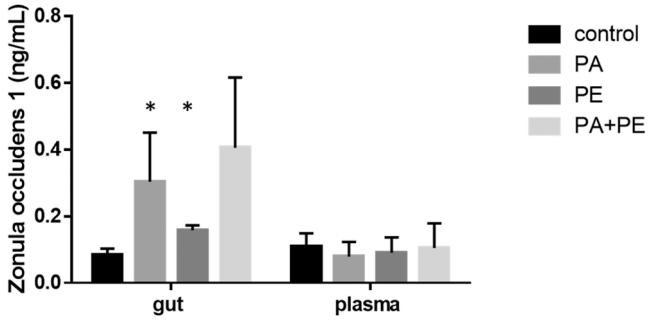

Fig. 4. Concentration of the tight junction protein zonula occludens protein 1 in rat duodenum (left) and plasma (right) in control animals (n = 6), and animals fed with similar diets containing PA (n = 6), PE (n = 6) and PA+PE (n = 6), measured by ELISA. Results are presented as mean ± SEM. Differences at $p < 0.05$ are indicated by asterisks.

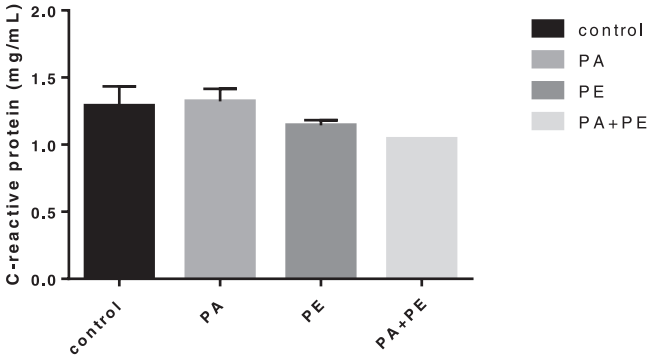

Fig. 5. C-reactive protein (CRP) concentration in rat plasma obtained by cardiac puncture after 5 weeks ingestion of MP (PA: polyamide, PE: polyethylene, PA+PE: polyamide and polyethylene), 0.1% (w/w) in the feed, measured by ELISA. No significant differences were observed among the groups. Results are presented as mean ± SEM.

observe any effect on weight gain or well-being of the animals. Slight and non-significant changes of intestinal permeability after MP feeding in the Ussing chamber experiment, and higher expression of OCLN in duodenum were observed. In addition, higher zonula occludens protein 1 levels in duodenum after exposure to MP was observed, but no difference of expression of inflammatory proteins in duodenum nor of CRP plasma concentrations was registered. Presence of MP in the gastrointestinal tract of the rats was proven by fecal analysis which revealed measurable concentrations of both PA and PE as expected.

Although the effects of MP exposure have been extensively studied in fish and other aquatic organisms (Kögel et al., 2020), earthworm (Boots et al., 2019), lugworms (Wright et al., 2013; Green et al., 2016) and copepods (Cole et al., 2019; Bai et al., 2021), there are only a few studies that investigated oral MP exposure in terrestrial mammals such as mice or rats (Jin et al., 2019, 2021; Yong et al., 2020; Hou et al., 2021; Ijaz et al., 2021; Wei et al., 2021). Uptake of 5 μm or 20 μm PS particles in gut, liver and kidney was observed (Jin et al., 2019), and described effects include changes in intestinal mucosal barrier (Jin et al., 2019; Li et al., 2020; Sun et al., 2021), oxidative damage in the liver (Deng et al., 2017), cardiotoxicity (Wei et al., 2021), effects on the ovaries (An et al., 2021; Hou et al., 2021) and testis (Xie et al., 2020; Hou et al., 2021; Ijaz et al., 2021; Jin et al., 2021) or no effect on oxidative stress (Stock et al., 2019). Of note, the vast majority of these studies used oral gavage for MP application, in contrast to the continuous exposure with feed used in the present study, which better reflects natural conditions. Administration by feed will ensure a continuous intake, while the oral gavage will be comparable with a bolus dose. It is known in pharmacology that this may lead to different absorption kinetics (Turner et al., 2011). Since rats are nocturnal animals, MP given through feed follows rats' natural eating habits, as opposed to MP administered through oral gavage, which is usually administered during the daytime. Additionally, most of these studies that use oral gavage as an exposure route treat the MP with other components in order to keep it homogenized in suspension. The administration through feed without chemical treatment is considered closer to real life.

Similar to our results, the MP exposure in previous studies had no effect on weight gain in mice (Deng et al., 2017; Jin et al., 2019; Stock et al., 2019; Hou et al., 2021) or rats (Ijaz et al., 2021), although the majority of studies did not report weight after exposure (Jin et al., 2019; Yang et al., 2019; Li et al., 2020; An et al., 2021; Hou et al., 2021; Sun et al., 2021; Wei et al., 2021), which would be desirable to be reported in all studies as a sub-acute, easy-to-measure endpoint.

Most published studies in rodents used polystyrene (PS), one PE (Sun et al., 2021). To our knowledge, none had investigated PA so far. However, PE exposure by (Sun et al., 2021) was different from that in the present study, as they used MP with a size of 1–10 μm, applied by oral gavage at a dose of 0.2 μg/g/day. Changes in colonic expression of IL-8, IL-10 (upregulated) and IL-1β (downregulated) were observed. This is in contrast to our study, where rats in the MP groups received 0.1% MP of 15–48 μm size in feed, which corresponds to about 25 mg/day MP intake through 25 g feed , thus a higher mass but lower particle number exposure. In our setup, IL-10 expression was unchanged. However, due to the above-mentioned differences, it is difficult to directly compare these results.

Gut permeability was not previously tested in rats or mice. However, a study investigated the expression of tight junction proteins and applied a Ussing chamber in rainbow trout after PS exposure (Ašmonaitė et al., 2018). Rainbow trout were exposed to both virgin and harbor-water exposed PS particles (100–400 μm size) through their feed and the MP

intake was about 10 mg/fish/day. No change in either molecular or ion permeability, neither in the proximal nor distal intestine was observed. In addition, no changes in expression of pro- or anti-inflammatory mRNA (IL-1β, TNF-α, IL-17, IL-10) or in tight junction protein mRNA (ZO-1, occludin, tricellinin) were observed. As this protocol is quite close to our study, this study also points towards that under the investigated conditions, MP exposure did not alter gut paracellular permeability, in line with that expression of tight junction proteins were largely unaffected.

The factors that may explain the differences from our findings to the above cited studies in rats include the polymer type (PA and PE versus PS), the size of the particles (15–20 and 40–48 μm versus ≤20 μm), and the application with the feed versus oral gavage. Differences due to the application route should be further investigated.

In the present study, no change in the expression of the selected pro-inflammatory proteins was observed, and plasma concentrations of CRP were not influenced by MP administration. Thus, our study does not indicate that MP may cause intestinal inflammation in rats under the investigated conditions.

Results on the expression and concentration of TJ proteins are more difficult to interpret. While the permeability in the Ussing chamber was slightly increased after MP ingestion, but not reaching statistical significance, the expression of the OCLN gene and the concentration of zonula occludens protein 1 in duodenum were increased. Literature on the connection of those proteins with gut permeability is not conclusive. Some studies that investigated gut permeability have found decreased concentrations of occludin and zonula occludens protein 1 associated with increased permeability (Elamin et al., 2014; Little et al., 2018), while other studies reported increased OCLN and ZO-1 expression with increased permeability (Dokladny et al., 2006; Hudson et al., 2020). This could be interpreted as a compensatory effect and should be studied further.

Our study had several strengths and limitations. First, Wistar rats are an established model for the human gut. We performed a controlled feeding experiment including both sexes with two different polymers of different size ranges PA (15–20 μm) and PE (40–48 μm), where MP were taken up with the feed, thus ensuring a constant intake of particles. Among the limitations, we have to acknowledge that pristine PA and PE MP have been used, and that the feeding lasted only 5 weeks. Therefore, the MP do not represent all environmental MP and no conclusions on potential long-term effects can be drawn. Regarding permeability, we only investigated the duodenum, but not other parts of the intestinal tract or other organs which should also be included in future studies.

5. Conclusion

PA and PE particles had limited effects on the permeability of the duodenum, expression of pro-inflammatory protein genes and tight junction protein genes in the duodenum of Wistar rats. However, rats fed with MP displayed a higher duodenal permeability after exposure in Ussing chamber experiment, as well as higher OCLN gene expression in the duodenum and zonula occludens protein 1 concentration in gut homogenates. In light of the emerging MP pollution, more studies with longer exposure time to MP, use of environmental MP of small size and diverse shape and more outcomes are needed.

CRediT authorship contribution statement

Benuarda Toto: Conceptualization, Methodology, Validation, Formal analysis, investigation, Writing-Original Draft, Writing - Review and Editing. **Alice Refosco:** Validation, Formal analysis, Investigation, Visualization. **Maria O'Keeffe, Øyvind Halås Barkhald**: Investigation, Writing original draft, Writing review and editing. **Aurora Brønstad, Gülen Arslan Lied:** Conceptualization, Writing review and editing, funding acquisition **Fekadu Yadetie:** methodology, investigation, Writing review and editing. **Anders Goksøyr:** Conceptualization, writing – review and editing, Funding acquisition. **Tanja Kögel:** Conceptualization, Methodology, Validation, Writing - Review and Editing; Funding acquisition. **Jutta Dierkes:** Conceptualization, Methodology, Validation, Formal analysis, Resources, Writing - Review and Editing, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Tanja Koegel reports a relationship with Netherlands Organisation for Health Research and Development that includes: consulting or advisory.

Data availability

No data was used for the research described in the article.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.toxlet.2022.09.002.

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