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Investigation of nanoplastic biological effects on Caco-2 cells

Valentina Prota, Chiara Ritarossi, Barbara Rossi, Isabella De Angelis, Simonetta Palleschi, Cristina Andreoli
Dept Environment and Health, Istituto Superiore di Sanità, Rome, Italy

BACKGROUND

Due to their extraordinary characteristics, plastics are used worldwide in thousands of everyday products. In 2019, global plastics production was estimated at 370 million tons, and huge amounts of plastic waste are released into the environment every year. Micro and nanoplastics (MNPs), mainly derived from plastic waste degradation processes, are solid polymers made of particles with an average size of less than 5 mm and 100 nm, respectively. MNPs are ubiquitous in the environment contaminating water, air and soil, and becoming part of the food chain. This widespread presence of MNPs has raised public concern for their potential hazard to human health

AIM

Since ingestion is thought to represent the main route of human exposure to MNPs, the use of human intestinal cells allows investigating relevant aspects of their interaction with the intestinal compartment.
The present sudy is aimed to investigate the potential toxicity of 100 nm commercial polystyrene latex beads (PLBs), was investigated in the Caco-2 human colon carcinoma cell line at different time and doses of treatment

MATERIALS and METHODS

Polystyrene beads suspension characterization

100 nm polystyrene latex beads (PLBs) were obtained from Sigma Aldrich Company LTd. PLBs were first characterized by Dynamic Light Scattering (DLS) both in aqueous solution and in cell culture medium, at two concentrations, at the beginning and at the end of treatments.

Cell cultures and treatment

Caco-2 human colon carcinoma cell line were cultured in DMEM HG with 10%FCS. Confluent cells were treated with 25, 50 and 100 µg/ml of PLBs for 4h and 24h in cell culture medium. The effect of acute (30 min) treatment with 40mM $KBrO_3$ is also reported as positive control. At the end of treatment, cell viability were measured by Trypan blue.

Comet assay

After treatment cells were suspended in 0.7% Low Melting Agarose and assay was basically performed as described in Andreoli et al. (Mut Res 1997,377:95-104). Measurements of DNA damage, as single (ssb) and double (dsb) DNA strand breaks, were performed using Metafer (MetaSystem) and parameterized as tail moment values, the product of tail length and the fraction of total DNA in the tail.

Oxidative stress

Thiols concentrations were measured in both cell extracts and spent medium as previously described, with minor modifications in Palleschi et al., 2017. One-way ANOVA with Holm- Sidak post hoc correction (SigmaPlot 12, Systat Software Inc, US) was applied to analyze the differences among treatments within the same incubation time (4h or 24h).

RESULTS

Polystyrene beads suspension characterization

PLBs were characterized by Dynamic Light Scattering (DLS) to measure the hydrodynamic diameter (Z-average) and the polydispersity index (PDI) of the samples both in aqueous solution and in culture medium, at 10µg/mL and 100µg/mL, at the beginning and at the end of treatments,. Results showed the presence of particles in nanosize range and a good monodispersion state for all tested conditions (Table 1).

Table 1	Tempo 0		Tempo 4h		Tempo 24h	
	Z-average	PDI	Z-average	PDI	Z-average	PDI
1µg/mL in H_2O batch	117.6 ± 1.091	0.037 ± 0.013	n.d	n.d.	n.d.	n.d.
10µg/mL in DMEM	96.86 ± 1.464	0.328 ± 0.005	92.63 ± 0.9051	0.327 ± 0.005	92.07 ± 0.971	0.326 ± 0.004
100µg/mL in DMEM	135.40 ± 1.823	0.092 ± 0.013	130.20 ± 1.065	0.095 ± 0.019	127.70 ± 0.9617	0.094 ± 0.010

Comet assay

The possible DNA damage induction, as single and double strand breaks (ssbs and dsbs), after treatment with PLBs was investigated by the alkaline version of Comet assay protocol. Preliminary results (Figure 1) showed a slight, not significant, dose related increase of DNA damage after 24h of treatment (R^2= 0.93; regression analysis). No dose related response after 4h of treatment was observed. The DNA damage was quantitated by the Tail Moment values. The Tripan blue test showed cell viability was always above 90%.

Figure 1

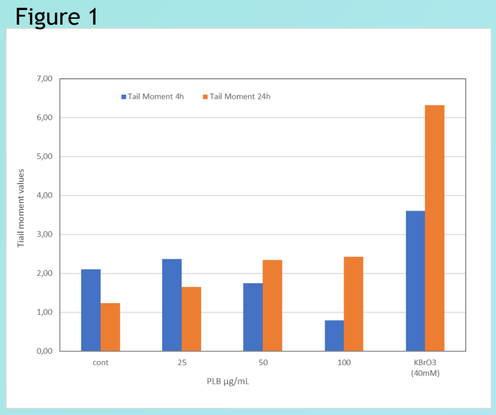

Figure 1 DNA damage on Caco-2 cells after 4 and 24 h of treatment with PLBs, evaluated as single and double strand breaks by the alkaline comet assay protocol. $KBrO_3$ (40mM) is reported as positive control. A dose related response (R^2= 0.93; regression analysis) after 24h of treatment was observed.

Oxidative stress

Caco-2 cells treated for up to 24h with PLBs ≤ 50 µg/ml did not show any significant change in total intracellular glutathione (GSH) (Figure 2, panel A) or cell redox status (Figure 2, panel B). However, the highest PLBs dose tested (100 µg/ml for 24h) induced a modest, though significant decrease of total intracellular glutathione, while the cell redox status remained seemingly unchanged. Moreover, treating cells for 24h with PLBs doses ≥ 25 µg/ml induced a dose-dependent decrease of the extracellular cysteine concentration (Figure 2, panel C). At difference, extracellular concentrations of glutathione, cysteinylglycine and homocysteine did not change to a significant extent at any of the time/dose combinations tested (data not shown).

Figure 2

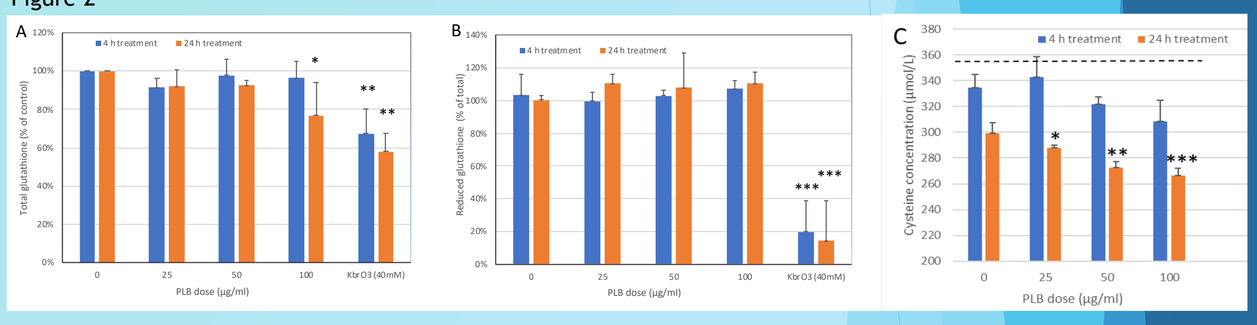

Figure 2 Effect of PLBs on Caco-2 intracellular concentration of total glutathione (A), percentage of reduced vs total intracellular glutathione (cell redox status, B) and extracellular concentration of cysteine (C) after 4 and 24 h of treatment. KBrO3 (40mM) is reported as positive control. Bars represent standard deviation (n =3). *p<0.05, ** p<0.01 and ***p < 0.001 vs control, one-way ANOVA with Holm-Sidak post-hoc analysis. Dashed line in panel C represents the nominal cysteine concentration of fresh cell medium (DMEM). Extracellular cysteine concentration in $KBrO_3$ treated cells was not analyzed due to the oxidant interference with the measurement method.

COMMENTS

The PLBs oxidative stress effects on Caco-2 cells:
- ✓ At the highest dose tested (100 µg/mL), reduction of the intracellular concentration of glutathione in the absence of a concomitant change of the cell redox status,
- ✓ Dose-related decrease of the extracellular concentration of cysteine, the availability of which is a rate-limiting step in glutathione synthesis, in the absence of a significant effect on extracellular cysteinylglycine, a GSH degradation product whose concentration increases as a cell response to oxidative stress.

Taken together, our preliminary results suggest that PLBs reduce cell antioxidant defenses without overtly induce cell oxidative stress.

The PLBs DNA damage activity on Caco-2 cells:
- ✓ No increase of DNA damage after 4h of treatment,
- ✓ Slight dose related increase of DNA ssbs and dbs after 24h of treatment.

These preliminary observations are in line with the knowledge about the DNA damage activity of PBLs at prolonged exposure time.

Further studies are in progress to investigate more in depth PLBs effect on medium cysteine concentration and verify a causal role for medium cysteine depletion in PLBs-induced decrease of cell glutathione. In addition, further experiments to shed light to the nature of DNA damage observed will be carried out.

REFERENCES

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