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Effects of lead and PET nanoplastic particles on metabolism and cell stress markers in murine RAW 264.7 macrophage cells

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Abstract

Nanoplastics (NPs) and heavy metals are two prominent environmental pollutants. They can be found in water sources and in the soil. This means we can actively take up these particles either by ingestion or inhalation. The most common NP found in the environment is polyethylene terephthalate (PET) and a high level and important heavy metal contaminant is lead (Pb). NPs are known to adsorb and desorb many contaminants including lead. PET and Pb separately have been shown to cause changes in cellular stress pathways in several mammalian cell types. However, it is not known whether both PET and Pb together can exacerbate the individual effects of PET and Pb. This research focused on determining if the presence of Pb and PET separately and combined affects cell viability and the expression of pathway markers for cellular stress in murine RAW 264.7 macrophage cells. Cells were exposed to PET treatment (100 µg/mL), Pb treatment (0.5 µM), or PET + Pb combined treatment (100 µg/mL and 0.5 µM, respectively), or a control treatment. A metabolic activity assay using Resazurin dye was performed to determine cell viability in treatments. There was a significant difference in metabolic rate between PET and Pb only treatments. A qRT-PCR was performed to determine expression of cell stress pathway markers including KRAS, COX-2, IL-1β and ERK. KRAS, COX-2 and ERK showed significant increase in expression but not IL-1β for all treatments relative to control. Overall, the data do not support the idea that the presence of PET + Pb combined have a greater effect than PET or Pb only. There is a slight indication in our data that PET + Pb combined show slightly reduced effects in metabolism and marker expression in the RAW 264.7 cells. This is interesting because PET only takes up Pb as a heavy metal and might act to decrease the effects of Pb by sequestering it from the cell.

Introduction

- Plastics can come from primary (manufactured) or secondary (degraded by nature) sources [1]
- Plastic particle sizes can range from the micro (5mm but >1µm) to the nano scale (<1 µm) in size [1]
- The most common plastic in the environment is polyethylene terephthalate (PET) [2,3]
- Nanoplastic (NP) particles may be ingested or inhaled into the body where they can pass into tissues. [4]
- Several cell types can uptake environmental plastic particles including macrophages as one of the first lines of defense in our body. [4]
- NPs, including PET, have been shown to cause activation of common markers for cell stress such as MAPKs pathways, and inflammatory pathways. [2,5]
- PET has low affinity for adsorption overall, but it can still adsorb Pb at high concentrations. [6,7]
- Pb is heavy metal that is toxic at low and high concentrations that can be found in many environments [8]
- Pb has also been shown to activate some of the same cell stress pathways as PET. [9]

Experimental Design and Materials

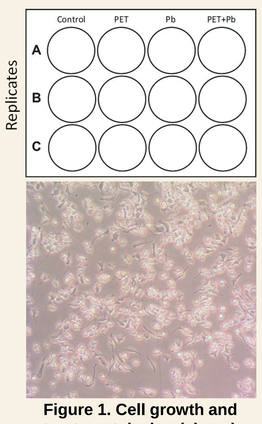


Figure 1. Cell growth and treatment design (above). RAW 264.7 mouse cells in culture (bottom).

- Treatments
  20,000 – 40,000 cells per well
  PET concentration = 100µg/mL
  Pb concentration = 0.5µM
  PET + Pb treatment included both
- RNA Extraction
  Rneasy Mini Kit (QIAGEN)
- RNA to cDNA
  High-Capacity RNA-to-cDNA™ Kit (Applied Biosystems)
- qRT-PCR
  Custom primers (IDT)
  Sso Advanced Universal SYBR Green supermix (BioRad)
- Resazurin
  Resazurin Cell Viability Kit (Abcam)

Results

Amplification Plot for qRT-PCR Assay

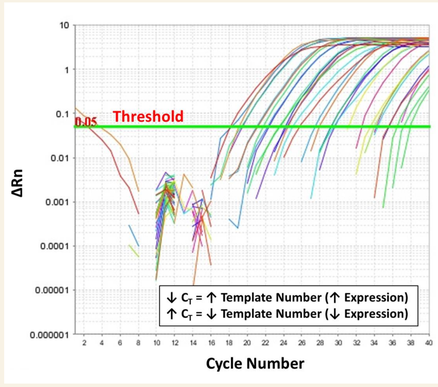


Figure 2. qRT-PCR results for cell stress markers in RAW 264.7 cells exposed to all treatments. Data for $\Delta\Delta C_T$ calculations are from the threshold (green line) cycle numbers.

Average $\Delta\Delta C_T$ for Expression of Four Cell Stress Targets

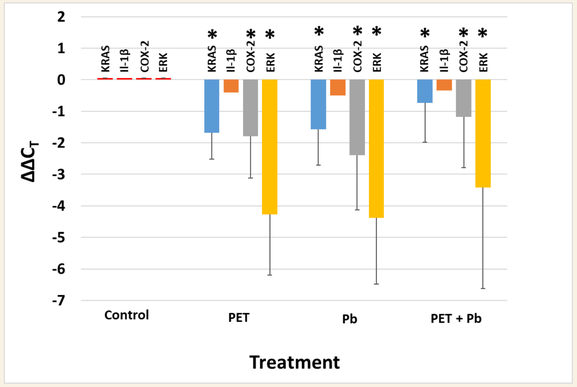


Figure 3. Graph of $\Delta\Delta C_T$ for four cell stress targets (KRAS, Il-1β, COX-2, ERK) in control cells and in treatment cells with PET, Pb, and PET + Pb. The asterisks indicate a marker showing significant activation relative to control. There are no significant differences in levels of activation among treatments for all markers.

Uptake of PET Nanoplastic Particles in RAW 264.7 Cells

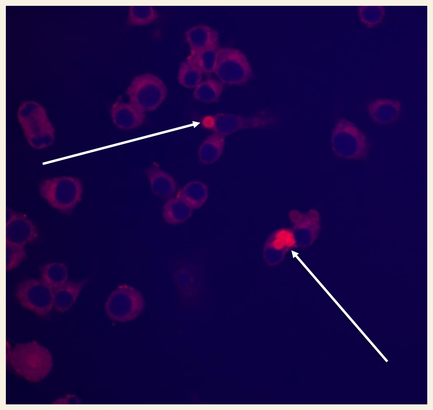
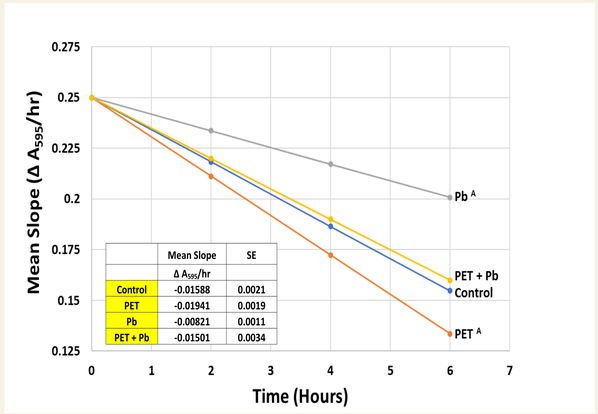


Figure 4. Fluorescent microscope image of RAW 264.7 macrophage cells showing uptake of Nile Red stained PET nanoplastic particles (white arrows). Nuclei are stained with DAPI (blue fluorescence).

Results Summary

- PET nanoparticles can be taken up by RAW 264.7
- Pb treatment showed slower metabolism than any other treatment and was significantly different lower than the PET treatment metabolism
- All the treatments showed activation of three cell stress markers but did not significantly activate Il-1β
- There were no differences in stress marker activation between treatments

Normalized Average Metabolic Curves for RAW 264.7 Cells



	Mean Slope Δ A_{595}/hr	SE
Control	-0.01588	0.0021
PET	-0.01941	0.0019
Pb	-0.00821	0.0011
PET + Pb	-0.01501	0.0034

Figure 5. Graph of normalized metabolic curves for RAW 264.7 cells from the resazurin assay (Abcam). Each line represents the rate of metabolism for a different treatment. There was a significant difference (A) in metabolic rate between the Pb and PET treatments.

Resazurin Assay Showing Color Change over Time

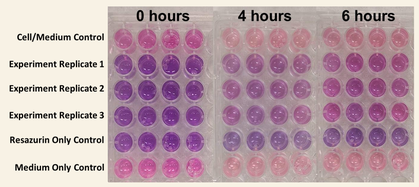


Figure 6. Picture of resazurin assay plate over time at 0, 4, and 6 hours.

Questions and Hypotheses

Questions:

1. Does the presence of Pb and PET nanoplastics affect growth and cellular stress responses in murine RAW 264.7 macrophage cells?
2. Are there synergistic effects of these two particles?

Hypotheses:

1. The presence of Pb and PET will decrease cell viability.
2. PET + Pb combined will decrease cell viability more than PET or Pb alone.
3. Cell stress target gene expression will increase with exposure to PET and Pb.
4. PET + Pb combined will increase target gene expression more than PET or Pb alone.

Conclusions

- PET is showing similar activation of cell stress markers as Pb.
- PET + Pb showed a lower metabolic effect relative to Pb but not PET treatments.
- PET + Pb is not showing the synergistic effect that was hypothesized.
- Future studies should focus on why this apparent decrease occurs and if Pb adsorption/desorption rate has an impact.

References

1. Yee, M. S., Hii, L., Looi, C. K., Lim, W., Wong, S., Kok, Y., Tan, B., Wong, C., & Leong, C. (2021) Impact of microplastics and nanoplastics on human health. *Nanomaterials, 11*(2), 496.
2. Aguilar-Guzmán, J.C., Bejtka, K., Fontana, M., Valsami-Jones, E., Meza Villezcas, A., Vazquez-Duhalt, R., & Rodríguez-Hernández, A. G. (2022). Polyethylene terephthalate nanoparticles effect on RAW 264.7 macrophage cells. Microplastics and Nanoplastics, 2(9).
3. Dong, S., Cai, W., Xia, J., Sheng, L. Wang, W., & Liu, H. (2021). Aggregation kinetics of fragmental PET nanoplastics in aqueous environment: Complex roles of electrolytes, pH and humic acid. Environmental Pollution, 268, 115828.
4. Banerjee, A., Shelver, & W. L. (2021). Micro- and nanoplastic induced cellular toxicity in mammals: A review. Science of the Total Environment, 755, 142518.
5. Hu, Q., Wang, H., He, C., Jin, Y., & Fu, Z. (2020). Polystyrene nanoparticles trigger the activation of p38 MAPK and apoptosis via inducing oxidative stress in zebrafish and macrophage cells. Environmental Pollution, 269, 116075.
6. Goody, V., Blázquez, G., Calero, M., Quesada, L., & Martín-Lara, M.A. (2019). The potential of microplastics as carriers of metals. Environmental Pollution, 255, 113363.
7. Li, W., Zu, B., Yang, Q., Huang, Y., & Li, J. (2022). Adsorption of lead and cadmium by microplastics and their desorption behavior as vectors in the gastrointestinal environment. Journal of Environmental Chemical Engineering, 10, 107379.
8. Metryka, E., Kupnicka, P., Kapczuk, P., Simińska, D., Tarnowski, M., Goschorska, M., Gutowska, I., Chlubek, D., & Baranowska-Bosiacka, I. (2020). Lead (Pb) as a factor initiating and potentiating inflammation in human THP-1 macrophages. International Journal of Molecular Sciences, 21, 2254.
9. Metryka, E., Chibowska, K., Gutowska, I., Falkowska, A., Kupnicka, P., Barczak, K., Chlubek, D., & Baranowska-Bosiacka, I. (2018). Lead (Pb) exposure enhances expression of factors associated with inflammation. International Journal of Molecular Sciences, 19(6), 1813.

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