Nanomedicine2019
Milano, 2-3 May 2019

XX-AA

Pulmonary toxicity of antibacterial metal oxide nanoparticles: effects on *in vitro* lung models

[a]Rossella Bengalli, [a]Alessandra Zerboni, [a]Luisa Fiandra, [a]Paride Mantecca.
[a]*POLARIS Research Centre, Dept. of Earth and Environmental Sciences, University of Milan-Bicocca, Piazza della Scienza 1, 20126, Milano, Italy*
e-mail of submitter: rossella.bengalli@unimib.it

Metal oxide nanoparticles (MeO-NPs), thanks to their effectiveness as antibacterial agents, were recently used as coatings or additives in consumer goods, such as textiles, in order to prevent the insurgence of bacterial infections. Nevertheless, materials coated with NPs could be subject to mechanical stress, such as tear, wear, scratching and washing during their whole life cycle, leading to the unintentional release of NPs in the environment and consequent human exposure. Therefore, this massive production and use of NPs poses a growing risk of humans to new nanomaterials, making pivotal to investigate the effects of NPs on different biological models[1]. Airborne released NPs are particularly concerning, due to their high mobility in the air and the possibility of entering the human body through inhalation, which is the most critical route of exposure among various pathways, including skin contact and ingestion. For these reasons, the toxicity of water-based antibacterial copper oxide (nCuO) and zinc oxide (nZnO) NPs was assessed using different lung *in vitro* models.
nCuO and nZnO, produced by sonochemical process, were characterized by TEM and DLS. The mechanism of toxicity induced by NPs was investigated by using A549 cell line by analysing cell viability (MTT test), colony forming efficiency (CFE) assay and the release of the pro-inflammatory interleukin-8 (IL-8). Furthermore, preliminary experiments on lung barriers cultivated at the Air Liquid Interface (ALI) were also performed. Monocultures of A549 cells and co-cultures of alveolar and endothelial pulmonary cells seeded on Transwell inserts were exposed to the NPs and the lung barrier responses (including viability, damage and inflammation) were evaluated after 24 hours.
Data showed that exposure to nZnO resulted cytotoxic, inducing strong cell death in A549 monocultures starting from the dose of 10 ppm, while exposure to nCuO induced significant biological responses, but at higher doses (20 ppm). IL-8 release was increased by both NPs at the dose of 20 ppm.
CFE assay was performed as a promising standard test to study MeO-NPs toxicity. This test is an example of a robust cell viability method that has been implemented in the genotoxicity testing of chemicals (e.g. in the OECD TG.476)[2] and can give additional information on cell proliferation rate and colony morphology. Data from CFE assay showed that nCuO and nZnO both affected cell proliferation, inducing a reduction of cell colony formation.
Preliminary results from monocultures and co-cultures cultivated at the ALI and exposed to a thin liquid layer of NPs, showed that nZnO affected the functionality of the epithelial respiratory barrier. In particular, in the co-culture model, after nZnO treatment, a reduction of barrier integrity and increased cytotoxicity were observe, while no significant effects were observed with nCuO NPs in these more complex *in vitro* models.
In conclusion, MeO-NPs have great potential in the biomedical field, but their ability to induce also toxic responses may vary depending on the different properties of the NPs and the characteristics of the different *in vitro* model used. These findings on the effects of new NPs contribute to the development of safe(r)-by-design nanomaterials and pose the attention on the potential risk of MeO-NPs during all their life-cycle, from production to use, until their release in the environment.

References

[1] Mantecca P, Kasemets K, Deokar A, Perelshtein I, Gedanken A, Bahk YK, Kianfar B, Wang J. Airborne Nanoparticle Release and Toxicological Risk from Metal-Oxide-Coated Textiles: Toward a Multiscale Safe-by-Design Approach. Environ Sci Technol. 2017 Aug 15;51(16):9305-9317. doi: 10.1021/acs.est.7b02390.
[2] OECD, 2015. Test No. 476: In Vitro Mammalian Cell Gene Mutation Tests Using the HPRT and XPRT Genes. OECD Publishing. http://www.oecd-ilibrary.org/ environment/test-no-476-in-vitro-mammalian-cell-gene-mutation-tests-using-thehprt-and-xprt-genes_9789264243088-en;jsessionid=1h0nxo8t44eay.x-oecd-live-03.

Acknowledgments: This work was supported by EU funded H2020-720851 PROTECT (grant agreement No 720851) project.