Characterization of the toxicity of high-generation cationic PAMAM dendrimers towards a representative organism of aquatic ecosystems

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Nowadays, nanomaterials are widely used in many different fields as biomedicine. Dendrimers are nano-sized, radially symmetric molecules with well-defined, homogeneous, and monodisperse structure that has a typically symmetric core, an inner shell, and an outer shell; due to these characteristics, their use is being tested in the implementation of targeted therapies in biomedicine. Today, hundreds of products containing nanomaterials are used around the world which could end up in aquatic ecosystems [1].

This work has focused on analyzing the effect of poly (amidoamine) (PAMAM) G5-NH_2 and G7-NH_2 in an organism of great ecological importance such as the filamentous cyanobacterium *Anabaena* sp. PCC7120.

Nanoparticles have particular characteristics that depend on various physicalchemical properties such as size and aggregation. Firstly, physicochemical properties of both dendrimers were measured in water and culture medium. Both dendrimers in culture medium showed an elevated level of aggregation (measured with DLS) and displayed a positive charge (measured as z-potential).

Then, the effect of both dendrimers was studied. Both dendrimers significantly decreased the growth of the cyanobacterium. G7-NH_2 (EC_{50} = 0.072 ± 0.005 µM) was more toxic than G5-NH_2 (EC_{50} = 0.096 ± 0.004 µM). There was a clear relationship between dendrimer generation and toxicity, with higher toxicity for higher generation, as previously reported [2].

Physiological parameters were studied by flow cytometry using several fluorochromes to elucidate toxicity mechanisms. Cyanobacteria exposure to dendrimers resulted in significant increase in the formation of intracellular reactive oxygen species (superoxide anion, hydrogen peroxide and hydroxile anion), increase of lipid peroxidation, damage in membrane integrity, membrane potential depolarization, increase of metabolic activity, acidification of intracellular pH and alteration of intracellular free Ca^{2+} homeostasis.

Morphology of the cyanobacterium was studied by optical microscopy and flow cytometry. The results showed alteration in the morphology of both filaments and individual cells within filaments exposed to dendrimers.

Photosynthesis was analysed in the cyanobacterium exposed to dedrimers. Both dendrimers significantly increased photosynthetic O_2 evolution, as previously described in other organisms [3]. Furthermore, the in vivo analysis of pigments showed an increase in the fluorescence of chlorophyll a, phycocianin and carotenoids. Finally, the expression of genes related to oxidative stress response were analysed. Genes encoding superoxide dismutase (*sodA*) and peroxidases (*prxA* and *prxQ-B*) increased expression after exposure to both dendrimers.

Dendrims exhibited high toxicity towards *Anabaena* sp. PCC7120 and several physiological, morphological, photosyntethic and genetic paramenters were affected, so this study provides relevant data to advance in the field of nanomaterials environmental risk assessment.

References

[1] E. Navarro, A. Baun, R. Behra, N.B. Hartmann, J. Filser, A.J. Miao and L. Sigg, Ecotoxicology, 17(5), (2008) 372-386

[2] S. Gonzalo, I. Rodea-Palomares, F. Leganés, E. García-Calvo, R. Rosal and F. Fernández-Piñas, Nanotoxicology, 9(6), (2015)706-718

[3] Petit. A. N. Debenest. T. Eullaffroy. P. y Gagné. F., *Nanotoxicology*. *6*(3), (2012)315-326

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