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Genotoxic and cytotoxic potential of titanium dioxide (TiO_2) nanoparticles on fish cells in vitro

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Abstract Intrinsic genotoxic and cytotoxic potential of titanium dioxide (TiO_2) engineered nanoparticles (ENPs) were evaluated in a metabolically competent, established fish cell line derived from rainbow trout (*Oncorhyncus mykiss*) gonadal tissue (i.e. RTG-2 cells). Prior to evaluation of the toxic potential, mean size of the ENPs was determined using transmission electron microscopy (TEM). As a prerequisite, an extensive characterisation of the ENPs was carried out following sonication which enabled the synthesis of an efficient dosing strategy for the cells in which exposure in phosphate buffered saline (PBS) gave an optimal agglomeration effects compared to distilled water (H_2O) and minimal essential media (MEM). Interaction of the ENPs with cells under scanning electron microscope (SEM) was also studied. The genotoxic and cytotoxic potential of the ENPs were determined either alone or in combination with ultraviolet radiation (i.e. UVA). Whilst genotoxic potential was determined by evaluating DNA strand breaks using single cell gel electrophoresis (SCGE) or the comet assay and induction of cytogenetic damage using cytokinesis-blocked micronucleus (MN) assay, cytotoxicity was determined by measuring the retention of supra vital stain, neutral red, by the lysosomes using the neutral red retention (NRR) assay. In addition, while performing the comet assay, lesion specific bacterial endonuclease, formamidopyrimidine DNA glycosylase (Fpg), which recognises oxidised purine bases, was used to determine oxidative DNA damage. The results suggested that the highest concentration of the ENPs (i.e. 50 μg ml^{-1}) did not produce elevations in DNA damage over 4 h (comet assay), 24 h (modified comet assay) or 48 h (MN assay) exposures in the absence of UVA irradiation, although there was a significant reduction in lysosomal integrity over 24 h exposure (NRR assay). The induction of MN did not show any enhanced levels as a function of ENP concentration. A significantly increased level of strand breaks was observed in combination with UVA (3 kJ m^{-2}). In general, the NRR assay suggested elevated levels of cytotoxicity when the UVA exposure was carried out with MEM compared to PBS, although both showed an increase when in combination with the highest concentration of ENPs (i.e. 50 μg ml^{-1}). Overall, the study emphasises the need for adoption of an holistic approach while evaluating the potential toxic effects of ENPs in which appropriate measures should be taken to avoid agglomeration or aggregation to facilitate efficient cellular uptake to evaluate potential biological responses.

Keywords Nanoparticles · Genotoxicity · Cytotoxicity · Photo-genotoxicity · RTG-2 cells · Comet assay · Micronucleus assay · Neutral red retention assay

Introduction

The large scale predicted manufacture and use of engineered nanoparticles (ENPs or NPs; materials that have at least one dimension in a range between 1 and 100 nm) have stimulated research activities to determine their physico-chemical properties, interaction with biological systems and resultant biological responses (Wiesner et al.

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