Genotoxicity assessment of TiO_2 nanoparticles in SH-SY5Y cells: suitability of the cytokinesis-block micronucleus test

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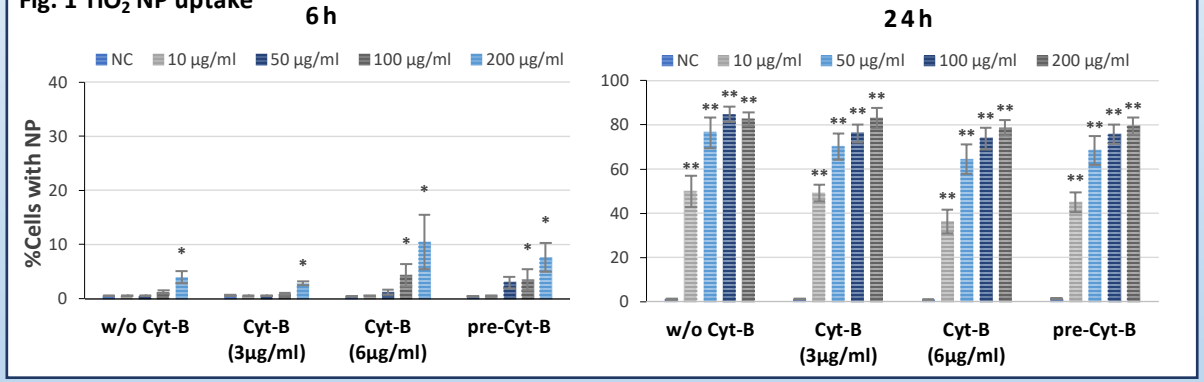
INTRODUCTION

Standard toxicity tests might not be fully adequate for evaluating nanomaterials since their unique features are also responsible for unexpected interactions. The *in vitro* cytokinesis-block micronucleus (CBMN) test (Test Guideline 487, OECD, 2014) is recommended for genotoxicity testing of pharmaceuticals intended for human use, but cytochalasin-B (Cyt-B) may interfere with nanoparticles (NP), leading to inaccurate results.

OBJECTIVE: To determine whether Cyt-B could interfere with micronuclei (MN) induction by TiO_2 NP in human SH-SY5Y cells, as assessed by CBMN test.

Fig. 1 TiO_2 NP uptake



METHODS

- **SH-SY5Y cells** (human neuroblastoma) were treated for 6 and 24 h with TiO_2 NP (10-200 µg/ml).
- **Cellular uptake** of TiO_2 NP was determined by flow cytometry (Suzuki *et al.* 2007) with some experimental adaptations: absence of Cyt-B, co-treatment with 3 or 6 µg/ml Cyt-B, and pre-incubation with 6 µg/ml Cyt-B for 1 h before adding the TiO_2 NP. Cell culture medium was used as negative control (NC)
- **Genotoxicity** was evaluated using the CBMN test. Two treatment options were compared to the standard co-treatment (simultaneous addition of NPs for 6/24 h and 6 µg/ml Cyt-B for 24 h): (1) delayed co-treatment (application of NP for 6/24 h, addition of 6 µg/ml Cyt-B 3/6 h later, and further incubation for 24 h), and (2) post-treatment (application of NP for 6/24 h, wash out and addition of 6 µg/ml Cyt-B for 24 h). Influence of Cyt-B MN induction as evaluated by flow cytometry (FCMN) in the presence or absence of Cyt-B was also assessed.
- For genotoxicity analysis, 1% DMSO in medium as negative control (NC) and mitomycin-C (10 or 1.5 µM for 6 or 24 h, respectively) as positive control (PC), were used.
- Three independent experiments were performed for each experimental condition. Data were expressed as mean ± standard error. *P≤0.05, **P≤0.01 comparison to the control; #P≤0.05, comparison to 100 µg/ml co-treatment; ††P≤0.01, comparison to 100 µg/ml post-treatment.

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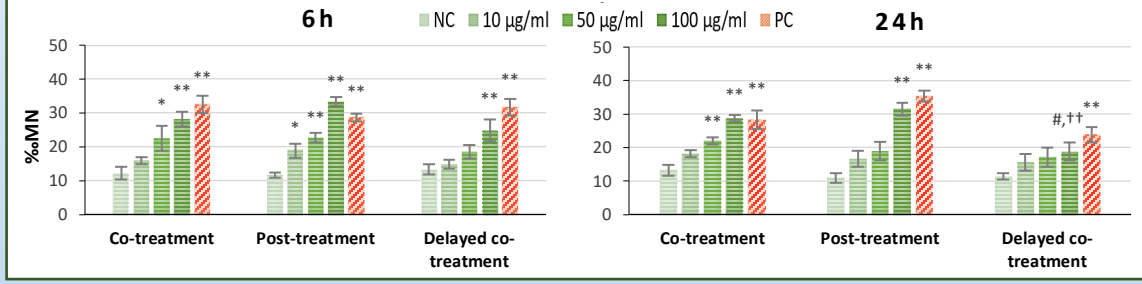
RESULTS

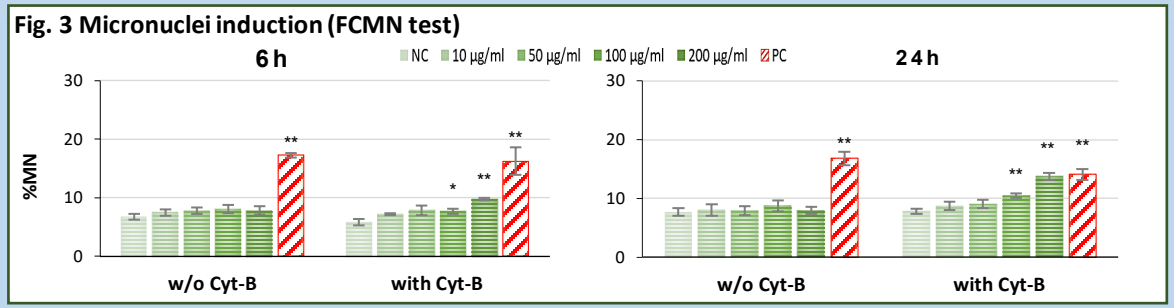
- TiO_2 NP were significantly internalized by cells, both in the absence and presence of Cyt-B, indicating that this chemical does not interfere with NP uptake. (Fig. 1).
- CBMN test showed dose-dependent increases in MN frequencies after 6 or 24 h treatments in the three experimental options. No differences between experimental options were obtained in most of conditions tested (Fig. 2).

Fig. 2 Micronuclei induction (CBMN test)



- FCMN evaluation showed progressive increases in MN frequencies after 6 or 24 h. FCMN assay only showed a positive response when Cyt-B was added simultaneously with TiO_2 NP, suggesting that Cyt-B might alter CBMN assay results. (Fig. 3).

Fig. 3 Micronuclei induction (FCMN test)



CONCLUSIONS

Post-treatment and delayed co-treatment of Cyt-B, proposed by OECD for CBMN test when applied to nanomaterials, seem not to be adequate alternatives to avoid Cyt-B interference under the specific conditions employed in this study. Consequently, further investigations are necessary to define additional protocol alternatives of CBMN assay for accurately assessing genotoxicity of nanomaterials.

References

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