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Titre 25 mots 25

DNA damage after *in vitro* exposure to very low concentration of combusted cerium nanoparticles (CoCeO₂NPs) and to CoCeO₂NPs + Benzo(a)pyrene in rat and human gametes.

Study question 25 mots 23

Do *in vitro* gametes exposures to very low concentration of CoCeO₂NPs and to CoCeO₂NPs + Benzo(a)pyrene induce significant and similar DNA damage ?

Summary answer 25 mots 22

In vitro gametes co-exposure to CoCeO₂NPs + Benzo(a)pyrene induced statistically higher DNA damage than exposure to CoCeO₂NPs alone in rat and human.

What is known already 100 mots 100

Cerium dioxide nanoparticles (CeO₂NPs) are widely used as diesel additive and are released in the air after engine combustion. The Organization for Economic Cooperation and Development included CeO₂NPs in the priority list of nanomaterials requiring urgent evaluation. *In vitro* exposure of human and mouse sperm and cumulus oocyte complexes (COC) to very low concentrations of pristine CeO₂NPs induce significant DNA damage. Benzo(a)pyrene (BaP) is also released in the air by diesel combustion and is known to induce DNA damage in sperm and COC. Nevertheless, pristine CeO₂NPs are modified by combustion; the impact of CoCeO₂NPs exposure and to CoCeO₂NPs+BaP remains unexplored.

Study design, size, duration 75mots 66

Pristine CeO₂NPs were extracted from EnviroxTM diesel additive and combusted at 850°C, (average combustion temperature in a diesel engine), to obtain CoCeO₂NPs. Crystalline structure of CoCeO₂NPs was analysed by X-ray Diffraction. Rat gametes were sampled in epididymis and oviducts after euthanasia of five mature males and four 4 weeks old females (after ovarian stimulation). Human frozen sperm from fertile donors were purchased from Germetheque biobank (France).

Participants/materials, setting, methods 75 mots 69

Human and rat gametes were exposed *in vitro* to very low concentrations of CoCeO₂NPs [1 to 1.10³ µg.l⁻¹] and human sperm to 1 µg.l⁻¹ CoCeO₂NPs + 25µMol BaP during 2 hours in Ferticult© Medium + 1% DMSO at 37°C, 5% CO₂. DNA damage was analysed by alkaline comet assay (ACA) and quantified by Olive Tail Moment (OTM) in oocytes and follicle cells and by % Tail DNA in sperm.

Main results and the role of chance 200 mots 199

In rat oocytes and follicle cells, exposure to 1 µg.l⁻¹ CoCeO₂NPs induced significantly higher DNA damage (mean±SEM OTM = 10.26±0.36 and 4.91±0.29, respectively) compared with unexposed controls (2.21±0.18 and 0.72±0.05, respectively) and with higher CoCeO₂NPs concentrations (p < 0.0001).

In rat sperm, exposure to 1 µg.l⁻¹ CoCeO₂NPs induced significantly higher DNA damage (mean %Tail DNA±SEM = 21.23 ± 0.37) compared with unexposed control (11.06±0.24) and with higher CoCeO₂NPs concentrations (p <0.0001).

In human sperm, exposure to 1 µg.l⁻¹ CoCeO₂NPs also induced significantly higher DNA damage (mean % Tail DNA±SEM = 32.06 ± 0.54) compared with unexposed control (11.96 ± 0.30) (p < 0.0001); co-exposure to 1 µg.l⁻¹ CoCeO₂NPs + 25µM BaP induced significantly higher DNA damage (mean % Tail DNA±SEM = 33.63 ± 0.67) compared with 25µM BaP alone (28.57 ± 0.54) and 1 µg.l⁻¹ CoCeO₂NPs alone (24.62± 0.52) (p < 0.0001).

As in our previous results, obtained in human sperm after *in vitro* exposure to various concentrations of pristine CeO₂NPs [10 to 1.10⁴ µg.l⁻¹], DNA damage was inversely proportional to the CoCeO₂NPs concentrations; we might hypothesize that our results are related to the lower aggregation states at low concentration, inducing a higher surface contact between cells and CoCeO₂NPs.

Limitations, reasons for caution 50 mots:

These results cannot be extrapolated to *in vivo* toxicity of CoCeO₂NPs after inhalation, but demonstrate that interactions between CoCeO₂NPs and germ cells induce significant DNA damage. Additional data should be needed to assess DNA damage in rat COC after *in vitro* exposure to very low CoCeO₂NPs concentrations and BaP.

Wider implications of the findings 50 mots 47

The co-exposure to very low concentrations of CoCeO₂NPs and BaP showed for the first time a cumulative genotoxic impact on human sperm. Potential impacts of diesel exhaust exposure in couples and pregnant women are a major concern for public health, highlighting the need for *in vivo* studies.

Trial registration number: Animal experiment agreement N° 15447-2018061110211950.