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Pascale Debersac, Michèle Bisson, A. Malo, F. Petitot, Jean-Marc Porcher, Claude Lambre

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Research on an Effect of a Short Term Exposure to Low Concentrations of Ozone on V79 Cells Using the Comet Assay

DEBERSAC P., BISSON M., MALO A., PETITOT F.*, PORCHER J.M., LAMBRÉ C.

INERIS, BP 2, F-60550 VERNEUIL-EN-HALATTE, FRANCE.

* CEA/DSV, BP 6, F- 92 FONTENAY AUX ROSES, FRANCE.

Introduction

During episode of atmospheric pollution in industrialized areas, the ozone concentration can reach up to 0.35 ppm for a few hours. These concentrations are beyond the protection thresholds for human health and vegetation recommended by EU directive 92/72 and based on the guidelines of the World Health Organisation. Ozone is not by itself a radicalar species, but most of its toxic effects are thought to result from radicalar reactions mediated by .OH or by nucleases activation. So far, most of the genotoxic effects reported have been observed *in vitro* at concentrations in the range of 1 to 10 ppm during 1 hour (Victorin, 1992), that is higher than the actual individual exposure.

Recent studies performed *in vitro* or *in vivo* at lower concentrations, namely between 0.2 and 1 ppm, have shown that the genotoxic effect of ozone is questionable (Kozumbo *et al.*, 1996, Lee *et al.*, 1996, Gabrielson *et al.*, 1994). Therefore, in an attempt to mimic more closely the environmental conditions, we have studied the consequences on cultured cells of an exposure to low concentrations of ozone for a short time. For this purpose, we used the V79 cell line for which extensive data are available regarding the genotoxicity of numerous compounds. Genotoxicity assessment using this cell line has been frequently done with the micronucleus and the comet assays. Due to the oxydative stress induced by ozone we decided, in a first instance, to use the comet assay which is considered as more relevant to assess efficiently this kind of injury.

Methods

The cell line used is V79 cultured in D-MEM medium supplemented with 20 % of foetal calf serum.

Treatments were performed at 37°C during 1 hour on cells at confluency. H₂O₂ was used as a positive control at concentrations of 75, 150 and 300 µM. Ozone was produced by photolysis of an oxygen flow (Sonimix 6023, LNI).

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Ozone concentrations were measured with an ozone analyser (O₃41M Environnement SA). The chambers of exposure were self-contained and regulated for CO₂, temperature and humidity. For ozone treatment, V79 cells were cultivated on petri dishes set on oscillating plates, in order to facilitate direct contact between ozone and cells.

The concentration of ozone were: 0.1 ppm, which corresponds to usual urban exposures and 0.3 ppm which is representative of an acute environmental exposure. A higher dose (0.9 ppm), irrelevant to reported exposures, has also been included in the study. Ozone was also used in association with HNO₃ (8000 µM) in order to mimic some aspects of NO₂ exposure.

Cytotoxicity was assessed with MTT or neutral red assay and cell viability was verified before each comet assay by the trypan blue dye exclusion test.

For the comet assay, the cells were embedded in low melting point agarose at 1 % and after cell lysis at pH 10 and DNA denaturation at pH 8, an electrophoresis was performed (Fairbairn et al, 1995). DNA was stained by propidium iodide before microscopic analysis. Tail moments were measured by image analysis. Image analysis system correspond to a CCD monochromatic camera adapted on a DMLB 100 microscope (Leica) and driven by FLUOVIEW MORPHOSTAR software (IMSTAR). The value of the tail moment was defined by Olive *et al.*, 1990 as a product of the percentage of DNA in the tail distribution and the displacement between the head and tail means.

Results and discussion

No toxic effect was detected for H₂O₂ after a 1 hour exposure at 75, 150 or 300 µM. A linear dose relationship was observed in the comet assay for the positive control for the doses up to 300 µM (Figure 1). These results are in agreement with those previously reported on V79 cells or on human fibroblasts by Olive *et al.*, 1994 and Singh *et al.*, 1991 respectively.

For ozone exposures at 0.1, 0.3 and 0.9 ppm no toxic effect was seen with both MTT and neutral red assays. Results obtained from the comet assay showed that ozone did not induce any DNA strand breaks for concentrations close to environmental exposures (0.1 ppm). For higher doses (0.3 and 0.9 ppm) no significant effects were shown under our experimental conditions by comparison with unexposed cells (Figure 2). Similar results were observed by alkaline elution on BEAS-2B cells after an exposure to ozone between 0.2 and 1 ppm during 2 to 4 hours (Gabrielson *et al.*, 1994).

Others studies on BEAS-2B cells using the comet assay, have shown that there is a significant increase of effects for 40 or 60 minutes exposure at 0.4 ppm or 1 hour at 0.1 ppm (Lee *et al.*, 1996). These results seem to confirm the hypothesis of differences in cell sensitiveness.

Adjunction of HNO₃, without modification of the pH of the medium during ozone exposure, did not change the result of cytotoxic evaluation. By comet assay, increasing concentration of HNO₃ up to 8 mM did not induce any genotoxic effects (Figure 3).

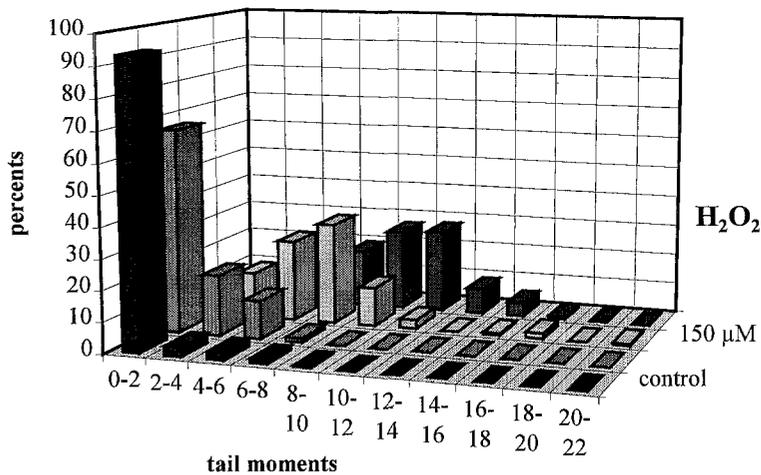


Figure 1 : Tail moments measured by the comet assay after a 1 hour exposure to H₂O₂.

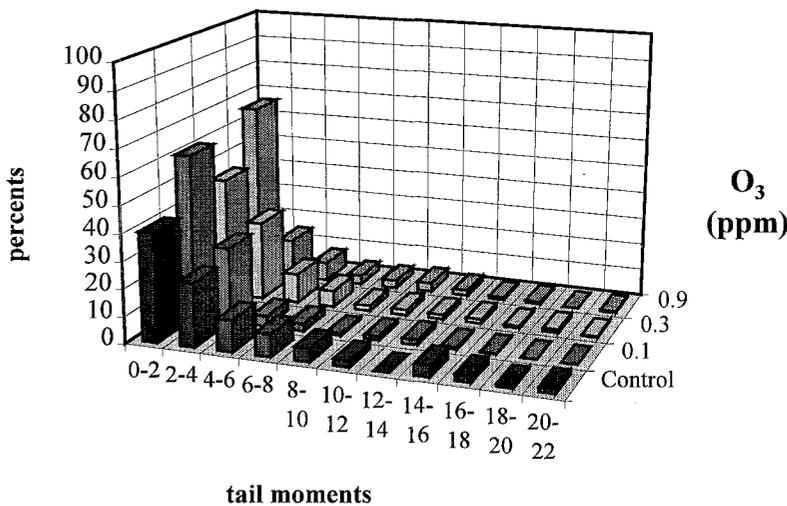


Figure 2 : Tail moments measured by the comet assay after a 1 hour exposure to ozone.

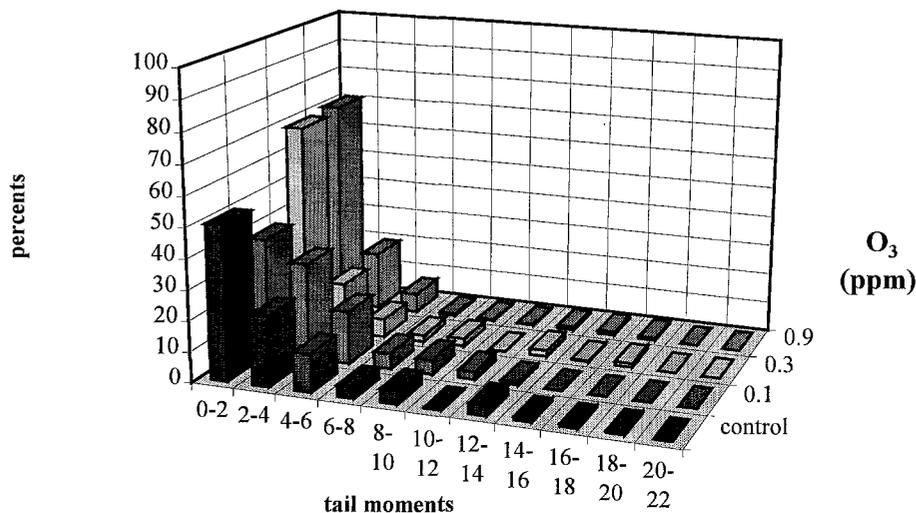


Figure 3 : Tail moments measured by the comet assay after a 1 hour exposure to both ozone and nitric acid.

Conclusions and perspectives

Comet assay appear to be fairly sensitive in the detection of genotoxic alterations induced on V79 cells by the oxydative stress produced by a 1 hour exposure to H₂O₂. Under the same experimental conditions, ozone alone or in association with HNO₃ did not induce any effects. As in the case of oxygen peroxide, ozone is known to induce DNA strand breaks through the radicalar reactions that are mediated by oxygen species. In this study we worked at low levels of ozone concentrations and short time of exposure (1 hour). One can assume that a prolonged exposure to ozone would have induced more DNA damages and in addition would be more relevant in order to mimic an environmental exposure. In the same way we have chosen not to modify the pH of the medium during HNO₃ exposure but these conditions are not representative of the reality of this kind of exposure since in the lung, a pH value may be around 6.8. The V79 is a well known cell line but it is not the most representative of the lung which is build up of a large variety of different cell types. Other cell lines such as BEAS-2B or epithelial cells in primary culture should be more representative. All these questions are currently under investigations.

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