A novel in vitro micronucleus (MN) test procedure after exposure of human derived lung cells to volatile chemicals at the air/liquid interface in vitro

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INTRODUCTION

A Vitrocell® *in vitro* exposure system (Fig. 5) offers the possibility of culturing human derived cells on plastic membranes permeable to culture medium, and of exposing them directly at the air/liquid interface. This method reflects inhalational exposure occurring in the in vivo environment. To develop a new bioassay for this exposure system, we optimized the conditions for the cytochalasin-block in vitro micronucleus (CB-MNvit) test procedure using commercially available PTFE cell culture inserts that facilitated preparation of fine specimens without undergoing complicating procedure, e.g. harvestings of cells. As a model we investigated MN induction of the volatile chemical formaldehyde (FA) in human alveolar epithelial cells (A549).

RESULTS & CONCLUSION

The optimal cell density ranged from 6 to 8 x 10^4 cells/insert seeded 24 h before experiments. The best condition for hypotonic treatment was incubation in 1.5 % tri sodium citrate at 37°C for 6 min, and for fixation of cells a fixation solution containing EtOH:acetic acid:FA 60:20:1 (v:v:v) at 4°C for 5 min. After drying, the plastic membrane was cut off from the PTFE insert and was fixed on a glass slide and stained with DAPI. Cells were analysed microscopically for the presence of MNBNC (Fig. 2.). Under these optimized conditions, experiments were conducted for the known genotoxin FA. This compound induced a significant concentration-dependent increase in the number of micronucleated cells in A549 after 1-h exposure (Fig. 3.), with maximal MN frequencies of 61.6 ± 2.5 MN/1000 BNC (at 250 mg/m³). On the other hand, the frequency of micronucleated cells exposed to clean air was stable at a low level (4.6 ± 2.1 MN/1000 BNC). Finally, the WST-1 assay showed, that FA reduced cell viability starting at a concentration of 6.25 mg/m³ (Fig. 1.).

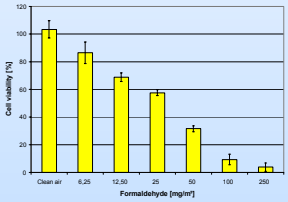

Fig. 1. Cell viability in A549 cells upon 1-h treatment with different concentrations of formaldehyde. Data represent mean ± SD from three culture inserts per concentration point. *p<0.05, **p<0.01 (student's t-test).

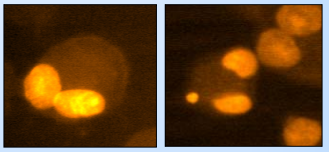

Fig. 2. Photomicrographs (400x) of a binucleated cell (BNC, left) and of a micronucleated binucleated cell (MNBNC, right) directly fixed on the PTFE membrane of the culture insert and stained by DAPI.

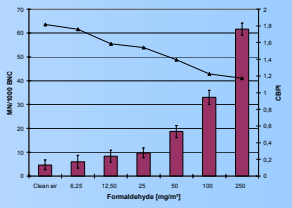

Fig. 3. Induction of MN, assessed by the CB-MNvit test, in A549 cells with 1-h exposure to different concentrations of FA. Positive control: Ethyl methansulfonate [43 mmol/L] Cytochalasin-block proliferation index (CBPI) is presented by the curve. Data are mean ± SD of three culture inserts per concentration; *p<0.5; **p<0.01 (student's t-test).

This novel MNvit procedure, in which the MN test was performed directly on PTFE inserts of a Vitrocell® exposure system, is a rapid, simple and sensitive technique to detect MN inducing activity of volatile chemicals in lung cells *in vitro* as shown for FA.

MATERIAL & METHODS

A549 human lung cells (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, DSMZ, Braunschweig, Germany) grown to confluency were seeded on polytetraflouroethylene (PTFE) cell culture inserts. Thereafter, cells were exposed for 1 h to different concentrations of FA (6.25 mg/m³ to 250 mg/m³) in a Vitrocell® exposure system at a constant flow rate of 5 mL/min. FA concentrations were determined by the DNPH-method using HPLC. After exposure, cells were evaluated for cell viability (WST-1 assay) and for the induction of micronuclei by the CB-MNvit assay. The issues concerning the new MN test procedure are 1) the required cell density on the PTFE membrane to obtain an adequate number of cells for observation of MN and 2) hypotonic and fixation treatment for the optimal cytoplasmic:nuclear area ratio.

Fig: 4. The FA atmosphere was generated in a generation chamber using a static technique. Liquid FA was introduced in the chamber and vaporized. The gaseous FA was diluted with clean air to obtain concentrations of FA of 6.25, 12.5, 25, 50, 100 and 250 mg/m³.

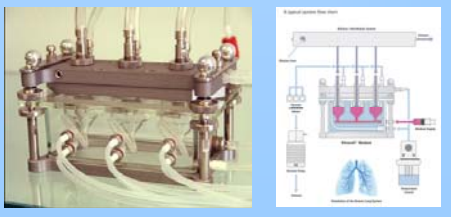

Fig. 5. The exposure system (Vitrocell®) offers the possibility to expose cells directly to a continous air flow. Cells were maintained in air/liquid interface.

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