Frequency of micronuclei in human bronchial epithelial cells induced by major diesel exhaust components and organic extracts from diesel emissions

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ABSTRACT

Despite substantial technological advance resulting in decreased fuel consumption and lower emissions, road traffic remains a significant source of air pollution, particularly in metropolitan areas. From a complex mixture of chemicals and particles produced by incomplete combustion of organic material, polycyclic aromatic hydrocarbons (PAHs) are the most important as they may bind to nucleic acids and proteins, cause their damage and/or loss of function and induce mutations. Nitro-derivatives of PAHs, characteristic for diesel exhaust, are persistent in the environment and highly mutagenic and carcinogenic in model systems. Recently, alternative fuels based on blend of standard diesel with biodiesel compound became popular.

However, little is known about genotoxicity of emissions of the alternative fuels. In our study, we compared DNA damage, measured as the frequency of micronuclei (MN) in human bronchial epithelial cells (BEAS-2B), induced by major diesel exhaust components [benzo[a]pyrene (B[a]P), 1-nitropyrene (1-NP), 3-nitrobenzanthrone (3-NBA)] and organic extracts from extractable organic matter (EOM) obtained from emissions from various blends of biodiesel with diesel fuels. The cells were treated for 28 h and 48 h with three non-cytotoxic concentrations of individual compounds and extracts. The frequency of MN was assessed in cytochalasin B-blocked cells using manual scoring. The samples were analyzed in triplicates; 1500 cells/sample were scored.

All the tested compounds and diesel emission extracts increased MN frequency above the control level (2% MN) in both time intervals; the MN frequency was generally higher after the longer treatment period. Among the individual compounds, 3-NBA was the most potent (the MN frequency reached almost 8%), followed by 1-NP and B[a]P. The extracts from emissions from diesel and biodiesel fuel blends did not differ in their ability to increase the MN frequency; for all these samples, the frequency of MN reached up to 4% after the 28 h treatment. After the 48 h treatment period, higher doses of individual compounds, as well as diesel fuel emission extracts tended to decrease the MN frequency probably reflecting negative impacts of tested compounds on cell viability.

In summary, our data demonstrate greater genotoxic potency of nitro-PAHs when compared with the parent compound. Moreover, genotoxicity of extracts from diesel and biodiesel emissions seems to be comparable regardless the content of alternative fuel.

AIMS

- **To optimize the conditions to analyze DNA damage by the micronucleus test in human bronchial epithelial cells (BEAS-2B) for broad genotoxicity testing.**
- **To analyze the genotoxicity of the engine emissions of the alternative fuels (biodiesel B30 and B100) in comparison to diesel fuel (B0).**
- **To evaluate the effect of exposure of the major diesel exhaust components [benzo[a]pyrene (B[a]P), 1-nitropyrene (1-NP), 3-nitrobenzanthrone (3-NBA)] on genetic damage in BEAS-2B cells.**

CONCLUSIONS

- **All aspects of the method: (i) cultivation medium optimal for this cell line, (ii) chamber system used allowing to save consumables, (iii) timing of experiment was optimize for broad genotoxicity testing (see Figures 1-5).**
- **The genotoxicity of the engine emissions from the diesel and alternative biodiesel are comparable and increase with the time of exposure during the cultivation. Contrary to these observations, CBPI moderately decrease with dose for longer cultivation time (see blue Graphs).**
- **Both nitro-PAHs compounds (1-NP and 3-NBA) demonstrated in case of tested conditions higher genotoxic potential in comparison with B[a]P (see black Graphs).**

METHODS

Cell line and cultivation: Non-tumorigenic human bronchial epithelial cells (BEAS-2B), 48 passage, were used for genotoxicity testing. BEBM™ and BEGM™ media (Lonza) were used as a base for coating of cultivation surface and cultivation medium preparation. Cultivation system Nunc® Lab-Tek® Chamber Slide™ for 8 independent experiments was used.

Tested chemicals: Extractable organic matter (EOM) from diesel exhaust particles obtained from emissions of three types of fuel (B0 - 100 % diesel fuel, B30 - a blend of diesel fuel and 30 % biodiesel, B100 - 100 % biodiesel) and the major diesel components (benzo[a]pyrene (B[a]P), 1-nitropyrene (1-NP), 3-nitrobenzanthrone (3-NBA)) were tested for their genotoxic potential. Tested doses were 1, 10 and 25 µg/ml for all fuels and 25, 100 and 200 µM for B[a]P and 1, 5, 10 µM for 1-NP and 3-NBA. Two exposure times, 28 and 48 h, were compared.

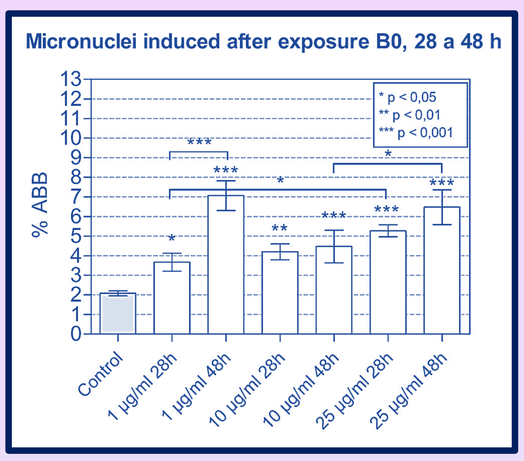
Collection of EOMs from fuel emissions: HiVol 3000 sampler equipped by Pallflex TX40HI filters was used for collection of particulate matter produced by Iveco Tector engine in laboratory operation. EOMs were extracted by dichloromethane and dissolved in dimethyl sulfoxide.

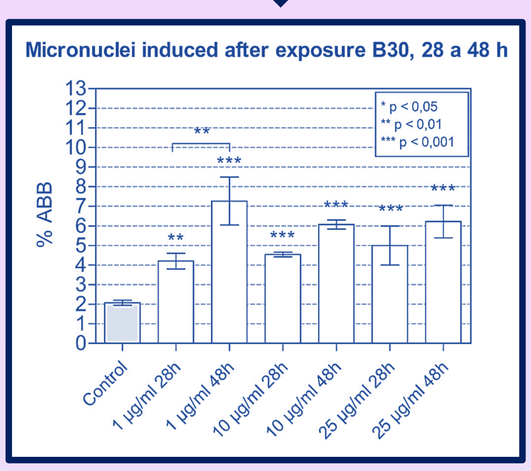
Binucleated cells induction: Cells were treated with 0.1 µg/ml of cytochalasin-B for 28 h before the end of cultivation to induce binucleated cells (BNC).

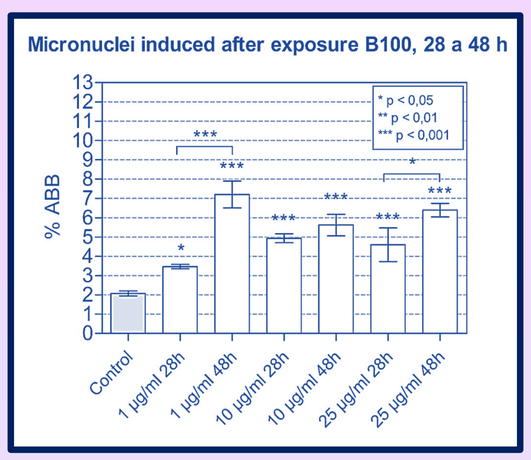
Fixation and staining: After the ending of cultivation, the cells were treated with hypotonic solution and fixed with a mixture of methanol: acetic acid (3:1). Slides were stained by 5% Giemsa solution.

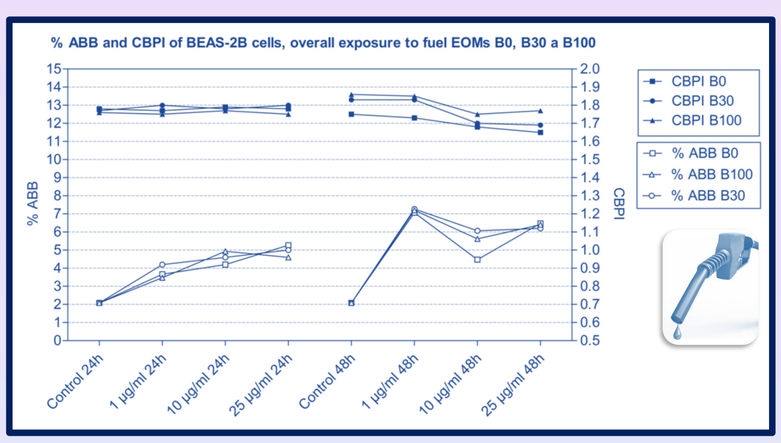
Microscopic analysis: Total 3x 500 BNC cells per each tested compound were evaluated with optical microscope. Cytokinesis-block proliferation index (CBPI) was calculated for controlling the cell division. The results were expressed as a percentage of aberrant binucleated cells with micronuclei (% ABB).

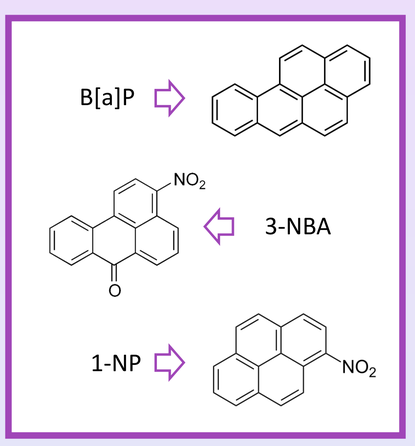
RESULTS

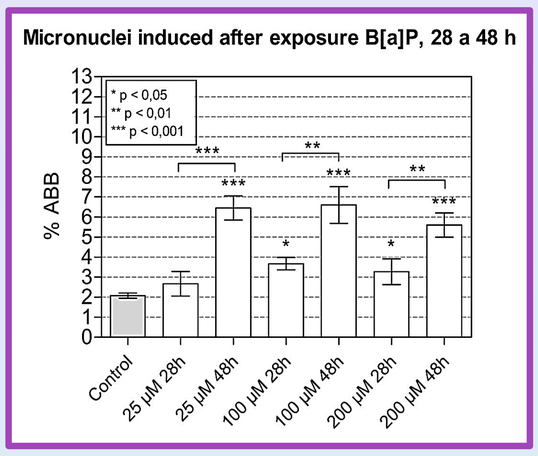


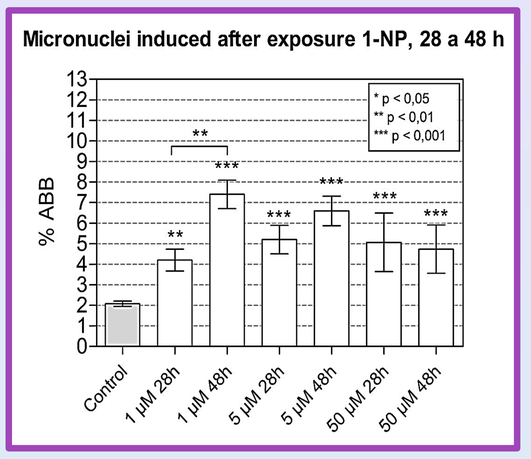


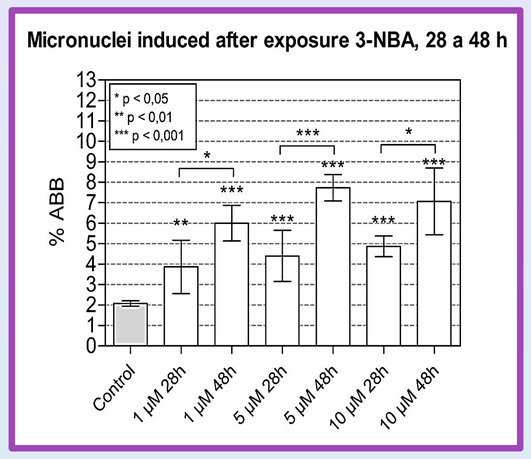












FIGURES

Figure 1:
Cultivation system Nunc® Lab-Tek® Chamber Slide™, 8 wells

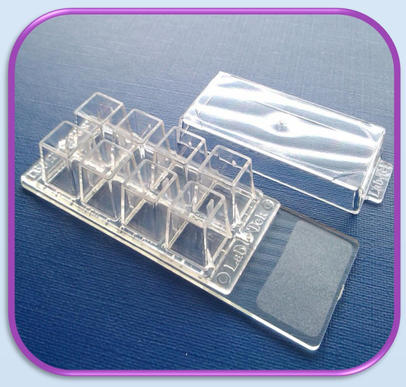


Figure 2:
BEAS-2B cells growing in the chambers

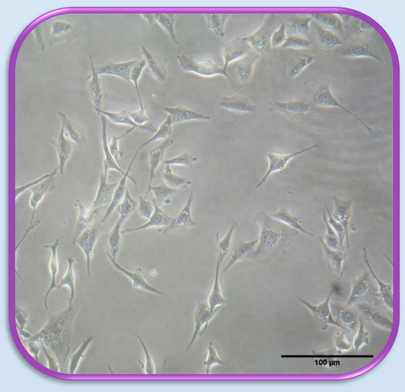


Figure 3:
Giemsa-stained binucleated and mononucleated BEAS-2B cells

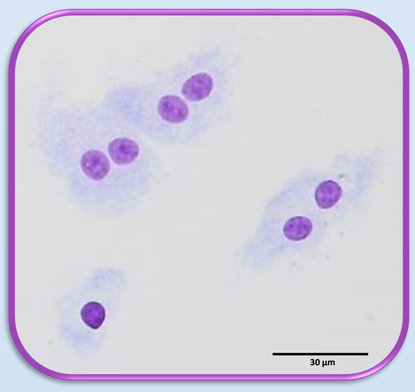


Figure 4:
Giemsa-stained binucleated BEAS-2B cell with micronuclei

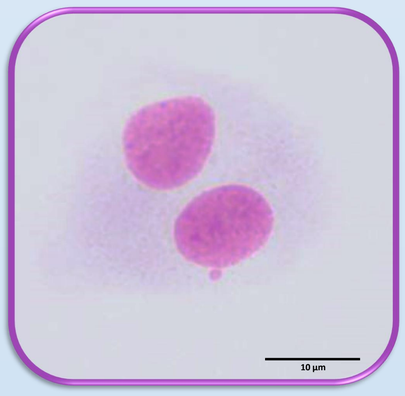
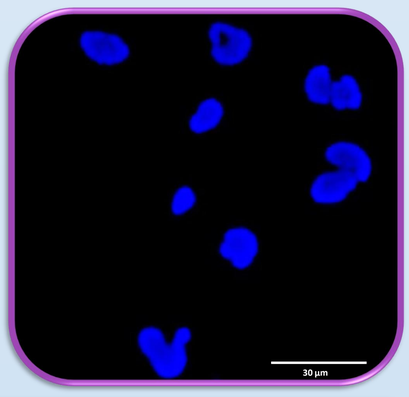


Figure 5:
DAPI-stained nuclei of BEAS-2B cells growing in appropriate medium



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