COMPARISON OF GENOTOXIC EFFECTS OF MAJOR DIESEL EXHAUST COMPONENTS IN HUMAN ALVEOLAR BASAL EPITHELIAL CELLS (A549)

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BACKGROUND

Internal combustion engines (ICE), including diesel engines, power most of the motorized road vehicles. ICE are a major source of air pollution in metropolitan areas. In the Czech Republic, they account for nearly half of the particulate mass (PM10). The particles emitted by ICE are very small and when inhaled, they readily deposit in human lungs, penetrate through cell membranes into the blood, and havea large and widespread detrimental effect on human health. Polycyclic aromatic hydrocarbons (PAHs) and their nitro-derivatives represent compounds responsible for most of genotoxic and other negative biological effects of ICE-emitted particles on living organisms.

To compare biological effects of major components of ICE-emitted particles, we analyzed damage to macromolecules induced by benzo[a]pyrene (B[a]P), a model PAH, and PAH nitro-derivatives [1-nitropyrene (1-NP) and 3-nitrobenzanthrone (3-NBA)] in a model human alveolar basal epithelial cell line A549. The assessed endpoints included oxidative stress markers (lipid peroxidation, protein and DNA oxidation) and bulky DNA adducts.

AIMS OF THE STUDY

1. To assess oxidative damage to DNA, lipids and proteins induced in A549 cells after treatment with benzo[a]pyrene (B[a]P), 1-nitropyrene (1-NP) and 3-nitrobenzanthrone (3-NBA), major components of diesel exhaust.
2. To analyze bulky DNA adducts formation in A549 cells treated with B[a]P, 1-NP and 3-NBA.
3. To compare levels of respective biomarkers after different treatment periods (4 and 24 hours).
4. To determine differences in biological effects of a model PAH (B[a]P) and nitro-compounds (1-NP, 3-NBA).

METHODS

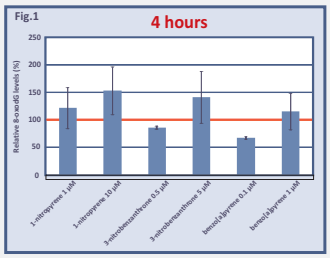
1. A549 cells were treated for 4 and 24 hours with different non-toxic concentrations of tested compounds (B[a]P: 0.1 and 1.0 μM; 1-NP: 1.0 and 10 μM; 3-NBA: 0.5 and 5.0 μM).
2. 8-Oxodeoxyguanosine (8-oxodG), a marker of DNA oxidation, was analyzed by HPLC-MS/MS; ELISA was used to analyze 15-F_{2t}isoprostane (15-F_{2t}-IsoP), a marker of lipid peroxidation, and protein carbonyls, a marker of protein oxidation.
3. Bulky DNA adduct levels were assessed using ^{32}P-postlabeling with nuclease P1 enrichment.

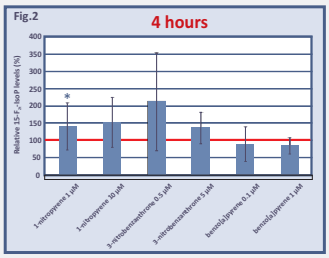
RESULTS

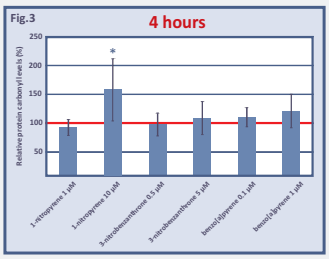
1. We observed no effect of B[a]P, 1-NP and 3-NBA on oxidative DNA damage regardless treatment period (Fig. 1).
2. Nitro-compounds increased lipid peroxidation; the effect was most pronounced after the 24h treatment with 3-NBA (Fig. 2).
3. Protein oxidation was induced by 1-NP after both 4h and 24h treatment periods (Fig.3).
4. 3-NBA induced highest levels of bulky DNA adducts. This compound was genotoxic even after the 4h treatment. The effect of B[a]P was evident only after the 24h treatment. 1-NP induced very low levels of bulky DNA adducts (Fig. 4, Fig. 5).

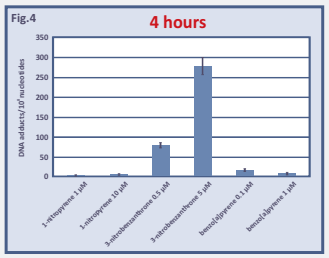
CONCLUSIONS

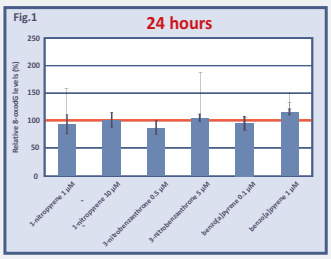
1. Tested compounds had no effect on DNA oxidation.
2. Oxidative damage to lipids and proteins is induced by nitro-compounds, but not by B[a]P.
3. 3-NBA exhibited strongest genotoxic potential even after the 4h treatment.
4. Our data highlight differences in genotoxic mechanisms of PAHs and their nitro-derivatives.

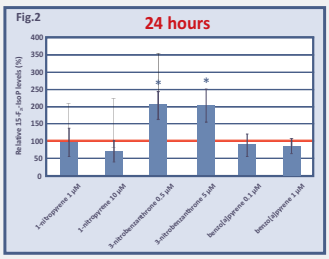


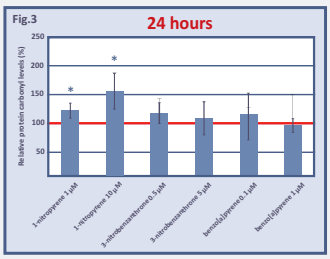












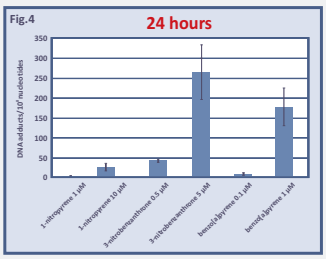


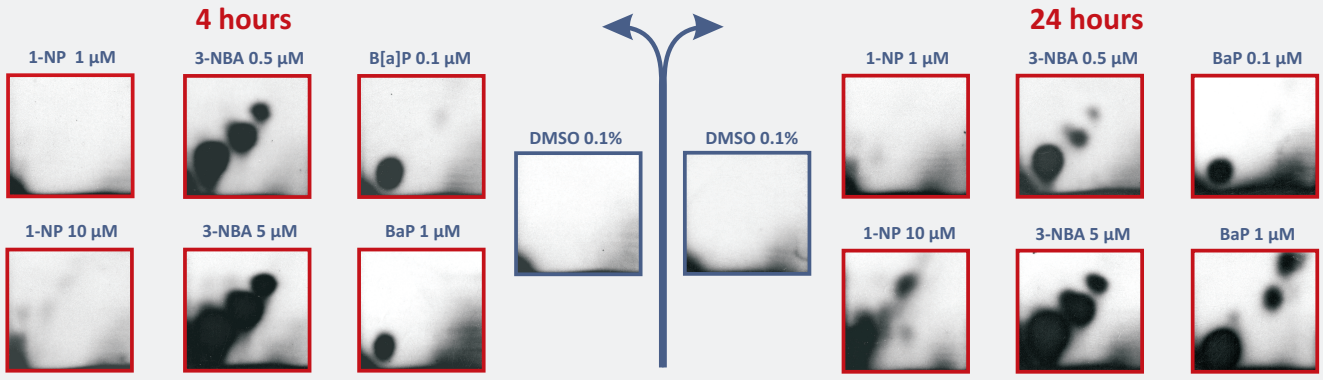
Figure 1. Relative levels of 8-oxodeoxyguanosine (8-oxodG) after treatment of A549 cells with tested compounds for 4 hours and 24 hours. Mean ± SD values from three independent experiments are shown. A bold horizontal line represents the baseline 8-oxodG levels in cells treated with DMSO.

Figure 2. Relative levels of 15-F_{2t}-isoprostane (15-F_{2t}-IsoP) after treatment of A549 cells with tested compounds for 4 hours and 24 hours. Mean ± SD values from three independent experiments are shown. Asterisks denote a significant ($p<0.05$) increase of 15-F_{2t}-IsoP levels. A bold horizontal line represents the baseline 15-F_{2t}-IsoP levels in cells treated with DMSO.

Figure 3. Relative levels of protein carbonyl groups after treatment of A549 cells with tested compounds for 4 hours and 24 hours. Mean ± SD values from three independent experiments are shown. Asterisks denote a significant ($p<0.05$) increase of protein carbonyl levels. A bold horizontal line represents the baseline protein carbonyl levels in cells treated with DMSO.

Figure 4. Levels of bulky DNA adducts after treatment of A549 cells with tested compounds for 4 hours and 24 hours. Mean from three independent experiments are shown.

Fig. 5.
Autoradiographs of TLC maps of ^{32}P-labeled digests after incubation of A549 cells with tested compounds for 4 hours and 24 hours.



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