

Poster: alternative testing methods for toxicity to reproduction

Assessment of DNA oxidation by nitroheterocyclic compounds using cultured YAC-1 cells

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Contradictory results have been published on the DNA damaging properties of the nitroheterocyclic compounds metronidazole and nitrofurantoin, antimicrobial agents widely used in human and veterinary medicine. In order to further study whether their interaction with cellular DNA is linked to the generation of reactive oxygen species we investigated the effects of metronidazole and nitrofurantoin on UV-C induced generation of 8-hydroxy-2'-deoxyguanosine (8-OH-dG) in molecular DNA in solution as well as in cultured murine hybridoma YAC-1 cells.

Calf thymus DNA (CT-DNA) dissolved in hepes-buffered saline or commercially available YAC-1 cells resuspended in hepes-buffered saline were treated either for 45 min or for 72 hours, respectively, with metronidazole, nitrofurantoin or histidine (as a nitrogenous control compound; 50 µg/ml each) before exposure to UV-C-irradiation (peak wavelength 253.7 nm; 15 min, 6 mJ/min x cm²) or sham irradiation. In some experiments, cells were incubated for a further 180 min period. In separate experiments, the free radical scavenging compound TEMPO (4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl) was added. A HPLC system equipped with UV and electrochemical detection was employed to measure the levels of 8-OH-dG in isolated cellular DNA.

Under the conditions chosen, UV-C caused a 123% increase in 8-OH-dG in molecular CT-DNA. Preincubation of CT-DNA with metronidazole prior to UV-irradiation led to significantly higher levels of 8-OH-dG compared to irradiated CT-DNA not treated with metronidazole (172.2 ± 36.1 µmol 8-OH-dG/mol dG). Preincubation with nitrofurantoin did not influence UV-induced 8-OH-dG levels; preincubation of CT-DNA with histidine prevented UV-induced increases in 8-OH-dG.

In untreated YAC-1 cells UV-C irradiation caused an increase in 8-OH-dG concentrations by 304%. Exposure of non-irradiated cells to metronidazole or nitrofurantoin for 72 hrs did not change the basal levels of 8-OH-dG. Incubation with histidine caused a significant decrease in 8-OH-dG concentrations by 72% in non-irradiated cells, while no such effect was seen in UV irradiated cells.

Pretreatment with metronidazole did not affect the induction of 8-OH-dG by UV-C in YAC-1 cellular DNA. In contrast, preincubation with nitrofurantoin led to a 57% decrease in 8-OH-dG levels in DNA of UV-C irradiated cells. The addition of TEMPO lowered the increase in UV-C induced concentrations of 8-OH-dG by 74% in untreated cells and by 78% or 74% in cells pretreated with metronidazole or nitrofurantoin, respectively.

In contrast to the findings using molecular CT-DNA in solution, metronidazole and nitrofurantoin did not affect 8-OH-dG levels in YAC-1 cell DNA and metronidazole had no effect on UV-C induced formation of 8-OH-dG. Whether nitrofurantoin exerts a factual "protective" effect on UV-C induced oxidation of cellular DNA is presently under investigation. The observed "resistance" of CT-DNA against UV induced oxidation following exposure to histidine suggests this substance as acting as a quencher of singlet oxygen (in cell free systems), whereas TEMPO probably exerts its known effect as a spin trap of UV induced ROS.

It is concluded that YAC-1 cells represent a useful tool for the investigation of the complex DNA damaging mechanisms of nitroheterocyclic compounds and, possibly, of other classes of compounds.

Keywords: 8-OH-dG, cellular DNA damage, metronidazole, nitrofurantoin