Supporting Information

Modulation of the Cytotoxicity and Genotoxicity of the Drinking Water DBP Iodoacetic Acid by Suppressors of Oxidative Stress

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Single Cell Gel Electrophoresis Assay

The day before treatment, 2×10^4 CHO cells were added to microplate wells in 200 µl of F12 + 5% fetal bovine serum (FBS) and incubated overnight. The next day the cells were washed with Hank's balanced salt solution (HBSS) and treated with iodoacetic acid alone or iodoacetic acid combined with the antioxidants in F12 medium without FBS in a total volume of 25 µl for 4 h at 37°C, 5% CO_2. The wells were covered with sterile AlumnaSeal. After the treatment the cells were washed twice with HBSS and harvested with 50 µl of 0.01% trypsin and 106 µM EDTA solution. The trypsin was inactivated with the addition of 70 µl of F12 + FBS. To measure acute cytotoxicity a 10 µl aliquot of the cell suspension was mixed with 10 µl of 0.05% trypan blue vital dye in phosphate-buffered saline. Approximately 100 cells were analyzed per treatment group. The number of stained (dead) cells and clear (live) cells were counted and the percent viability for each sample was calculated. SCGE data were not used if the cell viability was less than 70%. The remaining suspension from each well was mixed with 120 µl of molten 1% low melting point agarose (LMA) prepared with phosphate buffered saline, and 90 µl of the suspension were spread on each of two SCGE microscope slides. The microscope slides had been previously coated with a layer of 1% normal melting point agarose prepared with deionized water and dried overnight.

After the LMA solidified on an iced surface for 5 min, a final layer of 0.5% LMA was applied to the slides. To remove the cell and nuclear membranes the slides were placed overnight at 4°C in freshly prepared lysing solution (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris, 1% sodium sarcosinate, 1% Triton X-100, and 10% DMSO). The slides were placed in an alkaline buffer (1 mM Na_2EDTA, 300 mM NaOH, pH > 13.5) in an electrophoresis tank, and the DNA was allowed to denature for 20 min. The slides were electrophoresed at 0.72 V/cm (25 V, 300 mA) for 40 min at 4°C. The gels were neutralized with 700 mM Tris buffer, pH 7.5. The slides were rinsed in cold deionized water, dehydrated in cold (4°C) methanol for 20 min, dried for 20 min at 50°C and stored at room temperature in a covered slide box. For analysis the slides were hydrated in cold deionized water for 20 min, placed on a tray and stained with 65 μl of ethidium bromide (20 μg/ml) for 5 min. The slides were dipped in cold water to remove excess stain, a cover slip was placed over the gel, and the slides analyzed with a Zeiss fluorescence microscope with an excitation filter of BP 546/10 nm and a barrier filter of 590 nm. A computerized image analysis system (Komet version 3.1, Kinetic Imaging Ltd., Liverpool, UK) was used to measure various SCGE parameters (i.e. % tail DNA and tail moment) of 25 randomly chosen nuclei per microgel. The tail moment (integrated value of migrated DNA density multiplied by the migration distance) was used as the primary measure of DNA damage. The digitalized data were automatically transferred from the CCD camera to a computer-based spreadsheet for statistical analysis (*18, 24*). The SCGE experiments were repeated 3 times.