REDUCTION OF OXIDATIVE DNA DAMAGE WITH N-ACETYLCYSTEINE

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Cancer is thought to be the result of accumulated mutations in the DNA. A major contributor to these mutations is the damage due to oxidative stress caused by free radicals. Strategies that reduce oxidative stress on cells should result in less damage to DNA, less mutations, and thus less cancer. Prevention of cancer could include the consumption of antioxidants, both in foods and in supplements.[1] Our studies aim to gather quantitative data to support the notion that antioxidant supplementation reduces cancer by protecting the DNA from oxidative damage. Our hypothesis was that an antioxidant supplement can reduce the damage to DNA caused by oxidative free radicals.

To test our hypothesis, we chose to subject cell cultures to oxidative damage with H_2O_2. Both the control and the experimental culture received 1 mm H_2O_2 for 4 h, but the experimental was preincubated for 24 h with 6 mM N-acetylcysteine (NAC), a potent sulphur-containing antioxidant. At the end of the oxidative stress period, DNA was extracted and analyzed for damage. We also ran controls for no-treatment and treatment with NAC alone.

DNA damage was assessed to detect abasic (AP) sites in DNA, which, in the extracted DNA, is a direct measurement of damage. Our preliminary data suggest that cultures exposed to NAC show less AP sites and that NAC reduced the number of AP sites resulting from insult with H_2O_2.

BACKGROUND

The current paradigm of carcinogenesis presents it as the result of accumulated mutations in the DNA. These mutations come from damage to the DNA from several sources. Oxidative stress caused by free radicals derived mainly from oxygen metabolism is considered a major contributor to the DNA damage that eventually gives rise to many cancers. It follows that strategies that reduce oxidative stress on cells should result in less damage to DNA, less mutations, and thus less cancer. Consumption of antioxidants, in foods or in supplements should prevent cancer.[1] Sales of antioxidant supplements on the assumption that they will protect from cancer form the basis of a multimillion dollar business. The data published is full of controversy but relatively empty of quantitative data. Our studies aim to gather quantitative data to support the notion that antioxidant supplementation reduces cancer by protecting the DNA from oxidative damage. Our hypothesis was that an antioxidant supplement could reduce the damage to DNA caused by oxidative free radicals.

N-acetylcysteine (NAC) is a potent and popular sulphur-containing antioxidant. It is an immediate precursor of glutathione, a very important natural antioxidant. NAC has been shown to scavenge hydroxyl radicals.[2,3] Our preliminary data show that NAC reduces the oxidative damage to DNA, as judged by a reduction of abasic (AP) sites, after insult to cell cultures with H_2O_2.

METHODS

Cell cultures

Rat cells (Big Blue, Stratagene, California) were cultured in 50 mL flasks according to the instructions of the supplier. The cells were cultured for about 3 days, until confluent, prior to the experimentation. Experimental cultures were exposed for 24 hours with 6 mM NAC. All control cultures were mock treated.

Preliminary tolerance tests

To adjust the doses of H_2O_2 to a level where most cells would survive, cultures were treated with several concentrations of the peroxide. After the treatment, remaining live cells were determined using the MTT assay.[4] We demonstrated that 1 mm H_2O_2 for 4 hours would kill about 30% of the cells.

Peroxide treatment

To prevent NAC from reacting with the incoming peroxide in the medium, all cultures were washed with phosphate saline and replenished with fresh medium just prior to exposure to peroxide. In this way, the relevant NAC is intracellular. The cells were treated with 1 mm H_2O_2 for 4 hours, at 37°C in the incubator. At the end of the exposure period, the cells were washed and their DNA collected.

Extraction of DNA

DNA was extracted with the GenElute kit from Sigma (St. Louis, Mo), following the instructions of the kit. The kit is based on silica adsorption in spin columns. DNA concentration was determined spectrophotometrically.

The samples were kept refrigerated in Tris EDTA (TE) buffer.

Determination of AP sites

DNA was subjected to analysis with the DNA damage quantification kit, AP Site Counting, from Dojindo (Gaithersburg, Md). This kit covalently bonds biotin to the aldehyde that remains reactive in an AP site. The biotin tagged AP sites can be quantified with streptavidin conjugated with horseradish peroxidase, and read in a microplate reader at 655 nm. The calibration curve was made with the standards supplied in the kit.

RESULTS

The no treatment (-H_2O_2, -NAC) control showed about 4 AP sites per 10 kb. This is in keeping with the literature supplied by the supplier. The controls indicate that the procedure was done correctly. It appears that NAC can reduce DNA damage even in the absence of oxidative stress and that NAC reduces the damage to DNA by hydrogen peroxide. Because the extracellular NAC was washed away before the addition of the hydrogen peroxide, the mechanism affording protection must be intracellular.

NAC reduced the AP sites in untreated cells from 4 to 2 per 10 kb, and in peroxide-treated cells from 9 to 5 kb.

CONCLUSION

Under the conditions tested, intracellular NAC reduced the oxidative damage to DNA of cells in culture. This finding supports the strategy to raise the levels of antioxidant in the body to reduce the risks of developing cancer. A note of caution: these data are preliminary and should not be taken as the basis to suggest specific diets.

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