INVESTIGATION OF THE EFFECTS OF TREE FRUIT SUPPLEMENTATION ON THE REPAIR OF OXIDATIVE DNA BASE DAMAGE IN MOUSE EXTRACTS

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SUMARY

Our DNA is constantly being attacked by damaging agents, either exogenous such as various chemicals we are exposed to, or endogenous such as reactive oxygen species generated as by products of normal metabolism. These agents inflict a variety of different types of DNA modifications that could be citotoxic and cause cell death, or mutagenic and cause changes in the genetic information. To counteract the deleterious effects of DNA damaging agents, living organisms have evolved complex systems to repair DNA. One such system is the **b**ase **e**xcision **r**epair (BER) pathway. BER is the major repair pathway for the removal of small covalent modifications (such as oxidation and alkylation products) and single strand breaks, which are discontinuities in the phosphate backbone of one of the two strands of the double helix DNA (for review see (Demple and Harrison, 1994)).

Oxidative DNA damage accumulates at high levels in almost all organisms living under aerobic conditions. For example, it is estimated that 100-500 8-hydroxyguanines (a product of oxidative attack to guanines) are generated per day in a single human cell (Lindahl, 1993). Accumulation of oxidative damage, particularly in the mitochondrial DNA, has been causally linked to cancers, neurodegenerative diseases and aging (Souza-Pinto and Bohr, 2002). In mammalian cells, repair of oxidative DNA damage is initiated primarily by one of two DNA glycosylases, Oxoguanine DNA glycosylase (Ogg1) or Endonuclease III homologue 1 (NTH1), depending on the nature of the damage. These enzymes catalyze the release of the damaged base by cleaving the N-glycosyl bond between the base and the deoxyribose. They also provide the specificity of the BER pathway. The observation that oxidative DNA damage levels increase in pathological conditions allows us to speculate that any intervention that up-regulates BER capacity may have a beneficial and/or preventive effect.

A diet rich in fruits has long been thought to have beneficial health effects. Because fruits generally contain high levels of dietary fibers and small molecular weight antioxidants, it was hypothesized that consumption of large amounts of fruits prevents development of cancers and other illnesses such as cardiovascular diseases. Various epidemiological studies have addressed

this question, however with mixed results, and the role of diet on disease prevention still remains ill-defined (McCullough et al., 2002; McCullough and Giovannucci, 2004). Recent results with a blueberry rich diet have suggested that this diet may exert protective effects against the behavioral deficits associated with Alzheimer's disease, a neurodegenerative diseases that displays various biomarkers of elevated oxidative stress (Joseph et al., 2003). Although it is still unclear, it was proposed that these protective effects related to decreased levels of oxidative damage. Therefore, we asked the question of whether a peach/nectarine (P/N), fruits that also contain high levels of antioxidants, rich diet could modulate BER activities, thus decreasing the levels of oxidative DNA damage. This proposal was designed to address this question, and it consisted of three specific aims, as presented below. In this final report we present and discuss the results obtained for each specific aim.

SPECIFIC AIMS

1. Investigate the effects of different varieties of P/N extracts on DNA repair activities *in vitro*. Investigate the effects of fruit extract supplementation on mitochondrial DNA repair in four different organs, liver, brain, heart and kidney from mice.

Results obtained: In order to evaluate the direct stimulatory effects of P/N extracts on BER activities we utilized an *in vitro* assay that measures the activity of specific DNA glycosylases. This assay quantifies the incision of an oligonucleotide substrate containing a single base lesion at a defined position. By using different DNA substrates containing different specific lesions, we monitored the activities of 3 DNA glycosylases: Ogg1, with the 8-oxodG containing substrate; NTH1, with the 5-hydroxycitosine containing substrate; and uracil DNA glycosylase (UDG), with the uracil containing substrate. We compared the effects of different concentrations of 3 fruit extracts (O'Henry peaches, Elegant Lady peaches and Fire Pearl nectarines) on Ogg1 activity in mouse liver mitochondria (Figure 1). All three extracts stimulated 8-oxodG incision at concentrations of 375 or 750 μg. However, only Elegant Lady extracts significantly increased 8-oxodG incision (panel B). Then we investigated whether Elegant Lady extract could stimulate another DNA glycosylase involved in the repair of oxidative DNA lesions, NTH1. Figure 2 shows that increasing concentrations of extract, up to 750 μg, stimulated incision of a 5-OHdC containing substrate to similar levels as to the incision of 8-oxodG. Thus we concluded that Elegant Lady extracts have a stimulatory effect on the repair of oxidative DNA damage in mouse liver mitochondria.

Fire Pearl nectarine extracts showed a modest stimulation of 8-oxodG incision in mouse liver mitochondrial extracts. Since we previously showed that mitochondria isolated from different mouse tissues have distinct repair capabilities (Karahalil et al., 2002), we investigated the effects of this extract in mitochondria from mouse brains and hearts (Fig. 3). Similarly to liver mitochondria, 8-oxodG incision in brain mitochondria was slightly stimulated by the extracts. In contrast, incision in heart mitochondria was significantly (almost twofold) stimulated by Fire Pearl extracts. Sugar Lady Extract also stimulated 8-oxodG incision in heart mitochondria (Fig. 4). These results, along with those presented above, led us to conclude that peach/nectarine

extracts may stimulate DNA glycosylase activities in mitochondria. However, the effects were fruit and tissue specific.

We then investigated whether the fruit extracts could stimulate the activity of another DNA glycosylase, UDG, which is responsible for the repair of deaminated cytosines. None of the extracts tested stimulated UDG incision activity, and in fact, Summer Bright and O'Henry extracts significantly inhibited uracil incision in mouse liver mitochondrial extracts (Fig. 5). Similarly, NTH1 incision activity in MLM was inhibited by Summer Bright and O'Henry extracts (Fig. 6). And while Elegant Lady extracts had previously stimulated this incision activity in a concentration dependent fashion (Fig. 2), it failed to stimulate here. Sugar Lady Extract, however, showed some stimulatory effect. It is important to point out that those last experiments were performed with a second batch of extracts received from CTFA. The lyophilized extracts were prepared and stored in a similar manner to the 1st batch. Thus, we concluded that possible variations in the extracts could account for the differences we observed in 5-OHdC incision stimulation by Elegant Lady extract. This observation suggests that some of the stimulatory effects we have observed previously may be associated to some labile factors that were missing from the second batch of extracts.

2. The experiments presented above only evaluate the direct effect of the extracts on one particular DNA repair activity. Because the cellular responses to DNA damage involve the concerted action of several pathways to protect the integrity of the genome we proposed to investigate the effects of the fruit extracts on cellular survival after treatment with DNA damaging agents.

Results obtained: Because many of the protective effects of fruit-enriched diets have been observed in the central nervous system we chose the rat pheochromatocytoma cell line PC12 for the cell viability studies. These cells are a widely used model of neuronal cell culture and can be differentiated in culture into neuronal-like cells. We initially investigated if the extract itself showed any cytotoxic or proliferative effects. Using the reduction of the tetrazolium slat MTT (Roche) to its formazan salt to monitor cell viability we observed that varying concentrations of Summer Bright extract, up to 500 μg/ml, did not reduce or increased cell viability over a 24 hr exposure (Fig. 7). Thus, we chose this concentration for the protection studies. We used two different oxidants to induce cell death, hydrogen peroxide and menadione. Both agents cause severe oxidative stress and accumulation of oxidative DNA damage. However, addition of 500 μg of Summer Bright extract during the exposure times to the oxidants failed to protect the cells from viability loss (Figures 8 and 9), since cell survival rates were similar in presence and absence of the extracts. Likewise, Elegant Lady extract did not protect the cells against menadione-induced cell death (Fig. 10).

In order to confirm the previous results we utilized a more sensitive assay for cell survival, in which we measured the clonogenic capacity of individual cells after the exposure to the citotoxic agents. As we had previously observed with the MTT assay, Summer Bright extract alone did not show any cytotoxic effect (Fig. 11). Here again, it failed to protect the PC12

cells against menadione-induced cell death (Fig. 12). Thus, we concluded that the fruit extracts alone cannot prevent cell death caused by oxidative stress. However, it is possible that the protective effect of these extracts depends upon metabolic activation of some of their components or is limited by transportation into the cells where they could directly affect DNA repair enzymes.

3. In order to test the hypotheses presented above we proposed to test the effects of feeding mice with large amounts of fruit concentrates on mitochondrial DNA repair efficiency and on mtDNA oxidative levels. 2 groups of 24 C57Bl/6 mice each were fed a control diet or a diet containing 8% (weight) of peach lyophilized extracts for 3 months. Both diets were isocaloric and contained all the same nutritional characteristics with the exception that the peach extract substituted for corn meal in the control diet.

Results obtained: the mice underwent the feeding regiment without any noticeable problems. The average growth chart per group (Fig. 13) showed that both groups gained weight in a similar fashion, indicating that the peach diet was well accepted by the animals, and provided similar nutritional support as the control diet. At the beginning of December the study was terminated and all the samples are now being analyzed for DNA repair activities and DNA damage levels.

CONCLUSIONS

Our results from the in vitro DNA repair assays suggest that one or more components in the fruit extracts may have a stimulatory effect on DNA repair enzymes for oxidative DNA damage. However, we found considerable variability among the different fruits and even between two different batches of the same extracts. Further investigation is necessary in order to tease out whether these variations are due to preparation/storage of the extracts or to characteristics of the fruits used to prepare each particular batch, such as location of the crop and ripeness. These results may provide additional insights into the nature of the stimulatory components.

Although the results with the cell culture system suggest that the extracts have limited protective effects, several factors may have contributed for that, such as cell types and time-course of the experiments. Our own results have suggested that the stimulatory effects of the extracts vary in mitochondria obtained from different tissues. It is clear to us, however, that the most informative results from this study will come from the diet manipulation experiments. All the analyses for these samples are underway.

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