ANTIOXIDANTS AND BIOLOGICAL RADIATION PROTECTION

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ABSTRACT

Antioxidants and antioxidant enzymes, by combatting oxygen radical-mediated radiation-induced oxidative stress, may prevent the accumulation of damage involved in tumor initiation, promotion and progression, and thus serve to protect us against ionizing radiation. We are testing the possible role of dietary antioxidants, and other biological response modifiers, in determining individual radiation response. These experiments use the fluorescent protein beta-phycoerythrin as a target and biomolecular marker for radiation-induced oxidative stress. Antioxidants are ranked according to their radioprotectiveness by their ability to compete with beta-phycoerythrin for radiolytic oxygen radicals. Samples of blood serum from cancer patients have been analyzed using this technique. There is a trend towards decreasing antioxidant levels with increasing donor age, and this is consistent with data showing an increasing radiosensitivity with age.

We are presently monitoring antioxidant and antioxidant enzyme levels in atomic radiation workers and the general public, in order to assess whether they influence individual radiosensitivity. Knowledge of this source of biological response modification will be useful in applying radiation protection practices to those individuals or groups most at risk, and for estimating individual risks associated with radiation exposure.

INTRODUCTION

The risk of ionizing radiation is 10 cancers per 100 exposed persons per Sv [1] This number is a current estimate obtained by averaging data from a wide range of studies. It is predominantly weighted towards large cohort studies from people who have received relatively large doses of ionizing radiation. The overall cancer incidence of occupationally exposed persons is at worst not significantly different from non-exposed workers, and at best slightly less than other workers. Why should this be? What are the factors that modify this risk? Certainly, our genetic makeup is an important factor in determining our individual cancer risk. But, we are subject to our environment and there are a series of other factors that are also determinants. A basic knowledge of the radiation chemistry and cancer biology of the interactions of radiation and biological material is required. Exposure to ionizing radiation produces oxygen-derived free radicals in tissue [2]. These reactive oxygen species include the hydroxyl radical ($^{\cdot}OH$) and the superoxide radical anion ($O_2^{\cdot-}$), as well as other oxidants such as hydrogen peroxide H_2O_2. Damage caused by these same reactive oxygen species produced metabolically has been implicated in the development of cancer and in the ageing process [3].

Free radical damage can be prevented by hydroxyl radical scavengers and antioxidants. Hydroxyl radical attack on critical cellular constituents is prevented by the presence of free radical scavengers which compete with the target molecule for that radical. Once the radical has been transferred to the target, only electron donating antioxidant compounds, such as glutathione and vitamin C (ascorbic acid), can restore the target molecule's function by donating a hydrogen atom back to the target radical, which results in 'chemical repair', before oxygen can react at the site forming a peroxide [4]. Because this 'repair' reaction is much slower than hydroxyl radical scavenging, lower levels of antioxidants (30-100 times lower) can protect efficiently. The principal target molecule is the all important DNA, but other molecules are also damaged by radiation, including proteins and membrane lipids. After the free radicals have attacked the biological system and the damaged sites have been permanently oxidized or "fixed" by oxygen, then the biological repair systems come into operation. It may be surprising that cells have repair systems against radiation induced damage, but chemically, the damage is indistinguishable from that generated by normal aerobic metabolism. Some estimates for the quantity of this metabolically derived damage run close to the radiation-equivalent of 100 Gy hr^{-1}. This is mainly concentrated in the metabolically active centres e.g. mitochondria, but some damage to nuclear DNA is inevitable. Indeed, many different types of DNA damage can be detected in resting, unstressed cells [5]. This very high background of oxidative stress would support a threshold type radiation risk model, as it would take some dose to be 'seen' above this background. Because our aerobic cells experience constant oxidative stress, they have evolved defence mechanisms to produce a battery of antioxidant compounds that can protect against this damage. By eating quantities of fruits and vegetables, this level of protection is augmented by dietary antioxidants such as vitamin C and bioflavonoids [6]. These antioxidants are highly effective in protecting against acute radiation doses.

THE BPE ASSAY

Described here are measurements made using β-phycoerythrin (BPE), a highly fluorescent protein, as the target for radiation-generated free radicals. The level of oxidative damage can be monitored by measuring changes in the fluorescence of BPE solutions after irradiation. A 50 μL volume of different concentrations of a test sample in phosphate buffered saline (PBS) pH 7.4 was placed in each well of a clear polystyrene Nunc 96-well plate with v- shaped bottoms. To each well,

200 μL of a stock β-phycoerythrin (BPE) 340 ng mL^{-1} solution in PBS was added. The final concentration of BPE was 272 ng mL^{-1}. The fluorescence of each well was measured using an IDEXX Fluorescence Concentration Analyzer (FCA) set on a gain of 5, measuring at an excitation wavelength of 545 nm and emission wavelength of 575 nm. The plate was then placed in an AECL Gammacell 220 cobalt 60 irradiation unit for 30 s at a dose-rate of 0.6 Gy s^{-1}. The plate was immediately remeasured in the FCA and the percentage fluorescence remaining calculated for each concentration. The radioprotective effectiveness was calculated using the half maximal protection level.

Different classes of water soluble antioxidants, as well as biological samples, have been examined in this study. The aim of the research was to determine how radioprotective different antioxidant compounds and biological samples are.

RESULTS AND DISCUSSION

Using the BPE assay, vitamin C was almost 40 times more protective than simple sugars. Fifteen antioxidant compounds were measured using this technique and the results relative to vitamin C are shown graphically in Figure 1. This clearly shows that the compounds are highly effective and can be grouped into three main antioxidant classes.

Sulfhydryl compounds, such as glutathione, range from 1.27 to 2.85 times more effective than vitamin C. These compounds contain a hydrogen donating sulfhydryl group, the degree of radioprotection is a function of side groups on the molecules involved. The most effective phenol (compounds which contain a hydrogen donating OH group) was catechol (7.17 times more effective than ascorbic acid) and the least, trolox (a water soluble vitamin E analog) with an effectiveness 3.2 times that of vitamin C, indicating that these compounds are more effective than sulfhydryls in terms of the radioprotective effect. Catechol derivatives are found in high quantities in tea. Indoles range from 6-9 times more effective than vitamin C. The best indole measured was melatonin. Unfortunately this compound is neuroactive, presently precluding its use as an emergency preventive measure.

These antioxidant compounds also protect against cancer induced by oxidative damage. Interestingly, if the compounds are added after the damaging event e.g. after radiation exposure, they also protect cells against becoming neoplastic [7]. This suggests that the biology of tumor formation is also dependent on oxidative stress, and that antioxidants can protect against it, by inhibiting reactive oxygen-mediated

steps in the progression of the disease.

Many indices of oxidative damage increase during ageing and, at the same time, we also become less protected against oxidative damage [6]. Using the BPE assay to determine radioprotective effect, human plasma from twenty cancer patients were measured. Figure 2 shows the results obtained with the blood plasma of the twenty cancer patients for whom age data were available. The total radioprotective units normalized for total protein are plotted against donor age. The results for the twenty samples varied by a factor of two over a forty year age range.

There is an inverse trend between total radioprotective effect and age. The spread of data is quite large about the first order regression line which is described by: Radioprotective unit =980±130 unit -6.30±2.12 unit yr^{-1} ($r=0.56$; $P<0.01$). This shows that sensitivity to radiation increases with age, that is, samples from older patients are less protected against radiation damage. Organisms tend to become more oxidized as they age and this seems to be related to the metabolic rate of an organism (except for birds). Several caloric-restriction experiments, which reduce metabolism, have shown that a 30% reduction in energy intake can significantly decrease cancer incidence, while at the same time increasing life-span by almost 30% [8]. One possible explanation for the apparently higher Atomic Bomb Survival Data compared with their non-exposed neighbours is that the cohort was calorically restricted at the time of exposure.

CONCLUSION AND SUMMARY

By using the BPE assay to determine the radioprotective ability of different compounds, potential compounds can be identified as risk modifiers. Indole compounds, such as melatonin were found to be the most effective (almost nine times more effective when compared with vitamin C), followed by phenols and thiols. Radioprotective effectiveness was found to be inversely correlated with age, with a reduction of about half over a 50 year age range. In addition, possible risk modifiers associated with changes in oxidation status can be identified.

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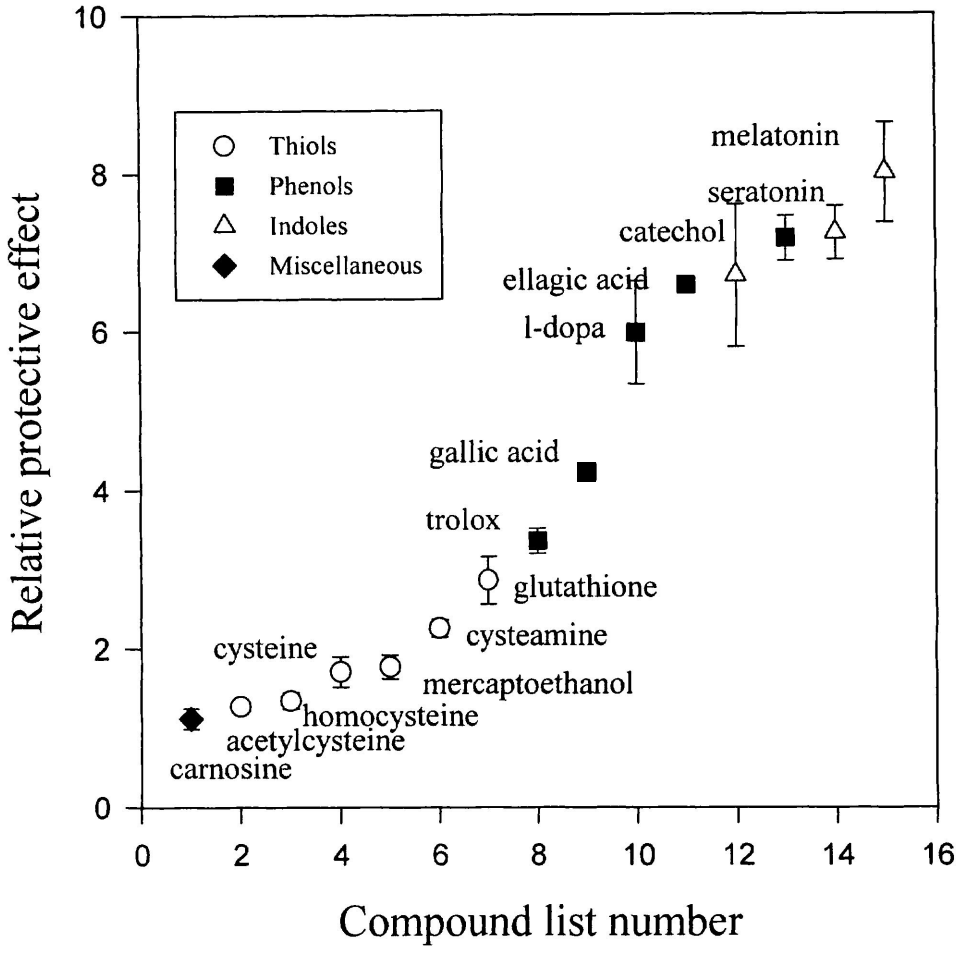

Figure 1: The relative radioprotective effects (compared to ascorbic acid) of thiols, indoles, phenols and miscellaneous compounds in order of effectiveness.

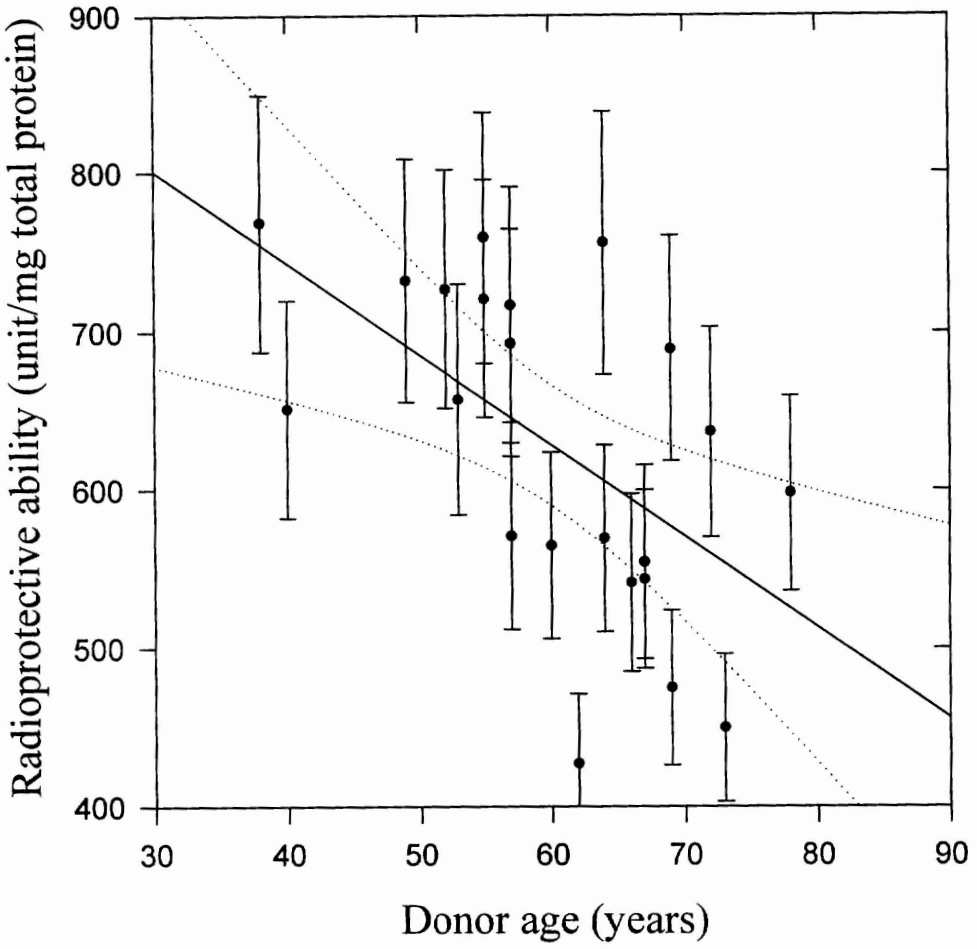

Figure 2: The radioprotective ability of cancer patient blood plasma and its relationship with donor age. The dotted lines show 95% confidence limits.