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ScienceDirect
Journal of Nutritional Biochemistry 18 (2007) 168–171
Journal of Nutritional Biochemistry

Oxidative stress and antioxidants: a link to disease and prevention?

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Received 28 February 2006; accepted 13 December 2006

Abstract

The thrust of this presentation takes a more programmatic approach and gives an overview of the programs at the NIH and the NCI that have a broad nutritional and basic science undercurrent and outline. Also discussed briefly are some areas of general concern that are under investigation in the nutrition group and are included in the group's outreach efforts among professional and academic organizations. The overarching focus of these efforts is to stress the importance of nutrition as a potential modulator of health/disease risks associated with genetic predisposition and environmentally induced disease from diet, lifestyle and exposure to pollutants.

Keywords: Antioxidants; Beta-carotene; Cancer; Environmental pollutants; Prevention

1. Introduction

The NIH is deeply involved in a number of groundbreaking "omics" projects that are being applied to nutritional research questions through a number of mechanisms. These include genomics, proteomics, metabolomics and the supporting bioinformatics and imaging techniques. These are being showcased as unprecedented opportunities that exist for the expanded understanding of the use of foods and food components to achieve genetic potential, to increase productivity and to reduce the risk of disease. Unfortunately, we cannot now identify who will benefit most or be placed at risk from bioactive nutrients due to incomplete data sets and a lack of understanding of the mechanisms of gene nutrient interactions. "Omics"-associated research can also provide an opportunity for exploring interactive mechanisms of nutrition as a modulator of disease risk factors associated with exposure to and metabolic transformation of environmental pollutants and naturally occurring toxins.

2. Antioxidants, genes, nutrition and cancer prevention

It is estimated that a third of all cancer deaths can be attributed to dietary factors. This is due to both a lack of intake of "protective" natural components in an individual's diet, such as polyphenols, stanols, flavonoids, stanols, sterols and carotenoids, as well as exposure to natural carcinogens in the diet, such as aflatoxin, fumonisin and heavy metals. Bioactive food components can influence a number of physiological processes: apoptosis, metabolism, cell differentiation and growth, DNA repair, hormone regulation, inflammation, etc. Using new techniques such as the "omics" to focus on targets themselves plus applying nanotechnology, bioinformatics, structural and computational biology and the use of molecular libraries and imaging may help define the building blocks, pathways and networks. In this way, it should be possible to decipher the actual sites of action and pathways of transduction, apoptosis, DNA repair, phytoestrogens and hormone metabolism as well as the specific pathways involved in folate and vitamin D defects. Proteomics and genomics seem to be the most obvious choices to begin with at this time and are receiving the most attention.

Presented at the Nutrition and Superfund Chemical Toxicity Workshop held at the University of Kentucky, Lexington, November 18, 2005, and sponsored by the Superfund Basic Research Program and the University of Kentucky.

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doi:10.1016/j.jnutbio.2006.12.003

It is important to consider the marked changes in nutrition, health and lifestyle that have occurred over the last century. In 1900, many foods were scarce, expensive and seasonally available, and food production and employment was local and labor-intensive. Dairy, egg and meat consumption was a focus of many diets; today, food is plentiful in the developed world, relatively cheap, available nearly year-round and internationally supplied. People now pay to exercise because work is not physically demanding; unfortunately, nutritional habits have not changed so markedly. This is unfortunate, as elevated fruit and vegetable consumption has been clearly associated with lower cancer risk in a majority of epidemiology studies; the decrease in relative risk is ranging from 1.3-fold to 1.9- to 2.8-fold for breast, colorectal and pancreatic cancer, respectively [1].

Gene expression studies are providing clues about molecular targets for food components and dietary modifications. Recent studies in mouse models of colon cancer, where the *p21* gene is sequentially knocked out, gives a very graphic example of the effects of a typical "Western" diet versus a more healthy and balanced AIN-76A diet. The survival in every case was lower with the Western diet, but the most marked effect was observed in the animals where both copies of the tumor suppressor *p21* gene were inactivated; survival was more than halved in this group [2].

3. Polymorphism

A number of genes have been linked to specific cancers when they are mutated. Several cytochrome P450s (Cyp1A1, 1A2, and 2E1), whose polymorphisms can effect activation of polycyclic aromatic hydrocarbons, common pollutants from combustion linked to lung, skin and many other cancers, as well as chlorinated solvents, polychlorinated biphenyls, nitrosamines and heterocyclic amines, have been linked to increases in lung, colon and gastric cancer, to name a few. Various dietary factors can induce P450s and stimulate metabolism. The polymorphic forms respond differently to induction and substrate specificity; nutrient substrates can also show differential response in their individual metabolic alterations by the P450s, most importantly isothiocyanates and organosulfur compounds. Polymorphism in the gene that utilizes glutathione to detoxify foreign compounds, glutathione transferase, enhances the protective effect of isothiocyanates in cruciferous vegetables, as their excretion rate is diminished. Polymorphisms in the *N*-acetyltransferases and the enzyme methyl tetrahydrofolate reductase are additional examples. Individuals in the Physicians Health Study who were rapid acetylators have shown a greater than fivefold increase in colorectal cancer compared with those who were slow acetylators [3]; this may be linked to differential handling of heterocyclic amines in cooked red meat. Polymorphism in the glutathione peroxidase gene markedly alters the enzyme's response to dietary selenium and its ability to detoxify hydrogen peroxide; the risk of lung cancer in subjects with the mutation in both copies of the gene is more than double [4]. Selenium has been shown to interact with the carcinogen arsenic, a widespread natural and industrial pollutant.

4. NCI nutrition

To stimulate this type of research, the NCI's Division of Cancer Prevention has organized a number of workshops and symposia primarily through the Nutritional Science Research Group (NSRG) in collaboration with a cadre of other groups, institutes and agencies as well as private scientific organizations. The complete list of these activities can be found on the NSRG Web site (http://www.cancer.gov/prevention/nutrition/events.html) but a couple of these topics merit specific mention — nutritionally based antioxidants and alterations in cancer risks (including etiology, prevention and treatment interaction).

5. Antioxidants

The conference "Free radicals: the pros and cons of antioxidants" was organized to summarize current understanding and identify major gaps in the knowledge of the physiological significance of antioxidants in cancer prevention and tumor biology in the presence and/or absence of conventional chemotherapy and radiotherapy [5]. This has continued to be a topic of discussion in the public and scientific press, and an extensive review of the importance and mechanisms of the class of antioxidant's involvement in health and disease, specifically cancer and heart disease, will be appearing in this journal in the very near future [6].

A myriad of compounds and some metals belong to the grouping classed together as "antioxidants." They can be subclassified into two main groups, the reducing agents (beta-carotene and vitamins C and E) and the nucleophiles (glutathione, *N*-acetyl cysteine, lipoic acid and selenium), but the general and somewhat naive understanding is that they are similar. From a structural standpoint, they are obviously quite different and the common similarity is that they all can interact with the oxidative state. They have many other properties and specific molecular targets and their association with the term antioxidants can detract from the fuller understanding of just how important antioxidants can be in disease etiology and prevention. One focus of the symposium was just how useful "antioxidants" can be in ameliorating the negative side effects of cancer therapy. This is a current and controversial topic that has been around for decades. While antioxidants have been investigated as radioprotectants from a military standpoint, there have been a number of small probing studies on their effects on chemotherapy and radiotherapy efficacy, some of which were presented at the symposium [5]. Just as chemotherapeutic agents interact at many different points in the cell cycle, antioxidants, even the same one, would a priori have different effects in combination with different drugs or in

different tumors. This is indeed the case and, in summary, we can make no summary on the usefulness of these compounds. It must be investigated on a case by case basis considering the specific antioxidant, the specific antitumor agent and the specific tumor site. The incomplete picture at this point shows that in some cases there is a positive effect, while in others it can be deleterious. One such example that highlights the conundrum is a recent study where topical vitamin E reduced the apparent side effects of radiation for head and neck cancer [7]. The observed reduction in side effects was reproduced in the short run, but oral vitamin E and beta-carotene increased the incidence of tumor recurrence in the long run [8]. These results are temporally based in the same patient group so the complexity introduced by multiple tumor types and multidrug treatments in the larger picture is obvious. A summary cautionary slide from several follow-up talks given by members of the NSRG probably says it all.

- Many phytochemicals have antioxidant properties in vitro, but these effects are not directly related to their many other effects on cellular signaling pathways, gap junctions and metabolic enzymes.
- Phytochemicals interact with the cell in unique ways both synergistically with related and unrelated compounds and through activation of metabolic enzymes.

6. Cancer prevention

One very poignant example of the state of confusion over dietary antioxidants has been highlighted by the confusion and changing level of evidence for the efficacy or deleterious effects of beta-carotene. Why was it thought that beta-carotene would prevent human cancer? Epidemiology pointed to an inverse dietary relationship with many epithelial cancers, including lung. It seemed a logical extension of epidemiology and the reported levels of carotenoids in fruits and vegetables. Early mechanistic data reporting that beta-carotene could be a prooxidant at high oxygen tension and in higher than physiological concentrations did not figure in the later determinations of clinical trial design [9].

Trials were begun and were successful in populations with low consumption of yellow and green fruits and veggies and resulting low blood levels of beta-carotene. Blot et al [10] reported significant protection in cancer incidence and mortality with an antioxidant mixture including beta-carotene. Later trials in smokers using substantially higher daily beta-carotene doses led to elevated lung cancer risk in both the ATBC and CARET studies [11–13]. This led to the removal of beta-carotene from a number of intervention trials and a general aversion toward its use by the public. A 2003 French study of an antioxidant mixture showing a marked reduction in several cancers in men with low background serum levels [14] did little to allay fears of the use of beta-carotene. This was in spite of hints from reexamination of CARET data showing a nonsignificant (low power) 20% reduction in the lung cancer risk in former smokers This observation has recently been repeated in a much larger cohort of smokers and nonsmokers showing a substantial (>50%) reduction in lung cancer in nonsmokers and a doubling in smokers with supplemental beta-carotene consumption [15].

6.1. Cancer risk

Although not a direct focus of the nutrition program in the Division of Cancer Prevention, much attention has been given within the NCI to the association of nutrients, antioxidants and environmental pollutants with increased cancer risk. These risks can come from food-borne “pollutants” such as the aflatoxins associated with liver cancer in developing areas with inadequate grain storage, the heterocyclic amines formed during the cooking of meat and other natural carcinogens. The risk can also come from contamination with environmental pollutants such as pesticides, metals and solvents. Many of these compounds are activated or detoxified by the same phase I and II enzymatic systems as described above. More recently, folate deficiency, being counteracted by fortification of many grain products, can accentuate the effects of arsenic, as the primary excretion pathway involves methylation and deficiency enhances the biological half-life. Arsenic exposure can also lead to a decrease in methylation capacity and changes in DNA methylation patterns, key in turning on and turning off gene expression and linked to several cancers [16] (see also review by Ross [17]). There are a number of other examples of nutritional modulation of pollutant-initiated cancers that may also be shown to be affected by bioactive food components, i.e., that nutrition can modulate the toxicity of environmental pollutants and thus affect health and disease outcome associated with chemical insults.

7. Conclusions

So what conclusions can be drawn on antioxidants? Should they be considered for use in ameliorating cancer risk? For prevention, maybe at physiological doses if one has low baseline levels, antioxidants may lower oxidant stress and reduce the chance of cell transformation, but since we do not know what effects to expect at which stage of transformation, in most cases it is not advisable without hard data supporting efficacy.

For cancer treatment, it is not advisable without clear indications from clinical trials demonstrating that specific antioxidants can lower oxidative stress in the malignant cell and may increase the antiapoptotic phenotype. Supporting data for this are lacking at this time.

Does beta-carotene cause cancer? In smokers, it is clear, maybe by acting as a prooxidant at elevated doses, that it at least stimulates lung cancer development and may interact with tobacco carcinogens enhancing metabolic activation or

protect early transformed cells from oxidative-initiated apoptosis. In nonsmokers, it clearly lowers risk [15], and in former smokers, it may also lower the relative risk. Supplementation appears to be most effective in the lowest baseline groups and when serum levels are in the normal range. It also appears to be effective with other antioxidants.

Exposure to environmental pollutants, and especially to some persistent organic pollutants, may provide similar risks towards cancer like those seen in smokers. Some data suggest that dietary antioxidants can down-regulate the procarcinogenic risks of environmental pollutants by interfering with activation or stimulating detoxification in some instances and enhancing the pollutant's activity in others.

Considering the larger picture, we can obtain some clear take-home messages, which though not profound can be summarized as follows:

- Studies at the "population" level may not hold for particular individuals.
- For example, some people seem to be able to smoke with impunity; for others, a "bad" diet seems to have little impact on risk.
- A similar general statement can be made for environmental carcinogens, one size does not fit all as far as risk and potential for intervention goes.

We might also conclude that

- Some people may not have to alter their diets to stay well.
- Increases in fruit and vegetables may benefit some but not all people.
- We still do not understand the complexity of diet and cancer and its interactions.

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