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Defence Against Reactive Oxygen Species*

ANTHONY T. DIPLOCK

International Antioxidant Research Centre, Guy's, King's College and St Thomas's Hospitals Medical and Dental School (University of London), Guy's Hospital, London, SE1 9RT, UK

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As part of the European Commission Concerted Action on Functional Food which was managed by the International Life Sciences Institute (Europe) a series of Theme Papers was produced which examined the 'state of the art' with respect to the subject matter and made recommendations for research. This paper is a summary of the paper concerned with Defence Against Reactive Oxygen species. Having reviewed the scientific literature the authors concluded that certain stringent criteria, which they identified, would need to be satisfied in order to be able to conclude that free radical events are involved in certain human diseases, and that antioxidants are capable of modulating these events and thus reducing the risk of disease. Although there is some evidence that would lead to this conclusion the authors demonstrated that there is at present insufficient evidence available on which to base a firm conclusion that antioxidants are capable of reducing risk of disease, and very little evidence that addresses the important question as to how much of the nutrients concerned are required in the diet to achieve the objective of reducing risk. Research priorities address the need in particular for the development and validation of cellular markers of oxidative damage which are required before there can be new human studies that address the question. There is also a need for more information as to the pharmacokinetics of uptake from diet, distribution and cellular concentration of the antioxidants.

Keywords: Reactive oxygen, antioxidants, disease risk, research needs, European commission concerted action

INTRODUCTION

There are four main questions that require to be answered in providing a critical assessment of the ability of dietary antioxidants to maintain human health.

1. What is needed to establish that free radical events are involved in that detriment to human health that can eventually be associated with pathogenesis of an identified disease?
2. What is needed to establish that antioxidants have specific benefits in maintaining optimal health and wellbeing and reducing the risk of degenerative disease?
3. What is needed to make a claim about the functional, nutritional and health benefits of antioxidants?
4. What is needed to bring an antioxidant product to the market?

* An outline of the Individual Theme Group paper (by A.T. Diplock, J.-L. Charleux, G. Crozier-Willi, F. Kok, C. Rice-Evans, M. Roberfroid, W. Stahl, José Vina-Ribes). FUNCTIONAL FOOD SCIENCE IN EUROPE (ILSI/European Commission Concerted Action, 1998).

Although considerations of 'health' and 'well-being' are paramount, the concept of 'disease' is often the paradigm by means of which it is possible to assess the effect of the absence of a nutrient.

ESTABLISHMENT OF CRITERIA

1. The goal is to determine whether specified foods which contain antioxidants can be considered as 'functional foods' in that they may confer health benefit.
2. A 'functional food' is considered to be a food which delivers a physiological benefit. Description of it should convey unambiguous information that is without deception to the consumer about physiological or health benefit.
3. In order to substantiate a claim about health benefit it must be possible to provide evidence, substantiated from the world literature, that certain criteria are satisfied.
4. The Theme Group considered that there are six criteria that need to be individually established in the present context.

Criterion (i) A plausible and validated rationale for; (a) involvement of free radicals in processes leading to detriment to human wellbeing and health, and to specified diseases and (b) involvement of dietary antioxidants in the prevention of these processes involving free radicals. This should include results derived from studies carried out *in vitro*, in cells in culture, in *in vitro/ex vivo* models, and in animal models.

Criterion (ii) The existence of human population epidemiological data which demonstrate a statistically validated inverse relationship between intake, or preferably serum concentration, of individually specified antioxidants and the risk of, or mortality from, particular diseases.

Criterion (iii) The existence of statistically validated epidemiological evidence that links intake (or serum concentration) of identified antioxidants at an early stage of a disease process with risk of human disease which may develop some time after exposure to the antioxidants. Both intermediate endpoints, that have been shown unequivocally to predict subsequent disease, and final endpoints may be used.

Criterion (iv) The existence of markers for evaluating free radical events in human subjects, and the modulating effect of antioxidants on them. This will include validation of methodologically uncomplicated markers, by inter-laboratory studies of the same material which has been shown to give the same answers in different laboratories. The chosen marker must be directly relevant, that is it must have functional significance; the marker must have been shown by unequivocal techniques to have significance both to the physiological function, and to the health maintenance or disease risk, to which it is linked.

Criterion (v) The existence of statistically validated interventional human evidence in large groups of human subjects: (a) which clearly shows that enhancement of intake of specified antioxidants is associated with improvement in a valid index of wellbeing and health, or a lowered risk of subsequent disease; and (b) which demonstrates optimal levels of intake of antioxidants, using parameters measured by chosen markers. This implies that markers have been established which demonstrate a valid relationship between intake of the antioxidant and the parameter evaluated by the chosen marker(s).

Criterion (vi) The existence of clear evidence that the intervention that is proposed with an antioxidant nutrient is safe. This will include evidence that a conclusion as to safety applies with equal force to all groups in the population, including those that are indulging in behaviour which might be expected to increase the risk of the disease concerned.

These are clearly extremely stringent criteria. What is required in coming to a conclusion as to the health benefit of antioxidants is to evaluate the 'state of the art' with respect to them, and to come to a conclusion as to how and to what extent, these criteria are satisfied at present.

EVALUATION OF THE "STATE OF THE ART" WITH RESPECT TO THE CRITERIA

(1) Oxidative Damage, Oxidative Defence and Pro-oxidants in Disease

(i) Imbalance of reactive oxygen species and antioxidant defence systems may lead to modifications of biologically relevant macromolecules which provides a logical pathobiochemical mechanism for the initiation and development of disease states.

(ii) Experimental data obtained *in vitro* provide evidence that antioxidants function in systems that scavenge reactive oxygen species and that these are relevant to what occurs *in vivo*.

(iii) The relevance *in vivo* of these observations depends *inter alia* on knowledge of the uptake and distribution of the antioxidant within the human body, and on what tissue levels of the anti oxidant may be expected in relationship to dietary levels. The knowledge database in this respect is at present incomplete.

(2) Evaluation of Oxidative Damage to DNA, Lipids and Proteins

(i) There is some way to go until validated precise biomarkers of oxidative damage in human subjects *in vivo* under minimally invasive conditions are available.

(ii) Oxidative damage measurements in DNA using hplc and gas chromatography-ms techniques have merits and limitations. Oxidation artifacts can arise in preparation/derivatization/storage of DNA.

(iii) Lipid oxidation products in plasma are best measured as isoprostanes or as lipid hydroperoxides. Isoprostane measurement will advance specificity and precision and may give a measure of whole body lipid peroxidation.

(iv) Measurement of oxidative damage to proteins has some potential but such methods have not been effectively exploited.

(v) All these methods still need careful inter-laboratory validation.

(3) Nutritional Modulation of Oxidative Damage

(i) Epidemiological studies indicate that the major antioxidant nutrients vitamins E and C, and also β-carotene which may or may not be acting as an antioxidant *in vivo*, may play a beneficial role in the prevention of chronic disorders.

(ii) Human intervention studies using hard endpoints are the gold standard but trials are restricted mainly to the major antioxidants and do not allow firm conclusions because of inconsistent findings, an insufficient number of studies and the use of varying doses.

(iii) There is evidence that large doses of β-carotene may be deleterious to the health of certain subgroups of the population such as heavy habitual smokers.

(iv) Preventive trials with intermediate endpoints need to be developed for testing the efficacy of antioxidants.

(v) Bioavailability studies and dose-finding studies in combination with the development and application of validated biomarkers are required for a successful research strategy.

(4) Safety Implications of Antioxidant Nutritional Enhancement

(i) Vitamin C is safe at levels of supplementation up to 600 mg/day, and higher levels, up to 2000 mg/day, are generally thought to be without risk.

(ii) Vitamin E has a very low human toxicity and an intake of 1000 mg/day is without risk; 3200 mg/day has been shown to be without any consistent risk. The risk of long-term larger supplements has not been studied.

(iii) β-Carotene is safe at an intake up to 10 mg/day; larger amounts are viewed with caution because they have been shown to increase lung cancer risk in heavy habitual smokers.

(iv) There is little reliable information about the human toxicology of flavonoids. A key question is as to whether these substances are taken up by human subjects and distributed to the tissues in quantities sufficient to confer biological effect.

(5) Food Technology in Nutritional and Safety Aspects of Antioxidants

(i) The food industry has long experience in the control of oxidative damage in foods which can be used to advantage for the protection of beneficial food antioxidants.

(ii) Strategies for the protection in foods of vitamins C and E and β-carotene are already exploited by food technologies.

(iii) Other compounds with antioxidant activity may, or may not, have biological activity.

(iv) Oxidation during manufacture cannot be eliminated and efforts must be made to identify antioxidants which may be important to health and to discover in what form they are most useful.

(v) More information must be obtained in the fields of bioavailability and bioactivity.

(vi) With this information food technology strategies for the preservation of antioxidants which have been shown to be beneficial to health can be applied in a cost-effective manner.

CONCLUSIONS

(i) There is evidence that mechanisms that involve free radicals are implicated at some stage of the development of human diseases, and that the maintenance of wellbeing depends on the supply through the diet of antioxidant nutrients and possibly β-carotene, which modulate free radical processes *in vivo*. If it is shown that non-nutrient antioxidants are biologically available then they too may contribute to the total antioxidant effects of the diet *in vivo*, as well as contributing antioxidant potential during processing, storage and in the gastrointestinal tract.

(ii) Present epidemiological evidence is incomplete but in general it supports the basic hypothesis that antioxidant nutrients contribute to wellbeing and health. There is no evidence to the contrary which would negate this conclusion. There is minimal similar evidence with respect to the non-nutrient antioxidants.

(iii) The development of human study biomarkers is well advanced but these need critical evaluation for use in a new generation of human studies to examine and evaluate a quantitative cause and effect relationship between antioxidant intake and health benefit.

(iv) There are few reliable human intervention studies which establish cause–effect relationships, and none which show clearly the optimal amount of nutrient and non-nutrient antioxidants needed in the human diet. The identification and development of intermediate end points for evaluating the effect of intervention needs further careful work.

(v) Increasing human dietary intake of antioxidants is safe and without undesirable side-effects except in rare well-defined instances.

(vi) There is a need to ensure that measures adopted by food technologists in the processing of foods maintain the antioxidant content of the food, and that the antioxidants are still active at a suitable level when the food is consumed by the public. If it can be shown with certainty that a certain level of antioxidants in the food is associated with health

benefit it may be necessary for food producers to consider means of enhancing the content or form of the antioxidant up to or beyond that usually found in the natural food.

RESEARCH PRIORITIES

The principal research priorities are as under:

(1) The most urgent requirement is the validation of available biomarkers of oxidative damage before they can be used as biomarkers of intermediate endpoints in a new generation of human studies.

(2) Three types of validation are essential:
   (i) Comparison must be made of results that assess the degree of oxidative damage, obtained in the same laboratory on identical material using the same and complimentary methodologies.
   (ii) Comparison must be made of results obtained in as many different laboratories as possible of identical material which is exchanged between participating laboratories.
   (iii) Measurements made must be shown to be clearly linked to those phenomena which give rise to disease in human subjects. Is the DNA oxidative damage that is measured a real indicator of the involvement of such damage in mutagenesis and eventual carcinogenicity? Is the oxidation of LDL a reliable indicator of atherogenesis and eventual vascular disease?

(3) The validated and accepted markers will be used in a new generation of human studies. The use of intermediate endpoints should enable answers to key questions to be obtained considerably more quickly than in earlier studies whose endpoints were disease phenomena occurring many years after the initiation of the study. These new studies will provide for the first time rigidly controlled evidence of the benefit to be gained from antioxidants in the human diet, and will enable quantitation of the optimal levels of intake of antioxidants. In this connexion, care must be taken to ensure that the importance of the antioxidant contribution of the whole diet, as distinct from that of each individual antioxidant, will be evaluated.

References

[1] A.T. Diplock, J.-L. Charleux, G. Crozier-Willi, F.J. Kok, C. Rice-Evans, M. Roberfroid, W. Stahl and J. Vina-Ribes (1998) Functional food science and defence against reactive oxidative species. *British Journal of Nutrition* **80**, Suppl. 1, S77–S112.