CHAPTER 16

The Role of Antioxidants in the Prevention of Oxidative Damage to Nucleic Acids

Peter Møller* and Steffen Loft

Abstract

It is commonly assumed that ingestion of antioxidants is associated with low levels of oxidatively damaged DNA although this is far from conclusive in human intervention trials. A collective interpretation is difficult because many studies lack sufficient control and have unrealistically high baseline levels of oxidative DNA damage in human white blood cells (WBC). A survey of studies on the antioxidant hypothesis in terms of oxidative DNA damage excretion products in urine indicates that ingestion of antioxidant-rich foods may be more effective than single antioxidants. In WBC, there is some evidence of beneficial effects of ingestion of antioxidants and antioxidant-rich foods. This suggests intake of antioxidants either in tablet form or as natural ingredients of foods are associated with beneficial effects on oxidative stress status, but the effect is smaller than previously expected and supplementation of antioxidants to healthy and sufficiently nourished individuals may not be of large public health relevance.

Introduction

The role of antioxidants in the prevention of DNA oxidation can be investigated in a wide array of experimental settings, ranging from cell free systems, to large intervention studies. ‘Antioxidant’ is a widely used term that is difficult to define clearly in biological systems and the effect may depend on the experimental setting. We use the term antioxidant as a broad definition of any substance that can prevent oxidation of biomolecules, either directly by scavenging ROS, or indirectly by upregulating the antioxidant defense or DNA repair systems. The indirect antioxidant effect may be induced by xenobiotics or components in vegetables that are not scavengers or even considered potentially harmful e.g., isothiocyanates from cruciferous vegetables. The types of antioxidants can roughly be grouped into categories in relation to administration, as follows: (1) single antioxidants; (2) multiple antioxidants; (3) extracts or juices of natural food products. Antioxidants like vitamin C, vitamin E, carotenoids, and flavonoids have been identified in a large range of natural food products, but fruit and vegetables also contain a mixture of other antioxidants and bioreactive substances that are less well investigated in terms of antioxidant properties.

Cell free systems and cell cultures exposed to a ROS generating system are commonly used to investigate the scavenging effect of dietary compounds. The large number of investigations addressing the in vitro antioxidant potential of phytochemicals by far impedes a thorough

*Corresponding Author: Peter Møller—Institute of Public Health, University of Copenhagen, Building B, 2nd Fl., Øster Farimagsgade 5, Post Box 2099, DK-1014 Copenhagen K, Denmark. Email: p.moller@pubhealth.ku.dk

discussions. Both in vitro experimental settings have the advantage of being fast and reliable, but extrapolations to the effect in humans are often difficult, because the concentrations of antioxidants are unrealistic and the ROS-generating system can be disproportionate compared to the balance between antioxidants and ROS that exists in vivo. A further caveat is that antioxidants in high concentrations act as pro-oxidants; this in vitro effect is well known for vitamin C but also other phytochemicals show the same pattern e.g., quercetin. Besides establishing the scavenging ability of antioxidants, cell culture experiments can gain information about the regulation of gene expression of antioxidant proteins or DNA repair enzymes. Genetically altered cell cultures or gene silencing by small interfering RNA may be suitable model systems for mechanistic studies.

Animal experimental models offer several opportunities related to the understanding of the mechanism of antioxidants and this consequently strengthens the biological plausibility. An obvious advantage is the possibility of investigating the effect in the presumed target tissues such as colon and lung. More importantly, it is possible to test chemoprevention as interactions between antioxidants and known dietary genotoxic components, e.g., dietary supplementation with β-carotene suppressed generation of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-OHdG) by diesel exhaust particles in the lung of mice. Several rodent experimental models have shown that tea polyphenols inhibit carcinogen-induced DNA oxidation in various organs, whereas the effect on spontaneous oxidized DNA bases is limited. However, one should be aware that rodents differ in their requirement for antioxidants, which may hamper the extrapolation to humans. As an example, mice and rats synthesize ascorbate and α-tocopherol de novo, whereas humans and Guinea pigs lack this ability and require dietary vitamins C and E. Interestingly, using Guinea pigs as a model revealed no effect on hepatic 8-OHdG by variations in dietary vitamin C or E supplementation. This suggests that the pro-oxidant effect of vitamin C shown in vitro is difficult to reproduce in rodents or humans. It should also be stressed that the dose of antioxidants is crucial and that high-dose administrations of antioxidants may be toxic in some rodent organs, e.g., ingestion of Brussels sprouts extract in high doses diminishes 8-OHdG induced by 2-nitropropane in rat liver, but increases the endogenous level of 8-OHdG in the same organ. In fact, it is common that antioxidants in animal experimental models surprisingly reveal toxic rather than chemopreventive effects, e.g., higher levels of DNA oxidation have been observed in rat lymphocytes following supplementation with lycopene, quercetin, and resveratrol to the animals.

Intuitively, the most relevant way of exploring antioxidant effects in humans are supplementation trials, but measurements of oxidatively damaged DNA are often restricted to surrogate tissues such as white blood cells (WBC) and urine. The WBC are usually mononuclear blood cells encompassing lymphocytes and monocytes although some studies use total leukocytes. With the ability to detect 8-OHdG, the first of many antioxidant supplementation trials with focus on this lesion in WBC appeared at the beginning of the 1990s. At the same time, reliable detection of 8-OHdG in urine became possible, and this was soon followed by antioxidant trials with urinary 8-OHdG excretion as a key biomarker. However, by far the most popular assay in antioxidant intervention trials has been the comet assay, detecting DNA strand breaks (SB) or the enzyme-modified version of the comet assay, that detects oxidized purines (including 8-OHdG) and pyrimidines by formamidopyrimidine DNA glycosylase (FPG) and endonuclease III (ENDOIII), respectively. Also ex vivo exposure of cells to DNA strand breaking agents such as H₂O₂ or ionizing radiation has been used as a semiquantitative measurement of the donor’s antioxidative status. This modification of the comet assay is based on the notion that the intracellular content of antioxidants will affect the DNA breakage.

A brief survey of databases, like PubMed, reveals that more than 100 intervention studies have investigated the effect of antioxidants in human WBC and urine. Unfortunately, the results generated by many of the studies can be challenged because of poor design quality and