EDITORIAL

Exploring Antioxidants

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The antioxidant, reactive species, oxidative stress nexus

The term 'antioxidant' is used with ever-increasing frequency in both the popular media and biomedical literature. Notwithstanding its common usage, it can be a difficult term to define precisely. According to one current and useful definition from an authoritative source, antioxidant refers to "any substance that delays, prevents, or removes oxidative damage to a target molecule" (1). In the human body, antioxidants help to prevent, protect, or reduce damage to biological molecules from exposure to specific chemical substances, known as reactive species and which are often generated by endogenous metabolic processes. These reactive species comprise free radicals, such as the hydroxyl radical and superoxide radical, and non-radicals, which include hydrogen peroxide, peroxynitrite ions and hypochlorous acid (2). Notably, the range of biologically important reactive species is believed to be counteracted *in vivo* by an impressive array of antioxidant biomolecules. These include the antioxidant enzymes: catalase, superoxide dismutase and glutathione *S*-transferase; endogenously synthesized molecules such as glutathione and thioredoxin; and nutritionally derived antioxidants, such as vitamin C and vitamin E (3, 4). A severe imbalance between the generation of reactive species and their removal by antioxidants can result in a phenomenon typically referred to as oxidative stress (1). Oxidative stress, in turn, has been implicated in the pathogenesis of a wide variety of diseases, such as stroke, diabetes, breast cancer and severe acute malnutrition (5).

Complementary approaches to human antioxidant research

Perhaps the central challenge in understanding the potential contribution of antioxidants to human health and disease is to distinguish those situations where oxidative stress actually leads to disease, from those situations where it is merely a consequence of disease (arrived at *via* other means), or other situations where signs of apparent oxidative stress might, in fact, reflect augmented, albeit delayed and possibly insufficient, protection against disease. Accordingly, the possible roles played by antioxidants in human health and disease have been explored using a variety of different study designs. These designs generally can be assigned to one of four broad categories: epidemiological studies, intervention studies, studies using cellular models and *in vitro* characterization studies. The relative strengths and weaknesses of these designs ought to be kept in mind when interpreting the reports of studies based on them.

Antioxidant centred epidemiological studies encompass survey, case-control and longitudinal follow-up designs. Epidemiological studies can be used to establish a relationship between a proposed antioxidant and a particular disease condition and can also facilitate an estimate of the magnitude of any observed relationship (6, 7). Despite their unique strengths though, epidemiological approaches do not, by themselves, definitively establish causal relationships between a postulated antioxidant and a disease condition.

Clinical and field interventions investigate whether antioxidants can alter the course of particular health- or disease-related outcomes. For instance, some interventions are designed to discern whether antioxidant supplementation can change specific oxidative stress-related biomarker measures (8). Moreover, on occasion, interventional studies can concomitantly be used to test hypotheses pertaining to the contribution of a particular antioxidant to disease pathophysiology. However, interventional studies are not the design of choice for establishing disease aetiology.

In contrast, cellular models typically focus on the actions of antioxidants at the cellular and sub-cellular levels (9). These models can delineate the detailed mechanisms underlying the interactions among antioxidants, reactive species, and significant biomolecular targets (such as cell membranes and DNA) and their postulated effects on subsequent disease aetiology and pathophysiology. However, insights derived from such investigations often do not readily extend to allowing judgments as to whether the antioxidant of interest is important, or, indeed, relevant in influencing disease processes at the tissue, organ, or whole body levels.

Finally, *in vitro* antioxidant assessments can be invaluable for identifying and characterizing novel antioxidants obtained from natural sources. These assessments enable precise, quantitative estimates of antioxidant activity to be made and can be used to compare putative antioxidants with established antioxidants of measurable potency. A large and growing number of laboratory-based studies clearly demonstrate that chemical constituents derived from natural

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products can act as strong antioxidants *in vitro*. For instance, Costa Orsine and colleagues recently reported that extracts from the mushroom *Algaricus sylvaticus* exhibit potent antioxidant activity against the test oxidant DPPH (10). The results from this study present evidence consistent with the view that claims of the *Algaricus* mushroom's purported anti-atherogenic and anti-cancer properties might be attributable, at least in part, to its antioxidant properties. Nevertheless, the following caveat should be appended before extrapolating such promising findings to broader claims of potential health benefits: clearly demonstrable and reproducible *in vitro* antioxidant effects do not necessarily translate to clinically significant outcomes. More specifically, the myriad of appreciable differences between *in vitro* and *in vivo* environments means that drawing inferences from the test tube to living systems ought to be made cautiously. These *in vitro* studies do, however, provide a robust platform from which further characterization and investigation of putative physiologically relevant antioxidants can be launched.

CONCLUSION

The principal aim of this brief overview has been to provide a useable framework for interpreting findings from research on the role of antioxidants in human health and disease. In addition, it is hoped that it will help current and future researchers to select judiciously the best suited design(s) for answering their own particular antioxidant-related questions from among the existing repertoire of complementary research approaches. Suitably defined research questions and appropriately designed research studies should facilitate the combined efforts to generate and utilize sound, evidence-based knowledge of antioxidants for promoting health and protecting against acute and chronic disease.

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