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Review Article

Antioxidants as therapies: can we improve on nature?

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ABSTRACT

Although oxidative damage contributes to many pathologies the use of naturally occurring, small-molecule antioxidants as therapies for these disorders has not been successful. Here I discuss some of the reasons this may be so. Paramount among these are the difficulties in delivering enough of the antioxidant to the intracellular location required to decrease pathological oxidative damage and the challenge of assessing whether the intervention has actually decreased oxidative damage in the patient to a therapeutically useful extent. To develop effective antioxidant therapies the best strategy may be to create new chemical entities designed to detoxify a defined reactive oxygen species-dependent process that underlies a particular pathology, in the same way a conventional drug is designed to modulate a biochemical process, rather than applying antioxidants in an unfocused manner. In developing new antioxidants it will be useful to utilize endogenous processes to activate and recycle the molecules in parallel with the targeting of compounds to cells and organelles in ways that are not limited by the constraints that impair the distribution of endogenous antioxidants. In short, I suggest that the future development of antioxidant therapies should be viewed as an arm of drug development, utilizing focused approaches similar to those of medicinal chemistry and pharmacology, rather than as a branch of nutrition.

Contents

Introduction

As biomedical researchers in the oxidative stress and free radical fields, we all have an embarrassing secret. There is compelling evidence that oxidative damage contributes to cell death and dysfunction in a wide range of pathologies and that deficiencies in endogenous antioxidant defense and repair systems underlie many diseases—in fact it is difficult to think of a pathology in which oxidative stress does not play a role [1,2]. Consequently it is a short and obvious step to suggest that antioxidants should be an effective treatment for many diseases [2,3]. Furthermore, as many endogenous, small-molecule antioxidants (e.g., vitamin E, vitamin C, coenzyme Q) are cheap, orally bioavailable, safe in large doses, and absorbed and recycled within our bodies, they are excellent candidates for translation to the clinic [4]. However, the problem is that when we assess these antioxidants in controlled clinical trials they show very disappointing outcomes [5–8]. In fact, the larger and better conducted the clinical trail, the lower the therapeutic effect tends to be [5]. In this survey I consider some of the reasons trials of natural antioxidants have been disappointing and suggest ways of developing new chemical entities as antioxidants that may help overcome these limitations.

Why have natural antioxidants not been successful as therapies?

A large number of well-conducted clinical trials have been carried out using several different antioxidants on a range of pathologies with little improvement in clinical outcome for the

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patients [5]. Although it could be that if we hit on the right combination of disease, antioxidant, and treatment regimen a positive outcome would result, we should face up to the evidence and consider why antioxidants have been so disappointing clinically.

The first point to consider is whether these results are telling us that oxidative damage is not actually that important in disease pathology and is a poor therapeutic target. This goes against our instincts, as it is evident that high levels of reactive oxygen species and oxidative damage lead to cell death in vitro, in vivo, and in patients. However, do we know for sure that oxidative damage is a significant factor in the progression of a particular pathology? Of course, an increase in oxidative damage during a disease process could occur because it correlates with, rather than contributes to, the pathology. Thus, it is important to demonstrate that preventing oxidative damage actually leads to a clinically significant improvement [9,10]. We need to show not just that an antioxidant therapy decreases oxidative damage, but that the decrease in oxidative damage has a clinically significant impact. My view is that there will turn out to be many disorders in which oxidative damage does underlie the pathology and for which antioxidant therapies will be clinically useful. This view is supported by, for example, animal studies in which removal of antioxidant defenses increases pathology or overexpression of antioxidant enzymes improves the outcome [11–13]. However, this has to be demonstrated in patients for each disorder case by case. It is likely that in many cases oxidative damage will occur but not be directly causative [14], in which cases antioxidant therapy is unlikely to ever show benefit.

To address this critical point it would be good to measure changes in oxidative damage in patients and show that this damage correlated with the disorder and that prevention of the oxidative damage by the antioxidant treatment in question led to a clinical improvement [15,16]. At the moment the technical difficulties in assessing oxidative damage in vivo mean that we are often uncertain if the antioxidant therapy has actually led to a decrease in the extent of oxidative damage [14,17]. A corollary is that in many clinical trials it is difficult to determine if the lack of a success of an antioxidant is simply because it has not been delivered in sufficient amounts to prevent pathological oxidative damage. Alternatively, it may be that oxidative damage was prevented but that this had no impact on the pathology. Until these measurements are available, the outcome of many well-conducted antioxidant trials will remain ambiguous.

Another potential limitation to the development of antioxidants is whether they can block the many “positive” sides of reactive oxygen species, for example, by disrupting bacterial killing by neutrophils. More generally, the controlled production of reactive molecules such as hydrogen peroxide can act as a redox signal, linking metabolic processes by altering protein activity, for example, by the reversible modification of cysteine residues [18]. Consequently, the indiscriminate use of an antioxidant could do more harm than good by disrupting essential signaling processes. However, this concern can be addressed by measuring particular changes in reactive oxygen species and/or oxidative damage and by correlating these changes with the clinical outcome.

For an antioxidant to affect oxidative damage caused by a particular reactive species its activity must increase local antioxidant defenses sufficiently above background, endogenous levels to have an impact on oxidative damage. For many naturally occurring antioxidant compounds, such as vitamin E or vitamin C, the amount present within a particular tissue, cell, or organelle is regulated at multiple levels by alterations in absorption, distribution, uptake, and metabolism, with numerous feedback and homeostatic mechanisms to defend the levels present. Consequently, it can be difficult to increase many endogenous antioxidants much above the normal levels. Exceptions to this may occur in the rare cases in which antioxidant deficiency is due to a genetic defect in enzymes responsible for the synthesis of an antioxidant or in the proteins responsible for the absorption and distribution of a dietary antioxidant. In such cases dietary supplementation may work well to overcome the genetic defect. A related issue is that antioxidants do not act in isolation, but as an integrated defense network that combines the actions of small molecules with protective and repair enzymatic systems. All of these are subject to multiple layers of regulation in response to changes in reactive species and oxidative damage. Consequently, decreases in one antioxidant may be compensated for by upregulation of other defenses; conversely an exogenous antioxidant may cause a compensatory downregulation of endogenous protection, with no net increase in antioxidant defenses. Finally, and critically, the antioxidant is often relatively evenly dispersed throughout the body and cell, whereas the oxidative damage may be localized to particular cell types or organelles such as mitochondria. Consequently the overall antioxidant content within a tissue or cell may be adequate, but the local antioxidant concentration may be insufficient to deal with particular hotspots of oxidative damage. Thus there are many plausible reasons to explain the poor outcome of clinical trials of antioxidants.

How can we assess the efficacy of antioxidant therapies in vivo?

To improve the outcome of antioxidant trials in pathologies, there is a pressing need for methods to assess whether a particular antioxidant actually improves detoxification of a particular reactive species at a given stage in the pathology, at the right location, by increasing antioxidant defenses sufficiently above endogenous levels. This information, in conjunction with an assessment of the clinical outcome, is essential to determine if an antioxidant therapy is unlikely ever to be successful or whether improving the antioxidant efficacy is worth pursuing. To achieve this, the development of better markers of reactive oxygen species and oxidative damage in vivo are essential. This is a major weakness in our current investigations in experimental animal studies and is an even greater limitation in patient trials in which our options to assess antioxidant efficacy are far more limited. Ideally, we would like robust readouts of the levels of the particular reactive species within various tissues, cell types, and subcellular compartments and how these change over the course of the pathology. In addition we would like to be able to relate these changes to the levels of the exogenous antioxidants, the changes in posttranslational modifications to proteins, the levels of metabolites, the expression of genes at the transcriptional and protein levels, and also the amounts and types of oxidative damage. Clearly, we are a very long way from this ideal scenario, even in experimental animals, and the situation in patients is even less informative as analyses are restricted to a few oxidative damage markers in accessible body fluids. Even so, we need to assess as well as we can the effects of antioxidant administration so as to answer the critical questions: does the antioxidant intervention decrease a defined reactive species or type of damage at a particular location and time and does this improve the clinical outcome? There is an old saying about Christopher Columbus: “he didn't know where he was going when he set out, didn't know where he was when he got there, and didn't know where he had been when he got back.” Our technical limitations in assessing oxidative damage and reactive oxygen species in vivo mean that we are often in a similar, uncertain situation. The development of better biomarkers of oxidative damage is essential for the development of antioxidant therapies.