Biochemical and Biophysical Research Communications 393 (2010) 561–564

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Mini Review

Antioxidants: Molecules, medicines, and myths

John M.C. Gutteridge [a], Barry Halliwell [b,*]

[a] Potters Bar, Hertfordshire, UK
[b] Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

ARTICLE INFO

Article history:
Received 5 February 2010
Available online 18 February 2010

Keywords:
Free radical
Antioxidant
Supplements
Micronutrients
Vitamin E

ABSTRACT

There is an industry-driven public obsession with antioxidants, which are equated to safe, health-giving molecules to be swallowed as mega-dose supplements or in fortified foods. Sometimes they are good for you, but sometimes they may not be, and pro-oxidants can be better for you in some circumstances. This article re-examines and challenges some basic assumptions in the nutritional antioxidant field.

Introduction

The term antioxidant can be defined in multiple ways depending on the methods used to measure antioxidant activity and if the method changes the hierarchy of antioxidant activity will change [1]. Thus statements such as "*X* is the best antioxidant" or "*Y* is a better antioxidant than *Z*" mean nothing unless the assays used are described. To reflect this importance of methodology, we introduced a definition of an antioxidant as "any substance that delays, prevents or removes oxidative damage to a target molecule" [1]. Antioxidants can be complex molecules such as the superoxide dismutases, catalases and peroxiredoxins, or simpler ones such as uric acid and glutathione. As we shall further discuss, antioxidant activity may reside in cell structure.

The origin of antioxidants goes back to antiquity. In particular we are reminded of the remarkable technical knowledge of the ancient Egyptians in preserving dead bodies, in part due to the use of plant extracts rich in polyphenols. The oxidation or 'perishing' of rubber provided the incentive for systematic and detailed research into oxidation mechanisms and their prevention by simple chemical antioxidants. Although such studies were empirical in the late 1870s and early 1900s, by the 1940s free radical autoxidation mechanisms had been elucidated and some chain-breaking antioxidants identified (reviewed in [2]).

When Denham Harman proposed in the late 1950s that aging was a result of progressive changes caused by cumulative free radical damage, it immediately raised the possibility that antioxidant molecules might slow down the aging process and prolong lifespan. Feeding antioxidants, developed by the food and rubber industries, to rodents soon followed, and in several cases a statistically significant effect on lifespan was reported [3,4]. However, this has proven difficult to repeat, possibly because the antioxidants were correcting a life-shortening dietary deficiency rather than truly prolonging lifespan [1,5]. Nevertheless, these early experiments sowed the seeds of belief in the minds of both scientists and the general public that antioxidants might be the "elixirs of youth" that we had all been searching for.

The supplements and nutraceutical industries rapidly took these concepts on board. Not surprisingly, molecules with a simple structure dominate the nutritional supplements market because it is easy to make small molecular mass structures in quantity, put them in small bottles and sell them for large amounts.

Oxidation and reduction

Oxidation is a gain of oxygen or a loss of electrons, whereas reduction is a loss of oxygen or a gain in electrons. So called "pro-oxidants" promote oxidative damage in laboratory model systems whereas antioxidants inhibit. Standardisation of assay procedures is good practice in science. However, the assay procedures widely used to measure antioxidant activity *in vitro* often depend on the generation and trapping of non-biological radicals [1,2,6]. Such assays are useful when comparing the alleged *in vitro* antioxidant activities of berries or tea extracts but the results are usually biologically irrelevant [2,6]. Rarely do they translate into physiological effects [7–9]. Almost any chemical could affect the *in vitro* test systems currently used, leading to that chemical being classified as an antioxidant or pro-oxidant. Most research groups have

* Corresponding author. Address: National University of Singapore, Department of Biochemistry, 8 Medical Drive, Singapore 117597. Fax: +65 6872 0830.
E-mail address: bchbh@nus.edu.sg (B. Halliwell).

0006-291X/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2010.02.071

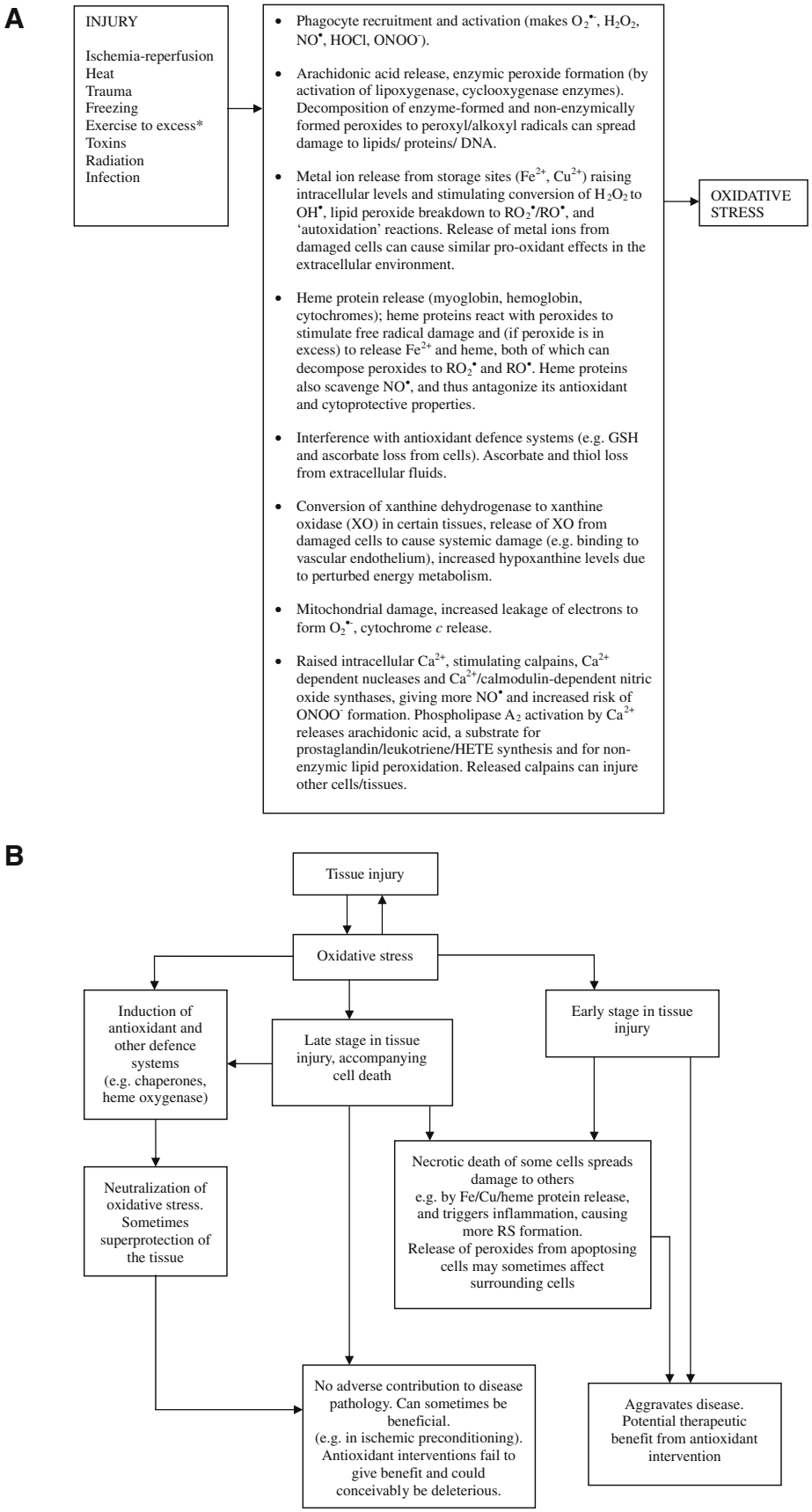

Fig. 1. Some of the reasons why tissue injury causes oxidative stress (A) and what oxidative stress means in the context of human disease (B). Adapted from [1] with permission of Oxford University Press. *To an extent that causes tissue damage/inflammation.

concentrated almost exclusively on antioxidants and oxidation. However, we should also be aware of the reductive side of the equation. Electrons must go somewhere [1], if something is oxidised by acting as an antioxidant then what will the oxidation product do next? Superoxide is a stronger reductant than it is an oxidant [1], and so we might consider superoxide dismutase to be an antireductant rather than an antioxidant enzyme, perhaps. Similarly, ascorbic acid (vitamin C) is certainly more of a reductant than an antioxidant, especially when low molecular mass iron is available [1,10,11]. In complex biological systems nothing works in isolation, if something is oxidised than something must be reduced and the electrons must balance.

Vitamins, trace elements, and micronutrients

Our Victorian grandparents' generation were rarely obese (unless as a result of a rare hormonal imbalance) and did not require the services of professional dietary advisors. Food was not processed and reconstituted to the degree it is today. Food processing and preserving for prolonged storage, transport, and enhanced shelf-life in supermarkets necessitates the adding of antioxidants to many products. Unfortunately, the public has become too familiar with the term 'antioxidant' and equates it to a chemical that is good for our wellbeing. Antioxidant foods, drinks (often in parallel unhealthily rich in sugars and colourants), cosmetics and creams abound.

As a result numerous vitamins, trace elements and micronutrients, often with well defined biochemical functions (such as ascorbate, a known cofactor for several enzymes) have been re-branded as 'universal antioxidants'. This implies that because they are antioxidants, the more one consumes the better it is for your health. The reverse could easily be true since over-consumption could down-regulate important endogenous antioxidants [1,12], depress parts of the immune system, or perhaps increase microbial damage or the normal cellular protective responses to tissue damage (Fig. 1) [12–16].

Worldwide, many tons of vitamin E have been consumed by the public with little or no beneficial effects to their health and even a suggestion of harm [17,18]. Perhaps the best we can hope for, as a result of this, is that the excreted vitamin E may have helped to decrease corrosion in our sewage systems, especially those using plastic piping [19]. Moderation rather than mega-consumption of purified nutrients is to be strongly recommended. Red wine is often held out as a tempting example of an excellent source of antioxidants. It contains flavonoids, other phenols and ethanol, the latter in high concentrations and with a second order rate constant for reaction with the hydroxyl radical of around $10^9\,M^{-1}\,s^{-1}$ [1]. How could too much red wine possibly be harmful? High alcohol consumption predisposes to many diseases however and polyphenols such as flavonoids are unlikely to be direct antioxidants *in vivo* [7,20].

Twenty-five years ago we pointed out that antioxidants would only significantly influence a disease process if free radicals or other reactive species caused or significantly contributed to the progression of the disease (Fig. 1) [21]. Then, and now [1,22], evidence supports the view that increased free radical formation is usually a consequence of tissue damage by a disease or toxin. Sometimes it contributes significantly, more often it does not and, in some cases, reactive oxygen species (ROS) may be exerting a protective role, e.g. by down-regulating inflammation (Fig. 1, also see [23–28]). Under such circumstances, assuming that an administered antioxidant could reach the site of increased ROS formation, it would at best marginally affect secondary tissue damage and could even be deleterious [1,17,18,23–25]. Sometimes pro-oxidants might be more beneficial [26–28]. Indeed, flavonoids may act just as much as pro-oxidants in the gastrointestinal tract and in cell culture systems as they do as antioxidants [20]. The biggest change in the past 25 years is public awareness of antioxidants. In 1984 only a handful of scientists were knowledgeable in this area whereas today antioxidants are a powerful part of everyday media advertising for foods and supplements.

Are free radicals and other reactive species really such villains?

Life evolved from an intense free radical-rich primordial 'soup', and without the simultaneous evolution of powerful antioxidants, life would never have got past the first cell division. The appearance of large quantities of molecular oxygen (itself a free radical) some 2.2 billion years ago provoked the need to further adapt to an aerobic environment, with a requirement for specialised protection against reactive forms of oxygen (reviewed in [1]). Antioxidant adaptation likely involved subtle changes to structural integrity and compartmentalisation of reactants (e.g. keep reactive oxygen species away from iron) within cells [1,10,11]. This is not such a complicated concept, just ask yourself which deteriorates faster and becomes rancid in the butcher's shop; a whole rabbit or minced rabbit? Unfortunately, antioxidants such as "structure" and "cellular compartments" cannot be simply measured in the laboratory. Because free radicals played such a major role in the evolution of plants and animals they are an essential part of the basic biology we study today [1,23,24,29,30]. Perhaps free radicals (at least for most of our lifespan) do not pose a great threat to our wellbeing unless we expose ourselves to an excess of free radical-generating agents such as cigarette smoke or ionising radiation. What we have adapted less well to, however, and does threaten our survival, is the ever-changing virulence of micro-organisms. The ability to sterilise a site of infection, by rapid production of reactive oxygen species ($O_2{\cdot}^-$, H_2O_2, HOCl, $ONOO^-$, etc.) can keep us alive. It does this now when we are challenged by a new pathogen and it certainly did before antibiotics and antivirals entered clinical medicine. The subjects who could mount a robust immune response with vigorous yet coordinated ROS production, would be selected for survival. During the 1980s, antibiotics were shown to be redox active and generate reactive oxygen species [1]. This led Gutteridge, Kovacic et al. in 1998 to propose that antibiotics mimic phagocytic calls by having a common mechanism of microbial killing using reactive oxygen species [31]. This concept has since been re-presented in detail [32].

Conclusion

Foods naturally containing antioxidants but not super-rich in calories, namely fruits, vegetables and grains, help maintain human health and delay disease onset. The contribution of antioxidants to these effects is uncertain. High-dose antioxidant supplements generally do no good and may cause harm [17,18,33]. Low dose mixtures, as in multivitamin/multimineral tablets, can sometimes do good, but may be beneficial only for those members of populations whose diet and lifestyle are so bad that they are deficient in certain micronutrients (reviewed in [1,22,34]). Perhaps we should spend more time exploring pro-oxidants as therapeutic [18,27], disease-preventing [1,22], and anti-aging [35–36] agents.

References

[1] B. Halliwell, J.M.C. Gutteridge, Free Radicals in Biology and Medicine, fourth ed., Oxford University Press, Oxford, 2007.
[2] G. Scott, Autoxidation and antioxidants: historical perspectives, in: Atmospheric Oxidation and Antioxidants, vol 1, Elsevier Science Publishers, Amsterdam, 1993, pp. 1–44.

[3] D. Harman, D.E. Eddy, Free radical theory of aging: beneficial effect of adding antioxidants to the maternal mouse diet on life span of offspring: possible explanation of the sex difference in longevity, Age 2 (1979) 109–122.
[4] A. Comfort, The biology of senescence, third ed., Elsevier Science Ltd., New York, 1979.
[5] J. Gruber, S. Schaffer, B. Halliwell, The mitochondrial free radical theory of ageing – where do we stand?, Front Biosci. 13 (2008) 6554–6579.
[6] G. Bartosz, Total antioxidant capacity, Adv. Clin. Chem. 37 (2003) 219–292.
[7] B. Halliwell, J. Rafter, A. Jenner, Health promotion by flavonoids, tocopherols, tocotrienols, and other phenols: direct or indirect effects? Antioxidant or not?, Am J. Clin. Nutr. 81 (2005) 268S–276S.
[8] C.Y. Lee, H.B. Issac, H.S. Wang, et al., Cautions in the use of biomarkers of oxidative damage; the vascular and antioxidant effects of dark soy sauce in humans, Biochem. Biophys. Res. Commun. 344 (2006) 906–911.
[9] R. Kelly, K.P. Yeo, H.B. Issac, et al., Lack of effect of acute oral ingestion of Vitamin C on oxidative stress, arterial stiffness or blood pressure in healthy subjects, Free Radic. Res. 42 (2008) 514–522.
[10] M.C.R. Symons, J.M.C. Gutteridge, Free Radicals and Iron: Chemistry, Biology and Medicine, Oxford University Press, Oxford, 1998.
[11] B. Halliwell, J.M.C. Gutteridge, Role of free radicals and catalytic metal ions in human disease: an overview, Meth. Enzymol. 186 (1990) 1–85.
[12] J.M.C. Gutteridge, Does redox regulation of cell function explain why antioxidants perform so poorly as therapeutic agents?, Redox Rep 4 (1999) 129–131.
[13] M. Ristow, K. Zarse, A. Oberback, et al., Antioxidants prevent health-promoting effects of physical exercise in humans, Proc. Natl. Acad. Sci. USA 106 (2009) 8665–8670.
[14] A. Childs, C. Jacobs, T. Kaminski, et al., Supplementation with vitamin C and N-acetyl-cysteine increases oxidative stress in humans after an acute muscle injury induced by eccentric exercise, Free Rad. Biol. Med. 31 (2001) 745–753.
[15] F. Leulier, J. Royet, Maintaining immune homeostasis in fly gut, Nat. Immunol. 10 (2009) 936–938.
[16] E.M. Ha, K.A. Lee, Y.Y. Seo, et al., Coordination of multiple dual oxidase-regulatory pathways in responses to commensal and infectious microbes in drosophila gut, Nat. Immunol. 10 (2009) 949–957.
[17] G. Bjelakovic, D. Nikolova, R.G. Simonetti, C. Gluud, Antioxidant supplements for prevention of gastrointestinal cancers: a systematic review and meta-analysis, Lancet 364 (2004) 1219–1228.
[18] G. Bjelakovic, D. Nikolova, R.G. Simonetti, C. Gluud, Systematic review: primary and secondary prevention of gastrointestinal cancers with antioxidant supplements, Aliment. Pharmacol. Ther. 28 (2008) 689–703.
[19] E. Oral, C.A. Godleski Beckos, A.J. Lozynsky, Improved resistance to wear and fatigue fracture in high pressure crystallized vitamin E-containing ultra-high molecular weight polyethylene, Biomaterials 30 (2009) 1870–1880.
[20] B. Halliwell, Are polyphenols antioxidants or pro-oxidants? What do we learn from cell culture and *in vivo* studies?, Arch Biochem. Biophys. 476 (2008) 107–112.
[21] B. Halliwell, J.M.C. Gutteridge, Lipid peroxidation, oxygen radicals, cell damage, and antioxidant therapy, Lancet 1 (1984) 1396–1397.
[22] B. Halliwell, The wanderings of a free radical, Free Radic. Biol. Med. 46 (2009) 531–542.
[23] M. Hultqvist, L.A. Olsson, K.A. Gelderman, R. Holmdah, The protective role of ROS in autoimmune disease, Trends Immunol. 30 (2009) 201–208.
[24] B. Halliwell, Phagocyte-derived reactive species: salvation or suicide?, Trends Biochem Sci. 31 (2006) 509–515.
[25] J. Hagenow, K.A. Gelderman, M. Hultqvist, et al., Ncf1-associated reduced oxidative burst promotes IL-33R$^+$ T Cell-mediated adjuvant-free arthritis in mice, J. Immunol. 183 (2009) 874–881.
[26] S.Y. Tang, I.K. Cheah, H.S. Wong, B. Halliwell, Notopterygium forbesii boiss extract, its active constituent phenethyl ferulate attenuate pro-inflammatory responses to lipopolysaccharide in RAW 264.7 macrophages. A "protective" role for oxidative stress?, Chem Res. Toxicol. 22 (2009) 1473–1482.
[27] M. Hultqvist, P. Olofsson, K.A. Gelderman, A new arthritis therapy with oxidative burst inducers, PLoS Med. 3 (2006) e348.
[28] J.W. Zmijewski, E. Lorne, Y. Tsuruta, et al., Antiinflammatory effects of hydrogen peroxide in neutrophil activation and actue lung injury, Am. J. Resp. Crit. Care Med. 15 (2009) 694–704.
[29] C.K. Sen, L. Packer, (Eds.), Redox Cell Biology and Genetics. Part A & B. Methods in Enzymology. Academic Press, New York, 2002
[30] B. Halliwell, Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life, Plant Physiol. 141 (2006) 312–322.
[31] J.M.C. Gutteridge, G.K. Quinlan, P. Kovacic, et al., Phagomimetic action of antimicrobial agents, Free Radic. Res. 28 (1998) 1–14.
[32] M.A. Kohanski, D.J. Dwywe, B. Hayete, et al., A common mechanism of cellular death induced by bactericidal antibiotics, Cell 130 (2007) 781–783.
[33] L. Melton, The antioxidant myth, Reader's Digest (2007) 56–62.
[34] S. Hercberg, K. Ezzedine, C. Guinot, et al., Antioxidant supplementation increases the risk of skin cancers in women but not in men, J. Nutr. 137 (2007) 2098–2105.
[35] S.I. Rattan, Hormesis in aging, Ageing Res. Rev. 7 (2008) 63–78.
[36] J. Gruber, L.F. Ng, S.K. Poovathingal, B. Halliwell, Deceptively simple but simply deceptive – *Caenorhabditis elegans* lifespan studies: considerations for aging and antioxidant effects, FEBS Lett. 583 (2009) 3377–3387.