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COMMENTARY

OXYGEN RADICALS, A FAILURE OR A SUCCESS OF EVOLUTION?

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Oxygen radicals are no doubt involved in the development of many pathological states. Nevertheless, the possibility that oxygen radical production was selected for during biological evolution in order to perform useful roles in relation to cellular metabolism is contemplated; previous data on this subject are briefly reviewed. The concept of an "oxygen radical cycle" is proposed as a useful theoretical model.

KEY WORDS: Free radicals, superoxide dismutase, catalase, glutathione peroxidase, superoxide, hydrogen peroxide.

INTRODUCTION

There is no doubt that oxygen radicals, and more generally free radicals, are involved in the development of various pathological states such as ischaemia-reperfusion injury[1] or inflammation.[2] Their implication in other degenerative diseases including cancer,[3,4] arteriosclerosis,[5] Alzheimers and Parkinsons disease,[6] is increasingly suspected. The wide range of diseases in which they seem to be involved, together with the enormous amount of literature that is published on the subject, sometimes leads to the global consideration of oxygen radicals as *deleterious* agents. One of the most consistent sources of oxygen radicals among tissues is the mitochondrial respiratory chain, specially at the ubiquinone[7] or NDAH[8] site. There, a small proportion (around 1%) of the oxygen consumption is partially reduced to $O_2^{\cdot-}$ and H_2O_2. That this happens is unquestionably true. Nevertheless, it is frequently assumed that this represents some kind of evolutionary "inefficiency". It is reasoned that no system can be 100% effective. Then, even though 99% of mitochondrial O_2 consumption is tetravalently reduced to water, it is considered as *unavoidable* that a small amount of O_2 be incompletely reduced to active oxygen species. Oxygen radical generation, according to that view, would be a representation of the incomplete "perfection" of living things.

I think that this view can be unfounded. We can prove that oxygen radicals are formed in the cell, but we can not know at present if their generation represents an evolutionary "failure" or, on the contrary, a physiological trait that was even selected for during evolution. An important suggestion (not a proof) concerning the evolutionary significance of mitochondrial oxygen radical generation can be obtained

from the mechanism of oxygen reduction at cytochrome oxidase. There, a sequential two electron path has been suggested, together with the existence of three intermediate states, oxy, peroxy and fully oxidized.[9] Nevertheless, no reactive oxygen intermediates are liberated to the medium at cytochrome oxidase and all (100%) the oxygen is reduced to water. This shows that the development of a system that reduces oxygen to water in various electron steps without releasing reactive oxygen intermediates is not an impossible task for the evolutionary process.

Thus, the possibility that the release of oxygen radicals at the mitochondrial respiratory chain is a controlled process cannot be discarded at present. The evolutionary significance of oxygen radicals could be one of serving useful purposes.[10,11] Their implication in pathological states will occur in situations in which the level of cellular oxidative stress (the balance between prooxidant factors and antioxidants) gets out of control. This can happen due to an exaggerated oxygen radical production, a decrease of antioxidants, or an increase in the amounts of macromolecules specially susceptible to oxidative damage. But oxygen radicals would not be "essentially" deleterious. This view has been proposed previously[12,13] as R. J. P. Williams has suggested: "If radicals had been so dangerous, surely during the process of evolution they would have been avoided; in fact they are used by all cells. The risks have always to be measured against the advantages in evolution, or elsewhere".[13] Thus, controlled production of oxygen-derived radicals could be used for metabolic purposes, even though this approach has been, with some exceptions,[10,11] rarely referenced.

POSSIBLE USEFUL ROLES OF OXYGEN RADICALS

What can be the nature of those purported useful purposes? There is no doubt that our knowledge about "benficial" effects of oxygen radicals is very limited. This can be related to the logically strong interest in the study of the causes of free radical related pathologies in humans. Nevertheless, some data are from time to time repeatedly appearing in the literature, suggesting useful roles for oxygen radicals. The following is a short summary of these findings.

Modulation of important cellular second messengers such as cyclic GMP has been reported to occur due to the effect of oxygen radicals,[14] $O_2^{\cdot-}$,[15,16] $OH^{\cdot}$,[17], H_2O_2,[18-20] hyperoxia,[21] or metabolism of H_2O_2 by catalase[22] upon guaylate cyclase activity. Organic radicals are thought to be involved in the synthesis of deoxyribonucleotides mediated by the enzyme ribonucleoside diphosphate reductase[23,24] and in the regulation of the endothelial-derived relaxing factor (EDRF).[25] In this respect, it has been recently showed that not only endothelial cells, but also neurons can produce $O_2^{\cdot-}$ and nitric oxide ($NO^{\cdot}$) leading to peroxynitrite ($ONOO^-$) generation.[26-28] The production of $O_2^{\cdot-}$ by the NADPH oxidase present in the cellular membrane and in the phagocytic vesicles of neutrophils, macrophages, monocytes and eosinophils is an important and well established part of the defensive systems of the body.[29-31] A similar membrane-bound H_2O_2 producing-NADPH oxidase, found in the membrane of rat adipocytes, can be involved in a proposed role of H_2O_2 as "second messenger" of insulin.[32-35] Production of $O_2^{\cdot-}$ by membrane NADPH oxidases has been recently demonstrated also in B-lymphocytes,[36-40] fibroblasts,[41-43] or in human glomerular mesangial cells.[44] Other chemical messengers whose synthesis has been related to organic hydroperoxides or H_2O_2 are thyroxine,[45-47] prostaglandins,[48-51] and

leukotrienes.[52] It has been even suggested that oxygen radicals are involved in fundamental and general processes such as development ad differentiation.[53,54] If this is true, the relationship of cancer with oxidative stress[3,55] could be due again to situations in which the normal effects of free radicals get out of control. Other important physiological processes which have been related to $O_2^{\cdot-}$ are: membrane potential,[56-58] the effect of vitamin K_1 on synthesis of prothrombin and coagulation factors VII and IX,[59] platelet aggregation,[60,61] metabolism of xenobiotics,[62,63] or 2-oxoglutarate-dependent hydroxylation.[64] All these are normal physiological processes important for the maintenance of homeostasis in different tissues and species. On the other hand, in addition to their involvement in many pathologies, free radical production and lipid peroxidation are normally and acutely stimulated physiologically during periods of high oxygen consumption, as it occurs in the muscle fiber during exercise[65-68] or in the brown adipose tissue during non-shivering thermogenesis.[69-72] In addition, more tissue or species-specific roles of free radicals have been also described in relation to: appearance of fertilization membrane in the sea-urchin egg in order to avoid polyspermy,[73,74] the development of bioluminescence in invertebrate animals,[75] the defense reaction of bombardier beetles against intruders,[76] the wound response of plant tissues,[77-79] the synthesis of lignin in plants,[80] or the synthesis of ATP in the hydrogenosomes of parasitic protozoa.[81,82]

THE "OXYGEN RADICAL CYCLE"

The "oxygen radical cycle" depicted in Figure 1 can help to remember the possibility that free radical production evolved for the development of useful purposes related to cellular metabolism. The cycle is completed with two well known enzymatic reactions, those of superoxide dismutase (SOD) and catalase (CAT). In both cases one or two of the products of the catalyzed reaction feeds back on the cycle. This is well known, but the organization of the drawing helps to stress this cyclic character. The O_2 production by SOD and CAT can be of minor relevance under basal conditions in relation to that released from hemoglobin at tissue capillaries; but this cyclic character could partially avoid a decrease of tissue pO_2 levels in situations in which O_2 radical production is greatly increased (if the system would not produce O_2, the O_2 radical burst followed by O_2 radical scavenging could quickly lead to local hypoxia, thus limiting many cellular oxygen-dependent functions). Nevertheless, it must be stressed that the balance between O_2 consumption and production during full operation of the cycle (the $O_2^{\cdot-}$ production plus the SOD and CAT reactions) is not complete since the stoichiometry of the reactions shows that only 3 molecules of O_2 are produced for every 4 molecules of oxygen consumed in each turn of the cycle, the fourth O_2 molecule being reduced to 2 molecules of water similarly to what occurs in the cytochrome oxidase reaction, but in this case without any coupled ATP production (see Figure 1 and its insert).

The cycle intermediates finally come from the environment (diet or respiration), and are eliminated by peroxidases such as glutathione peroxidases (GPx), which can then be fully considered as oxygen radical scavenging enzymes. All the antioxidant enzymatic systems can be regarded as regulators of the levels of oxygen radicals which can have their own physiological functions in the tissues. Some possible consequences or predictions of the cycle would be: 1) an increase in SOD not accompanied by a high enough increase in CAT can result in high H_2O_2

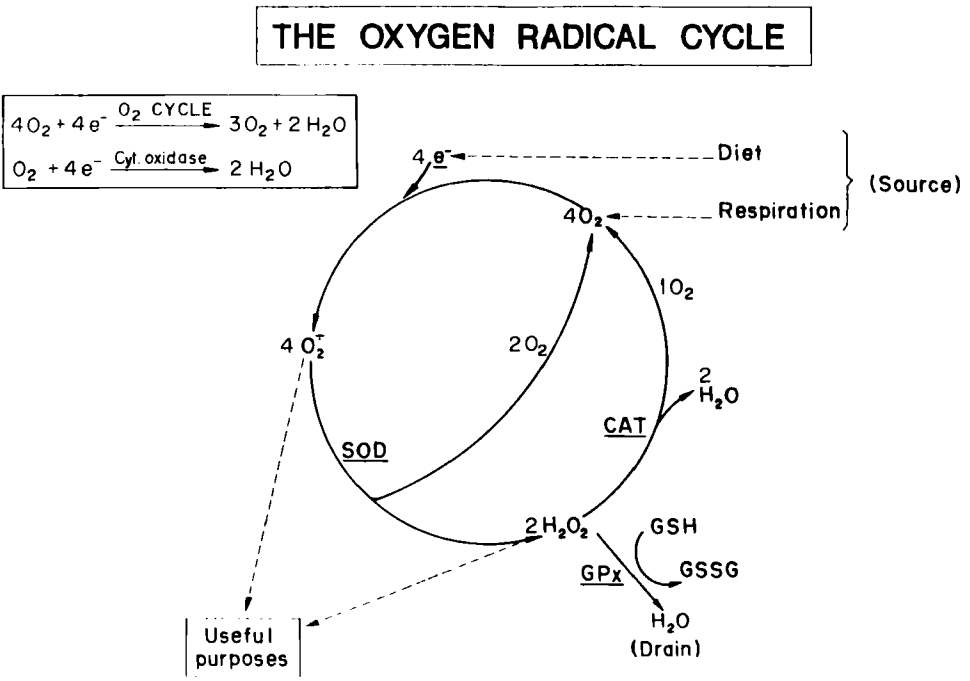

Figure 1 The oxygen radical cycle. Antioxidant enzymes regulate the levels of active oxygen species which can be involved in normal physiological processes when held at appropriate levels. SOD = superoxide dismutase; CAT = catalase; GPx = glutathione peroxidase; GSH and GSSG = reduced and oxidized glutathione; e^- = electrons.

concentrations, since SOD does not in fact eliminate cycle intermediates and produces H_2O_2; this can be related to the negative effects of strong SOD supplementation reported in various model systems, since H_2O_2 can produce oxidative damage through $OH^\cdot$ formation in the presence of a reducing agent such as ferrous iron. 2) an excess of CAT would not be so negative, initially at least, since it simply produces basal O_2. 3) a simultaneous and very strong increase of both SOD and CAT could expend metabolic energy, since it would reduce O_2 to water, using electrons from diet-derived substrates, without leading to ATP production. 4) an increase in the intake of food could theoretically elevate the tissue levels of $O_2^{\cdot-}$ and H_2O_2; if oxygen radicals are finally involved at the root of the aging process, this can be related to the fact that the only manipulation that unquestionably decreases aging rate is caloric restriction in the diet. 5) similarly, an increase in tissue oxygenation, or in oxygen consumption during exercise, can be the cause of the well known increase in oxidative stress trough an augmentation of cycle intermediates. All this is compatible with useful roles for oxy radicals if the concept of prooxidant-antioxidant balance, which is gaining acceptance nowadays, is held: if this balance is disrupted, excess concentrations of oxygen radicals results, leading to tissue damage. Finally, even if some of those predictions were wrong, I think that the study of possible useful roles of oxygen radicals merits further attention from the scientific community. A greater understanding of the causes and mechanisms of human pathologies is urgently needed.

But they would be perhaps highly clarified if we can manage to unveil previously unknown fundamental roles of free radicals in the tissues.

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