Antioxidants, DNA Damage and Gene Expression

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INTRODUCTION

Oxygen is the basis for mammalian life, as we know it. By four-electron transfer oxygen is reduced to water and the generated chemical energy is stored in a transportable form. Yet reduction of the ubiquitous oxygen can be incomplete and reactive oxygen intermediate species, ROS, are generated. Likewise, reactive nitrogen species, RNS, can be generated.1[1] ROS/RNS such as singlet oxygen, superoxide anion, hydrogen peroxide, the hydroxyl radical, peroxynitrite and hypochlorite all have dual actions from a biological point of view. On one hand, they pose a serious threat of deleterious effects by oxidising important structures and macromolecules in the cells and on the other hand they also function as part of defence and signalling mechanisms. Often this is referred to as the double edge sword nature of free radical chemistry. Pro- as well as eucaryotes have developed a complicated defence network system to control ROS/RNS. This network function includes enzymatic and non-enzymatic antioxidants, various repair
mechanisms and control mechanisms for elimination of severely but not mortally injured cells, i.e. apoptosis. It should be noted that in a chemical sense the term antioxidant is poorly defined, in chemistry there are oxidants and reductants. Therefore, the term antioxidant relates to a biological system and relates not to simple chemical reductants but to substances that are capable of performing a reduction-oxidation cycle and thereby transport electrons from very reactive molecules to less reactive molecules.

Recent developments in the construction of transgenic and knockout animals have brought more knowledge indicating the pathogenetic relevance of oxygen radicals. A transgenic mouse model for Huntington’s disease shows increased levels of malondialdehyde, 8-oxodG, 3-nitrotyrosine and heme oxygenase in areas of brain degeneration. Transgenic rats expressing glutathione S-transferase placental form have lower 8-oxodG levels in the liver and overexpression of Cu/Zn SOD or catalase also seem to protect against oxidative DNA damage. Furthermore, animals homozygous for specific glycosylase defects in OGG1 have high levels of 8-oxodG, however, only with a moderately elevated spontaneous mutation rate. A heterozygous manganese SOD knockout mouse with 50% decreased mitochondrial SOD activity showed increased oxidative damage. Collectively these data point to oxygen radicals as important pathogenic mechanisms.

OXIDATIVE STRESS AND ANTI-OXIDANTS

Antioxidants have attracted interest, particularly those that are dependent on dietary intake. An imbalance between oxidants and antioxidants, called oxidative stress, will lead to deleterious effects that are pathogenetically important for development of diseases. Regarding atherosclerosis the event is considered oxidation of LDL regarding cancer and ageing one important event is believed to be oxidative modification of DNA. These ideas led to huge intervention trials with antioxidants such as β-carotene and α-tocopherol. The first one showed a reduction in cancer incidence after supplementation with a combination of antioxidants. However, three large and long running trials were negative and one even indicated increased lung cancer from β-carotene supplementation. These trials have been disappointing in showing no effect, yet there is clear biochemical evidence of the effect of such antioxidants and of oxidants on the cellular signalling system and gene expression. Recently, this disappointment seems to be overcome. A combination of vitamins C and E proved efficient in reducing pre-eclampsia in women and an unpublished trial has demonstrated reduced arteriosclerotic progression from a combination of vitamins E and C supplementation. The present review will focus on gene expression and cellular signalling. Oxidative DNA damage has been reviewed extensively by us before.

SIGNAL TRANSDUCTION AND GENE EXPRESSION

During evolution aerobic organisms have evolved with an absolute dependence on oxygen. The restriction to live in an oxygen rich environment has provided no general need for oxygen sensors, except for special physiological functions, e.g. the carotid body. In contrast facultative and anaerobic organisms have general oxygen sensors in order to cope with shift from an anoxic environment to an oxygen rich environment. Mammalian cells require oxygen for most of their energy needs, for survival and for reproduction. The oxidative state, if sufficiently high, can be viewed as a sort of cellular beneficial state that could signal or allow critical biological events, i.e. mitogenesis. It appears that a variety of cellular signals can be elicited by both oxidants and mitogens. However, in contrast to specific mitogenic stimuli, oxidants, besides
stimulating mitosis, also have considerable toxicological implications.

From a review of the literature it is evident that in eukaryotes there are no transcription factors that exclusively are activated by reactive oxygen species, and it can be hypothesised that such transcription factors do not exist. Detailed review of the molecular biology of redox regulation of cellular signalling and gene expression can be found elsewhere.\textsuperscript{[29,30]}

The cellular response to a redox change appears to be highly variable. Upregulation and down-regulation can be seen in different systems and the same system can react differently depending on the magnitude of the redox change. This is rather the rule than the exception, as can be seen from Table I. As clearly indicated for instance by protein kinase C and the caspases, the same enzyme system may react differently to different levels of oxidative stress/antioxidant status. In Table II, some selected responsive elements are tabulated similarly and they also show a highly varying response from oxygen and antioxidants. In Table III, the list of signalling molecules also shows a highly variable effect of oxidants.

It can thus be concluded that although antioxidants can have a direct effect in reducing oxidative deleterious effects, i.e. act as antioxidants, they have pronounced secondary effects that come from a change in redox status. Presumably, the functional changes that come from changes in signal transduction and gene expression will need some time to become established. In the search for antioxidant effects \textit{in vivo} it is therefore important not only to look at immediate effects but also at the long-term effects. The redox status can be modulated from changes in antioxidant levels, however, also changes in oxygen consumption, e.g. from exercise, might be relevant, as reviewed earlier.\textsuperscript{[21] The effects of the increased oxygen consumption from exercise are highly variable, also with time.

A prime example of the unpredictable effects of modulation of response to changes in

<table>
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<tr>
<th>Table II</th>
<th>Oxidative stress and intracellular signalling</th>
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<tr>
<td>Elements</td>
<td>Effect of oxidants (O) and antioxidants (A)</td>
</tr>
<tr>
<td>NF-κb</td>
<td>Activation (O) blocking (A)</td>
</tr>
<tr>
<td></td>
<td>controversial</td>
</tr>
<tr>
<td>AP-1</td>
<td>Activated (O,A) blocking (O,A)</td>
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<tr>
<td>Ca\textsuperscript{2+}</td>
<td>Intracellular increase (O)</td>
</tr>
<tr>
<td>AP-1</td>
<td>Activated (O)</td>
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<tr>
<td>p53</td>
<td>Complex regulation</td>
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</tbody>
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<thead>
<tr>
<th>Table I</th>
<th>Oxidative stress and gene regulation/activity</th>
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<tr>
<td>Gene/enzyme/proteins</td>
<td>Regulation by oxidants</td>
</tr>
<tr>
<td>JUN kinase (JNK)</td>
<td>Induced</td>
</tr>
<tr>
<td>c-FOS</td>
<td>Increased transcription</td>
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<tr>
<td>MAP kinase</td>
<td>Stimulation</td>
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<tr>
<td>Tyrosine phosphatase</td>
<td>Inhibition</td>
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<tr>
<td>KAM-1, IL-1alpha, IL6, IL8, heme oxygenase</td>
<td>Induced</td>
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<tr>
<td>Metal binding proteins (MT-genes)</td>
<td>Induced</td>
</tr>
<tr>
<td>Heme oxygenase-1</td>
<td>Induced</td>
</tr>
<tr>
<td>Caspases</td>
<td>Induced</td>
</tr>
<tr>
<td>PKC</td>
<td>Induced</td>
</tr>
<tr>
<td>PKC</td>
<td>Inactivated</td>
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<tr>
<td>Ras</td>
<td>Activated</td>
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<tr>
<td>Phospholipase A and D</td>
<td>Activated</td>
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oxidants/antioxidants stems from the ATBC study with long time intervention with high doses of \( \beta \)-carotene.\(^{14} \) Unexpectedly, this trial demonstrated an increase in lung cancer risk from \( \beta \)-carotene intervention particularly related to smoking individuals. Following that observation animal studies looking at \( \beta \)-carotene supplementation and exposure to cigarette smoke revealed that expression of genes for retinoic acid receptors and activator protein-1, the latter encoded by the c-JUN and c-FOS genes, indicated suppression of RAB\( \beta \) gene expression and overexpression of activator protein-1, which combined with a strong proliferative response in lung tissue and squamous metaplasia could indicate a mechanism for enhanced lung tumourigenesis.\(^{31} \) These trials clearly indicate that modulation of oxidative stress by tobacco smoke – a substantial oxidative stress inducer in humans – combined with an antioxidant, have a highly differential effect on gene expression.\(^{31} \)

Since a major target of oxidative insult is DNA, it is of particular interest whether increased oxidative stress has any effect on DNA repair enzymes. A variety of specific repair enzymes for oxidative DNA modification has been described.\(^{32} \) In general there is a lack of quantitative methods for estimation of DNA repair, particularly in vivo and most of the data on repair of DNA oxidative products in vivo are indirect. In Alzheimer’s disease decreased repair has been indicated.\(^{33} \) High vegetable consumption has been shown to reduce the genetic damage, and this has been attributed to enhancement of cytosolic glutathione transferase and DNA repair proteins by substances in tomato and carrot juices.\(^{34} \) Indirectly, it is also suggested that cigarette smoking increases repair of 8-hydroxyguanine.\(^{35} \) In general it is believed that most of the DNA repair enzymes are housekeeping genes that are not subjected to regulation.\(^{32} \) However, it should be recognised that presently it cannot be ruled out that regulation of repair enzymes can occur.

Besides effects on DNA repair mechanisms, effects on a variety of antioxidant enzymes could be of interest, but so far there is no clear intervention study to demonstrate stimulation of these enzymes in vivo. However, it appears possible to develop gene therapy for oxidant induced diseases. In rats instillation of an adeno-virus vector encoding human superoxide dismutase or catalase c-DNA led to expression in the lungs of human catalase and CuZn-SOD that lasted for at least 3 days. Overexpression of SOD led to increased ischaemia–reperfusion injury whereas concomitant overexpression of catalase prevented this effect.\(^{36} \) Again this demonstrates the importance of multiple regulation by oxidants/antioxidants and the difficulties in predicting the outcome of intervention.
In conclusion there is clear evidence that antioxidants/oxidants are important for a multitude of different cellular functions and that there is a regulation of signal transduction pathway and gene expressions by the redox status for some cellular functions. The influence of, e.g., supplementation with an antioxidant is difficult to predict and ultimately controlled trials are needed to assess the overall effect.

References


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