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Overview

# Oxidative Stress and Antioxidants: Biological Response Modifiers of Oxidative Homeostasis in Cancer

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## Abstract

*Oxidative stress is defined as a misbalance in cell redox reactions resulting in the increase of reactive oxygen species (ROS) and/or decreased antioxidant defence. Crucial part of oxidative stress, which avoids protective effects of antioxidants, is lipid peroxidation (LPO). Since LPO comprises several chain reactions allowing the spread of ROS-caused damage to the macromolecules (proteins, nucleic acids and lipids) it is important in pathogenesis of various diseases such as cardiovascular diseases, neurodegenerative diseases, diabetes mellitus and cancer. On the other hand, research on physiology and pathology of lipid peroxidation revealed that not only ROS but also the LPO products such as 4-hydroxynonenal (HNE) are involved in physiological homeostasis of various tissues. This lead to conclusion that LPO and oxidative stress and not only pathological but also physiological processes. Accordingly, HNE was revealed as biomarker, growth regulating factor and signalling molecule.*

*In this light, both natural as well as synthetic antioxidants could be considered as »biological response modifiers« maintaining oxidative homeostasis. Accordingly, some antioxidants might eventually become important components of advanced individual and integrative biomedicine.*

## OXIDATIVE STRESS AND REACTIVE OXYGEN SPECIES

Oxygen is essential for aerobic organisms, but it can also be harmful because of formation of reactive oxygen species (ROS), and thereby oxidative stress. Oxidative stress is defined as a misbalance in cell redox reactions *(1)* which can be the result of either ROS overproduction or decreased antioxidant defence. As ROS are produced in cells under physiologic conditions, they induced evolution of different antioxidative mechanisms of cellular defence against oxidative stress (Figure 1).

### ROS and Lipid Peroxidation

Reactive oxygen species include radical species, such as superoxide anion ($\bullet O_2^-$), hydroxyl radical ($\bullet OH$), and also non-radical species, such as hydrogen peroxide ($H_2O_2$), singlet oxygen ($^1O_2$), hipochloric acid (HOCl) and ozone ($O_3$). Radicals are produced either in controled or uncontroled manner. One of the main sources of ROS is leakage of electrons from the respiratory chain *(2)*. Complex I and III of the respiratory chain are the source of superoxide anion *(3)*. Similar reactions

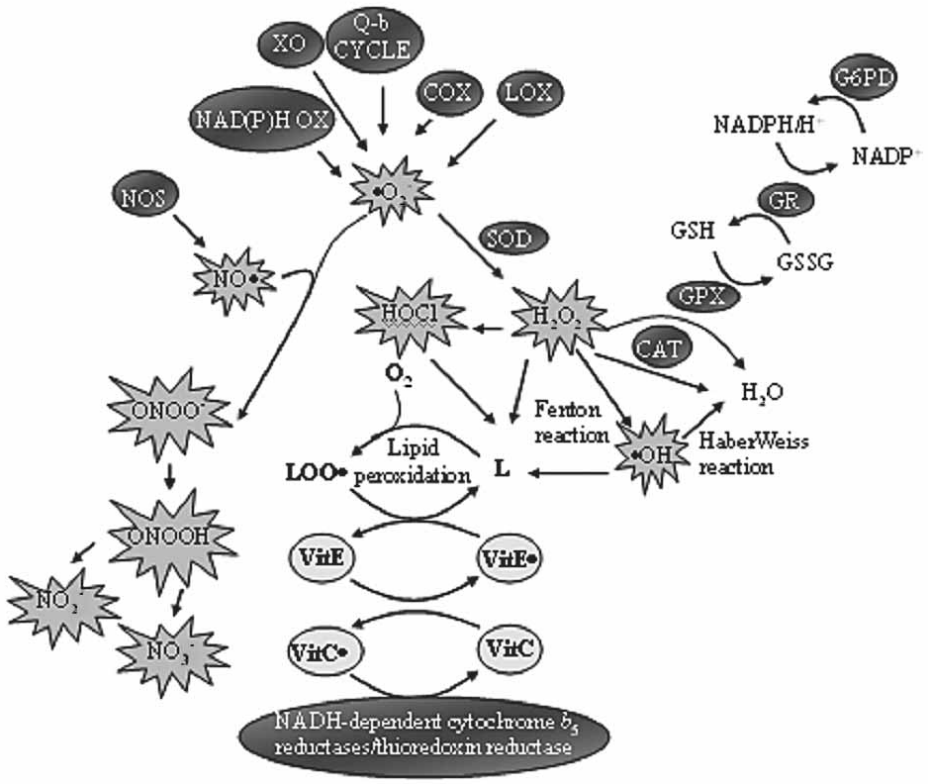


**Figure 1.** *Redox cycle of the cell and antioxidant defence from radical attack. Superoxide radical ($O_2^-$) is produced by NAD(P)H oxidase (NAD (P)H-OX), cyclooxygenase (COX), lipoxygenase (LOX), xanthine oxidase (XO), and by mitochondrial ubisemiquinone-cytochrome b (Q-b) cycle. Other radicals, such as $ONOO^-$, are formed in the cell are derived from other reactive oxygen or nitrogen species like nitric oxide (NO) and superoxide radical. NO is formed by NO synthase (NOS). $O_2^-$ is then transformed by superoxide dismutase (SOD) to hydrogen peroxide ($H_2O_2$), which can be further neutralized to water by catalase (CAT), glutathione peroxidase (GPX) or can undergo Fenton reaction. Oxidized glutathione (GSSG) from GPX reaction is regenerated by glutathione reductase (GR), which cycles with glucose-6-phosphate dehydrogenase (G6PD). Reactive oxygen species cause lipid peroxidation which is stopped in membrane by vitamin E, consequentially regenerated through vitamin C cycle.*

are generated by cytochrome P450. Group of cytochrome P450 enzymes (P450 or CYP) contain haeme in their active site, and are located in endopasmatic reticulum of hepatocytes *(4)*. Cytochrome P450 is known as one of the major enzymes involved in the metabolism of various drugs, and thereby metabolising and activating carcinogens *(5)*. Cytoplasm is potential source of radicals due to high content of iron and other transition metals.

Iron participates in several biological reactions and is essential for living organisms. Iron-containing proteins of the respiratory chain are involved in electron transport to provide the energy for cellular functional activities *(6)*. Iron is also required for cell growth and multiplication. However, the complex mechanism of the influence of iron on cell proliferation is not entirely understood. It is known that iron plays an important role in the activity of ribonucleotide reductase, a key-enzyme in DNA synthesis responsible for the reduction of ribonucleotides to deoxyribonucleotides *(6)*. This enzyme turns over rapidly and needs a continuous supply of iron to maintain its activity. On the other hand, metal ions, in particular those of iron, are required for formation of •OH which might mediate DNA damage induced by $H_2O_2$ and $•O_2^-$. Unlike these uncontrolled radical production, radicals are produced in »oxidative burst« of leukocytes *(7)*, iodide oxidation in thyroid gland *(8)*, and in prostaglandin synthesis from arachidonic acid *(9)*.

Oxidative stress affects all cell macromolecules, including DNA, causing mutations, proteins, causing inactivation, and lipids causing lipid peroxidation. Polyunsaturated fatty acids are especially sensitive to oxidative degradation caused by free radical reactions in cells *(11)*. This process results in the production of highly reactive aldehydes which are proposed to be »second toxic messengers« for the primary free radicals which initiated lipid peroxidation *(11)*. The highly reactive aldehyde 4-hydroxynonenal (HNE) appears to be one of the major cytotoxic products of lipid peroxidation that could be found in various tissues even under normal, physiological conditions *(12)*. Unlike free radicals or the other ROS, HNE has the unique feature to remain stable, not metabolised, by binding to macromolecules (such as proteins). Such HNE-macromolecular conjugates might be even required for the biological activities of HNE and could be detected by the use of monoclonal antibodies *(13)*. Furthermore, some of the results suggest that the modification of cell growth *in vitro* in presence of physiological concentrations of HNE (1μM) could further de-

pend on the presence of certain serum factors, but not serum albumin which is known to couple with HNE, thus decreasing the cytotoxic effects of the aldehyde *(14)*. Today, HNE is known to important in signalling pathways for cell cycle arrest, differentiation, regulation of gene expression (p53 and c-fos especially), and apoptosis *(10, 15)*. Consequentially, HNE acts as bifunctional (stimulating as well as inhibiting) regulator of the c-fos gene transcription thereby having an essential role in »turning inflammation against cancer«. In agreement with this are the findings of Poli and co-workers who showed a decrease of HNE and TGF-beta in patients with colon cancer, while Chron's disease (an aggressive inflammatory disease of the intestine) was associated with an increase of HNE and TGF-beta production *(16)*. These unique regulatory features explain presence of HNE in many diseases, such as cancer, neurodegenerative diseases, and autoimmune diseases *(17)*. Taken together, these findings are of general importance for the understanding of the mechanisms of the biological effects of not only HNE but of ROS and oxidative stress in general.

## Antioxidant Mechanisms

Due to deleterious effects of oxidative stress and lipid peroxidation, cells developed different mechanisms to cope with the challenge. Generally, mechanisms of antioxidative defence are grouped in two major groups: non-enzymatic and enzymatic systems.

Enzymatic mechanisms of ROS detoxification are intensively studied. These enzyme systems can be divided into two groups. One group reacts directly with ROS, while other act as redox regulators resulting in redox balance *(18)*. In addition, there are separate enzymatic systems with identical activity regarding different cellular compartments. Duplication of enzymatic systems ensures optimal protection against deleterious effects of oxidative stress, as well as different regulation and site specific antioxidant mechanisms. Non-enzymatic antioxidative systems are not as specific as enzymatic, but nevertheless, they are the first line of antioxidative defense, and therefore are certainly not negligible.

Superoxide dismutases (SOD) are the first line of defense against superoxide anions. SOD catalyses disproportion of superoxide anion to hydrogen peroxide and molecular oxygen. SOD has two isoforms, cytoplasmic, Zn/Cu SOD, and mitochondrial, MnSOD. The essential role of SOD is seen in diseases with disturbed SOD activity. For example, lowered activities of CuZnSOD are often, but not always, seen in tumors, suggesting that decreased antioxidant protection accompanied by increased ROS production could be not only essential steps in carcinogenesis, but many of the properties of the cancers cells in general *(19)*.

Hydrogen peroxide, generated by SOD, still represents a danger for the cell, and is therefore, substrate for another enzyme catalase. Catalase decomposes hydrogen peroxide in two ways: one acting as catalyst with water and oxygen as end products, and other acting as peroxidase *(20)*. Catalase is one of the most efficient enzyme, with reaction rate limited by the collision rate of enzyme with substrate *(20)*. This enzyme is present in all prokaryotes and eukaryotes, and is predominantly located in peroxisomes in mammalian cells. Overexpression of catalase renders cells and organisms (transgenic mice) less sensitive to oxidative challenge *(21)*. Interestingly, catalase deficiency yielded normal phenotype, which was described by Dr.Takahara in 1946. He described that acatalasemic patients had increased tendecy in developing oral ganagrene, presumably as a result of tissue damage by $H_2O_2$ produced by bacteria *(22)*. Also, there are reports that PEGylated catalase suppressed the growth of metastatic tumor cells after tumor removal by decreasing cytokines and their receptors such as EGF and EGFR. These finding indicate modulation of signal transduction by interfering with $H_2O_2$, which is thought to trigger the proliferation of dormant tumor cells *(23)*.

Peroxidases are group of enzymes involved in reduction of inorganic and organic peroxides. Unlike SOD and catalases, these enzymes are not dependent on metal ions in their active site, but have cysteine instead. Thereore, peroxidases require electron donnor from thiol groups. Taking this into consideration, two groups of peroxidases are distinguished: glutathione peroxidases (GSH as electron donor) and thioredoxin peroxidases (TRX as electron donor) *(18)*. GSH peroxidase uses GSH as electron donor resulting in oxidised GSH (GSSG), which is then regenerated by glutathione reductase. Thioredoxin peroxidase reduces hydroperoxides by using thioredoxin as electron donor, which is regenerated by thioredoxin reductase (uses NADPH) *(24)*.

Non enzymatic antioxidants are small hydro- and liposoluble molecules which protect against free radicals in aqueous as well as lipid cell compartments. These small molecules act as unspecific radical scavengers reacting as oxidants to ROS and thereby detoxifying them. Glutathione (GSH) is one of the most ubiquitary small molecules of antioxidant defence. Glutathione, γ-L-glutamyl-L-cysteinylglycine, reacts with its cysteine residue with oxidants thereby forming oxidised glutathione (GSSG) *(25)*. Other well-known small antioxidant molecules are vitamins, vitamin C, L-ascorbic acid, which protect the aqueous cell compartments, and vitamin E, α-tocopherol, which protect lipid compartments. Vitamin C, ascorbate, quenches radicals and forms ascorbyl radical, a stable radical which causes little oxidative damage *(26)*. Ascorbate regenerates through redox cycling, and this fact was used to selectively induce tumor cell death. Namely, menadione and ascorbate created a redox cycle which generated oxidative stress in tumor cells *(27)*. This approach is supported by following facts: cancer cells lack antioxidant enzymes, and thereby are more sensitive to oxidative stress; over-expression of GLUT transporters in cancer cells causes increased uptake of vitamin C, cancer cells show universal glycolytic phenotype, which is required for survival and invasion of surrounding tissue *(27)*. Eventually, oxidative stress caused by redox cycling of ascorbate and menadione results in

impairment of glycolysis and finally to cell death *(28)*. Vitamin E is a generic name given to a group of tocopherol and tocotrienols, of which α-tocopherol is the most abundant in human tissue and is of highest biological activity *(29)*. Vitamin E protects lipid compartments of cell by terminating the lipid peroxidation chain reaction or by inactivation of ROS *(30)*. Also, there are reports of pro-oxidant properties depending on cell environment. Vitamin E was shown to be involved in signal transduction by modulating specific enzymes such as protein kinase C (PKC), protein phosphatase 2A (PP2A), protein tyrosine phosphatase (PTP), protein tyrosine kinase (PTK), diacylglycerol kinase (DAGK), 5-, 12- and 15-lipoxygenases (5-, 12-, and 15-LOX), phospholipase A2 (PLA2), cyclooxygenase-2 (COX-2), and the mitogen activated protein kinase (MAPK) signal transduction pathway *(31)*, and also transcription factors like NFκB. Modulation of these proteins occurs via direct binding or by the interference with enzyme activation and enzyme redox regulation *(31)*. Finally, this alters cellular functions, such as apoptosis, necrosis, survival, adhesion, differentiation.

Antioxidants or agents metabolised to become antioxidants may function by variety of mechanisms: 1) quenching the formation of singlet oxygen, e.g. β-carotene, retinol; 2) scavenging ROS, e.g. polyphenolics; 3) scavenging or reducing lipid free radicals, e.g. α-tocopherol; 4) scavenging prooxidant metals, e.g. polyphenolics, flavonoids; 5) oxidizing ferrous iron, e.g. caeruloplasmin, apoferritin; 6) inhibiting prooxidant enzymes, e.g. allopurinol; 7) inducing or enhancing protective enzymatic defense against oxygen or oxydants, e.g. butylated hydroxyanisole; 8) sparing or renewing intracellular antioxiants, e.g. ascorbate, N-acetylcysteine; 9) stabilising membranes against lipid peroxidation, e.g. cholesterol, 17-beta--estradiol, tamoxifen; 10) reducing oxidatively stressed cells, e.g. ethanol, sorbitol, xylitol (NADH generators); 11) inhibiting enzymes that mediate gene expression as a result of oxidative stress, e.g. tamoxifen, methoxybenzamide. As mentioned above, antioxidants can also act as modulators of the cell proliferation and differentiation *(32)*. Of special interest is their induction of phase II enzymes in cancer development *(34)*: 1) glutathione transferases which conjugate mostly hydrophobic electrophiles with GSH; 2) NAD(P)H: quinone reductase (DT--diaphorase) which promotes two-electron reduction of quinones to hydroquinones; 3) UDP glucuronyltransferases which conjugate xenobiotics with glucuronic acid enhancing their extraction; 4) epoxide hydrolase which inactivates epoxides by hydration to diols. The most common plant antioxidant that is capable of inducing GSH-S-transferase both in vitro and in vivo (in particular in the liver) is ellagic acid which is present in strawberries, raspberries and grapes *(34)*, and could attenuate harmful effects of tobacco nitrosamines *(35)*. That might be particularly relevant if considered in the light of recent findings on the high relevance of GSH-S-transferases in cancer prevention and in general on the role of GSH in human pathology based on oxidative stress *(36)*.

The efforts to use natural pure antioxidants were assumed to offer a good option for the use of antioxidants in cancer prevention. Unfortunately, these ended in well known ATBC study (Alpha-Tocopherol Beta-Carotene) in 90' which showed that the use of beta carotene with the aim to decrease the incidence of lung cancer in smokers resulted in opposite findings of increased incidence of cancer in subjects using beta carotene *(37)*. Nowadays, it is clear that the use of beta carotene in the ATBC study resulted in the overload of beta carotene, which would on one hand cause misbalance of overall antioxidant mechanisms, while on the other beta carotene is decomposed under ROS attack (as in case of cigarette smoke) into mutagenic and potentially carcinogenic products *(38)*.

## Oxidative Stress, Antioxidants and Cancer

Various factors related to oxidative stress and antioxidants could play important role in development as well as in cancer therapies. Some of them are summarised in Table 1.

While many of such factors could have dual roles acting both as beneficial and as harmful factors, synthetic antioxidants that could act as desirable antioxidant and anticancer agents are almost entirely neglected. Synthetic antioxidants were in a way »collateral victims« of the studies that have shown that pure natural antioxidants could have undesirable side effects, mostly because under oxidative stress antioxidants could be metabolised in novel, cytotoxic free radicals, thus allowing the chain reactions and the spread of oxidative stress. Therefore, the efforts were raised to synthesize antioxidants that could under ROS attack from stable radicals ending oxidative stress. This feature gives a new insight in antioxidant function and therefore they could be considered as »biological response modifiers« maintaining oxidative homeostasis.

The example of oxidative stress/homeostasis in normal physiology is exercise. During exercise ROS and RNS (Reactive Nitrogen Species) are generated in muscle *(39, 40)*. As oxidative stress was considered to be harmful, the efforts were made to investigate the role of oxidative stress during exercise, especially during heavy exercise (for professional athletes). The numerous studies reported that antioxidant supplementation during exercise decrease oxidative stress parameters *(41, 42)*, but this did not turn out to be beneficial. In fact, it was shown that oxidative stress generated by exercise cause hormetic response leading to adaptation to oxidative stress and increasing organism's tolerance to stress *(43)*. These discoveries pointed out the necessity to maintain the natural oxidative homeostasis of the organism and the importance to help the organism to keep this homeostasis in illness, especially in tumor therapy. Tumor therapy is often based on oxidative stress and also, it is commonly accepted that there is persistent oxidative stress in cancer *(44)*. There are two general theories on the origin of cancer, and there are also two general theories on the carci-

**TABLE 1**

Some factors related to oxidative stress affecting cancer development.

| FACTOR | TARGET ORGANS (ORGANIC SYSTEMS) | ACTIVITY PRINCIPLE FOR: | |
|---|---|---|---|
| | | **Harmful effects** *(carcinogenesis)* | **Beneficial effects** (cancer prevention or therapy) |
| **Polluting Smoke** (cigarette, car & industry pollution, etc.) | respiratory system, genito-urinary system | complete chemical carcinogens and mechanical irritation by tar particles, hypoxia, immune suppression | none |
| **Minerals** (asbestosis, pro-oxidant pollutants) | respiratory system, gastrointestinal system | oxidative activity of iron, silicon oxide combined with mechanical irritation | possible free radicals scavenging activity of some minerals or miscellaneous anticancer effects due to the uncertain mechanisms |
| **Ionising radiation** (UV,X-rays) | skin, bone marrow, genito-urinary system | direct and indirect DNA damage | cancer radiotherapy due to the toxicity (DNA damage particularly of the proliferating cells) |
| **Toxic chemicals** (dyes, etc.) | skin, gastrointestinal system respiratory system, genito-urinary system | multiple, involving pro-oxidants and complete carcinogens | possible increase of the ROS detoxifying systems – uncertain |
| **Diet** (food restriction or abuse) | gastrointestinal system, genito-urinary system | mostly related to some plant tumor promoting components, mucose irritation and metabolic imbalances (fat, meat or glucose abuse) | plant antioxidants – possible way for the natural chemoprevention and therapy |
| **Medicaments** (drugs, complementary treatments, etc.) | gastrointestinal system, genito-urinary system, bone marrow | mostly affecting liver cytochrome P450, chemotherapeutic agents (cytostatic drugs) | chemotherapy of cancer due to the DNA damage, phototherapy of cancer, possible activity principle of some plant extracts |
| **Hereditary factors** (xeroderma pigmentosum, Down's syndrome) | skin, bone marrow | lower DNA repair system after mutagenic effects of ROS, lowered antioxidative capacity of the cells | uncertain |
| **Inflammation*** (chronic) | unspecific | cellular production of ROS combined with tissue damage | suppression of the tumor progression due to the inhibiting effects of suppressive cytokines (TGF-beta, TNF-alpha), unspecific immune response against cancer, changing tumor stroma – fibrosis |

* uncertain and not completely understood, but should be related to the mechanical irritation or response to particular tissue damage (such as inflammatory response to the tissue damage caused of radiation, asbestosis, smoke abuse, etc.)

nogenic effects of oxidative stress and anticancer activities of antioxidants *(45)*.

The genetic theory of cancer implies particular oncogene mutations as the cause of cancer and the epigenetic theory assumes that the structure of the active genes is normal, while the regulation of cell growth is altered in direction of less matured aggressive cells due to the activation of genes which should be quiescent and/or suppression of the tumor-suppressing genes. In case of somatic mutations, carcinogens are often metabolically activated by cytochrome P-450 (designated as phase 1 enzyme) causing oxidative damage of the DNA which is not lethal for the cells, but is irreparable and inherited by the progeny of the altered cell (mutation) *(46)*. Antioxidants should in this situation act as scavengers of ROS that could cause the damage of the genome. In case of epigenetic bases of cancer antioxidants should not only act as scavengers of ROS, but could, similar to oxidants and ROS, themselves act as modulators of the cell proliferation and differentiation *(47, 48, 49, 50, 51)*. In both genetic and epigenetic bases of cancer development antioxidants should primarily act in the initiation stage of carcinogenesis influencing the phase 1 enzymes. There are also phase 2 enzymes that could be induced by antioxidants *(43)*. They include: 1) glutathione transferases which conjugate mostly hydrophobic electrophiles with GSH; 2) NAD(P)H: quinone reductase (DT-diaphorase) which promotes two-electron reduction of quinones to hydroquinones; 3) UDP glucuronyltransferases which conjugate xenobiotics with glucuronic acid enhancing their extraction; 4) epoxide hydrolase which inactivates epoxides by hydration to diols.

Although there are many efforts to find as much as possible novel plant antioxidants, the problem of their instability during isolation or in physiologic conditions *(52)* are the major obstacle. Therefore, efforts are made to synthesize novel antioxidants, stable and with enhanced antioxidant activity. Some of the other character-

istics of these novel antioxidants are: antitumor activity – curcumin derivatives *(52)*, antidiabetic and cardioprotective effects – pyridoindole derivative *(53)*, calcium channel antagonists – diludin derivatives *(54, 55)*. Combination of antioxidants and other complementary treatment supporting conventional therapies could thus raise powerful tool in treatment of different diseases with disrupted oxidative homeostasis.

## Conclusions

Oxidative stress was for long considered as cause of different illnesses, but new findings indicate its importance in cell homeostasis. One of the most popular parts of redox biology and biochemistry in common life are antioxidants. Although antioxidants are beneficial they should be handled with care under physiological conditions and homeostasis. Oxidative homeostasis is disrupted in disease, especially in cancer. Chemo/radiotherapy is also based on oxidative stress, therefore, there is a need for novel antioxidants which would have antioxidant as well as tumor suppressing activity. There is rising number of antioxidants that are promising and their properties need to be thoroughly studied together with their mechanisms. These substances and their activity are of great importance in treating cancer and improving life quality.

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