Review Article

PATHOLOGICAL ROLES OF REACTIVE OXYGEN SPECIES AND THEIR DEFENCE MECHANISMS

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تشير الدلائل على أن العديد من الحالات الفسيولوجية والباثولوجية مثل التقدم في العمر، والإلتهاب، والعدوى الفيروسية، والأمراض التنكسية العصبية قد تحدث بفعل أنواع الأكسجين المتفاعل. وخلال العشرة سنوات الأخيرة، توفرت الدلائل بكثرة على أن عدداً كبيراً من المسارات المؤشرة داخل الخلية يتم تنظيمها بأنواع الأكسجين المتفاعل. وبإمكان عوامل نمو وسايتوكينات عديدة مرتبطة مع أنواع مختلفة من مستقبلات أغشية الخلايا أن تتسبب في زيادة الأكسجين المتفاعل داخل الخلية، ومنها مستقبلات السيتوكين، ومستقبلة التيروسين، والمستقبلات المقترنة ببروتين G. فعلى سبيل المثال، بالإضافة إلى تنشيط الأعضاء المختلفة للشلالات المؤشرة في نمو الخلية وتمايزها، فإن أنواع الأكسجين المتفاعل قد تنظم نشاط عوامل الإنتساخ أيضاً عن طريق التحويرات المؤكسدة للسيستايينات المحفوظة. ولقد تبين أن العديد من عوامل الإنتساخ حساسة لتفاعلات الخزلدة بما في ذلك P53. وفي الوقت الحاضر، فإن الباثولوجيات السببية لمرض باركنسون، ومرض ألزهايمر ومرض هنتنجتون غير معروفة. ومع ذلك فإن الفرضية الأكثر قبولاً هي الإجهاد التأكسدي الإنتقائي في الجهاز العصبي المركزي. وقد تم اقتراح أن الإنزيمات مثل الزانثين أوكسيداز هي المصدر الرئيسي لأنواع الأكسجين المتفاعل المتولد أثناء إعادة أكسجة الأنسجة الإقفارية. إن وظيفة معظم الأنظمة المضادة للتأكسد هي تحوير أنواع الأكسجين الشديدة التفاعل إلى أشكال وسطية أقل تفاعلاً والتي لا تمثل بعد ذلك تهديداً للخلية. إن الفوق أوكسيد ديسميوتاز يقوم بتحويل الفوق أوكسيد إلى الأكسجين الجزيئي وبيروكسيد الهيدروجين. نستنتج من ذلك، أنه يبدو أن التحكم في أنواع الأكسجين المتفاعلة بواسطة الأنظمة الدفاعية يحافظ على تراكيز قليلة وليس التخلص الكامل من أنواع الأكسجين المتفاعلة. وتهدف هذه المقالة على إلقاء الضوء على دور أنواع الأكسجين المتفاعلة على المستويين الفسيولوجي والباثولوجي.

Evidences indicate that many physiological and pathological conditions such as ageing, inflammation, viral infections, and neurodegenerative diseases may develop through the action of reactive oxygen species (ROS). During the last 10 years, increasing evidence has been provided that a large number of intracellular signaling pathways are regulated by intracellular ROS. Several growth factors and cytokines binding to different types of cell membrane receptors can elicit a rise in intracellular ROS. These include cytokine receptors, receptor tyrosine and G protein-coupled receptors. In addition to the activation of different members of signaling cascades involved in cell growth and differentiation, ROS may also directly regulate the activity of transcription factors through oxidative modifications of conserved cysteines for example. Several transcription factors have been shown to be redox-sensitive, including p53. At present, the etiopathologies of Parkinson's disease, Alzheimer disease and Huntington's chorea are unknown. However, the most accepted hypothesis is selective oxidative stress in the central nervous system. It has been also proposed that enzymes such as xanthine oxidase are the major source of reactive oxygen species generated during the re-oxygenation of ischemic tissues. The function of most antioxidant systems is to modify the highly reactive oxygen species to form less reactive

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intermediate which no longer pose a threat to the cell. Superoxide dismutase convert superoxide to molecular oxygen and hydrogen peroxide, the later is the converted to water and molecular oxygen by catalase, or to water only by glutathione peroxidase. In conclusion, control of reactive oxygen species by antioxidant defense systems appears to maintain low concentrations rather than complete elimination of reactive oxygen species. The aim of this manuscript is to shed light on the role of ROS both at physiological and pathological level.

***Key words*:** Reactive oxygen species, Free radicals, hydrogen peroxide, superoxide anion, antioxidant defense mechanisms.

Introduction

Reactive oxygen species (ROS) are oxygen-centered molecules which include the non-radicals, hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), hydroxyl anion (HO^-) and singlet oxygen (O_2); and the radicals, superoxide anion (O_2^-), hydroxyl radical ($HO^\cdot$), and nitric oxide ($NO^\cdot$). Although hypochlorous acid and nitric oxide contain oxygen, they will not be considered in further detail in this review because of the presence either a halogen or nitrogen atom. Reaction of hypochlorous acid or nitric oxide with superoxide anion generates hydroxyl radical and peroxynitrite under physiological conditions, respectively (1, 2).

Water (H_2O) is an oxygen centered molecule that can be split into ions (H^+ and HO^-) or free radicals ($H^\cdot$ and $HO^\cdot$) by heterolytic or homolytic fission, respectively. In fact, under physiological conditions pure water splits only by heterolytic fission which yields only 10^{-7} M of each H^+ and HO^-. Hence, water is a non-toxic molecule unless it exposed to a radiation source, which leads to homolytic fission with formation of free radicals (3). Free radicals possess an unpaired or free electron in their outer orbitals (4). The unpaired electrons in free radicals lead to their extremely reactive nature, which is a result of their high reduction potentials. Consequently, unpaired electrons may undergo either oxidation or reduction reactions with other reactants. When two unpaired radicals react, their unpaired electrons combine to form a more stable covalent bond. However, when an unpaired radical reacts with non-radical (paired electrons molecule), a new radical result, and a chain reaction can be initiated (4).

Superoxide anion and hydroxyl radical are free radicals in which the unpaired electron is localized to the outer orbital of an oxygen atom. Hydroxyl radical can react with other reactants *via* hydrogen abstraction, hydrogen addition or electron transfer, while superoxide anion reacts *via* only hydrogen abstraction and electron transfer (Section 1.2) (5).

Although, hydrogen peroxide and singlet oxygen are classified as ROS, they are not radicals and do not necessarily interact with tissue through radical reactions directly (6). Hence, they are not toxic *per se* but in presence of free radicals or catalysts (e.g. iron and copper) more reactive intermediates can be produced. Transition metals, such as iron and copper, which are found at the active site of many oxidase enzymes, e.g. cytochrome oxidase, have the ability to facilitate the transfer of single electrons to molecular oxygen to form free radicals (7).

ROS cause damage to cellular macromolecules. This is known as oxidative stress or "oxygen toxicity" (for more details see Sections 2.3-2.6).

1.1. Formation of ROS from molecular oxygen:

Reactive oxygen species are formed by the one or two electron reduction of molecular oxygen (8). The addition of one electron to molecular oxygen produces the superoxide anion while the addition of two electrons yields the peroxide ion (O_2^{2-}), which in biological systems is protonated to give hydrogen peroxide.

Hydrogen peroxide can then react with superoxide anion and ferric or cupric ions in the Haber-Weiss reaction, to produce the highly reactive hydroxyl radical (Figure 1). In the first step of this reaction, superoxide anion reduces ferric iron (Fe III) to ferrous iron (Fe II) or cupric (Cu II) to cuprous (Cu I), which in turn reduces hydrogen peroxide to form a hydroxyl radical and hydroxyl ion (9). The reduced metal ion undergoes re-oxidation during the second stage.

The hydroxyl radical is the most active ROS (10). This arises from a much higher reduction potential in comparison to other ROS. Its activity is minimized by removal of hydrogen peroxide and transition metals from the cell. As a result of its reactivity, the hydroxyl radical does not travel far and has a half-life of a few microseconds (10). However, hydrogen peroxide can cross cell membranes almost as readily as water while the

charged superoxide anion molecule can cross membranes only *via* transmembrane anion channels (11). Therefore, the presence of trace amounts of the transition metal ions Fe(III) or Cu(II) is a significant influence on the production of hydroxyl radicals in biological systems.

1.2. Mechanisms of ROS toxicity:

When free radicals react with a non-radical, other free radicals can be formed. This enables free radicals to induce chain reactions that may be thousands of events long; e.g. hydroxyl radical induces lipid peroxidation of polyunsaturated fatty acids *via* hydrogen abstraction (12). In addition, the reaction of hydroxyl radicals with aromatic compounds, such as the purine base, guanine in DNA, is processed *via* hydrogen addition (9). Thus ROS can act as both oxidant and reducing agents. Although the initial free radical produces only local effects, secondary radicals and degradation products can have biological effects distant from the site where the first free radical was formed. However, when two free radicals react with each other, a stable molecule may be formed (13). This explains the eventual termination of free radical-induced chain reactions.

The superoxide anion is potentially toxic. It may directly influence local homeostasis by oxidizing catecholamines, or it can be transformed into the hydroxyl radical *via* the Haber-Weiss reaction (Figure 1) (1).

In contrast, hydrogen peroxide, *per se,* is not especially toxic to the cell macromolecules, but it can cross cellular membranes and this feature is potentially important because the extracellular environment possesses few antioxidant defense mechanisms. In the presence of low concentrations of transition metal ions, hydroxyl radicals are formed from hydrogen peroxide, *via* the Fenton reaction (Figure 2). Alternatively, hydrogen peroxide can interact with superoxide anion to produce the hydroxyl radical, by Haber-Weiss type reactions (Figure 1).

Due to the charged nature of superoxide anion it is more concentrated in the intracellular compartment. As a result, hydroxyl radicals are produced predominantly from hydrogen peroxide by Haber-Weiss reactions in the intracellular compartment whereas the Fenton reaction is more important in extracellular compartments (14).

1.3. Sources of ROS:

Reactive oxygen species can be produced by a number of enzymatic reactions including oxidases and cytochrome P450s (15, 16). However, they are also produced non-enzymatically, often through redox cycling (17). Redox cycling is the cyclic reduction-oxidation of drugs or other xenobiotics, which can generate ROS. Several xenobiotics, such as adriamycin (a quinone derivative), are capable of redox cycling (Figure 3) (5).

As shown in figure 3, quinones can undergo a one-electron reduction reaction catalyzed by an enzyme such as the microsomal flavoprotein NADPH-cytochrome P450 reductase, resulting in the formation of the semiquinone free radical. This semiquinone metabolite is unstable in the presence of oxygen and is rapidly reoxidized to form the superoxide anion radical. In this process the parent quinone is regenerated leading to redox cycling of the quinone-semiquinone. The superoxide anion undergoes spontaneous or enzymatic dismutation to form hydrogen peroxide. Alternatively, in the presence of catalyst metals, excess superoxide anion can react with hydrogen peroxide to form hydroxyl radicals by a Haber-Weiss type reaction (Figure 1). Redox cycling also plays an important role in the toxicity of nitrofurantoin and bipyridyl compounds such as paraquat (4). In addition, redox cycling can induce lipid peroxidation and therefore, alterations in membrane permeability (12). Adriamycin and other anthracycline anticancer drugs can induce *in vivo* cardiac toxicity, which is associated with peroxidation of cardiac lipids (12). It has been proposed that this side effect is associated with hydroxyl radical formation, which is generated by redox cycling in the presence of catalyst metals (Figure 3b).

Xanthine oxidase generates superoxide anion and hydrogen peroxide during the re-oxidation of the enzyme with molecular oxygen (18, 19). Consequently, this enzyme is widely used to generate ROS *in vitro* (20). However, it has been reported that human xanthine oxidase serves as an NAD^+ dependent-dehydrogenase *in vivo* with minimal oxygen-reductase activity (21, 22). More recently, it has been shown that the dehydrogenase form may react slowly with oxygen to produce superoxide anion, although this activity is inhibited by NAD^+ (23). Hence, the importance of xanthine dehydrogenase *in vivo* as a source of ROS is not

proven. However, xanthine dehydrogenase-oxidase interconversions may occur in certain pathological conditions such as ischemia (22, 23). Nishino has proposed that under these pathological conditions, the protein environment surrounding the flavin is subjected to conformational changes that facilitate its reaction with oxygen and formation of ROS (22, 23). In addition, during ischaemia the NAD^+/NADH ratio decreases from 700 to 30, which increases the potential for ROS formation (22, 24).

Aldehyde oxidase, a closely related molybdenum hydroxylase, also generates superoxide anion and hydrogen peroxide but in contrast to xanthine oxidase, aldehyde oxidase seems to be a permanent oxidase, with no activity towards NAD^+ (25, 26). Studies on aldehyde oxidase and xanthine oxidase have shown that modulation of enzyme activities, cofactor availability, substrate concentration and oxygen tension all affect rates of intracellular ROS production (19, 27-29).

Cytochrome P450 (EC 1.14.14.1) is a family of haemoproteins found in high concentrations in mammalian liver and other tissues (29). It is the terminal oxidase of the microsomal electron transport chain responsible for oxidative metabolism of xenobiotics.

The main catalytic function of cytochrome P450 is to incorporate an oxygen atom into a lipophilic substrate (RH) molecule to give a more polar product (ROH) using NADPH as a cofactor (30) (Figure 4).

Activation of oxygen by cytochrome P450 involves the formation of partially oxidized enzyme-substrate intermediates. Certain substrates can divert electron flow to molecular oxygen thereby generating ROS, whereas most other substrates are tightly coupled to cytochrome P450 and do not produce ROS.

Usually, the uncoupling of the oxy-complex of cytochrome P450 generates superoxide anion although hydrogen peroxide can also be generated from uncoupling of the peroxy-complex (31). Uncoupling involves dissociation of the reducing equivalents from product formation, resulting in the formation of ROS instead of stoichiometric product formation as dictated by above equation (16). It is not well understood why some substrates 'uncouple' cytochrome P450 and divert electron flow to molecular oxygen, thereby generating ROS by-products, whereas other substrates are tightly coupled to cytochrome P450 electron transfer and do not produce these by-products (31).

In addition, the mitochondrial electron transport system generates ROS when oxygen tension is high and the respiratory chain carriers are highly reduced (32, 33). Under these conditions superoxide anion is produced and, following its dismutation, hydrogen peroxide (32, 34). In contrast, molecular oxygen is reduced to water under normal oxygen levels (Figure 5).

Brain metabolism can generate ROS, e.g. monoamine oxidase (MAO), a mitochondrial enzyme, catalyses the oxidation of amine-containing neurotransmitters, such as serotonin and dopamine, to yield hydrogen peroxide and the corresponding aldehydes (Figure 6) (35, 36). Aldehydes produced *via* MAO may be substrates for aldehyde oxidase and may generate further molecules of hydrogen peroxide and superoxide anion by the latter route (37).

Other sources of ROS include autoxidation of endogenous substrates, such as adrenaline and dopamine, in addition to other oxidase enzymes such as dopamine hydroxylase, D-amino acid oxidase, glucose oxidase and fatty acyl CoA oxidase (4, 34).

Although, the oxidative metabolism of xenobiotics or endogenous compounds is the most important source for ROS, reductive metabolism of xenobiotics may also contribute to ROS production. Cytochrome P450, NADPH cytochrome P450 reductase and DT-diaphorase can catalyze the reductive metabolism of xenobiotics, in the presence of electron donors, with the formation of ROS (38, 39).

DT-diaphorase, also referred to as NAD(P)H: quinone oxidoreductase, is a flavoprotein that catalyses the two-electron reduction of quinones to hydroquinones using either NADH or NADPH as an electron donor (39). Under aerobic conditions, the hydroquinone may be autooxidized back to the parent quinone with the formation of ROS (Figure 3a) (5, 40). Reductive metabolism of xenobiotics catalyzed by cytochrome P450 or NADPH cytochrome P450 reductase occurs *via* a one-electron reduction of a quinone to a semiquinone free radical. The semiquinone enters a redox cycle, with generation of parent compound and ROS (Figure 3b) (5).

Table 1: Mechanisms of enzymatic and chemical antioxidants (4)

Antioxidant	Reaction catalyzed / comments
1. Enzymatic	
Superoxide dismutase	$2O_2^- + 2H^+ \longrightarrow H_2O_2 + O_2$
Glutathione peroxidase	$H_2O_2 + 2GSH \longrightarrow GSSG + 2H_2O$
Catalase	$2H_2O_2 \longrightarrow 2H_2O + O_2$
Glutathione reductase	$GSSG + NADPH + H^+ \longrightarrow 2GSH + NADP^+$
Glutathione-S-transferase	Involved in the metabolism of many endogenous substrates including hydroperoxides.
2. Non-enzymatic	
α-Tocopherol (Vitamin E)	Scavenger involved in inhibition of lipid peroxidation.
Ascorbic acid (Vitamin C)	Functions as a reducing agent to scavenge ROS; Role in vitamin E regeneration.
Reduced glutathione (GSH)	Substrate for glutathione peroxidase; Functions as a reducing agent.
Thiol-containing compounds	Similar protective effect to GSH. Participates in GSH production.
Melatonin	Reacts with hydrogen peroxide to produce less reactive species, Stimulates antioxidant defense enzymes.
Metal ion chelators (iron and copper)	Inhibition of reactions leading to the formation of peroxides and the hydroxyl radical.

$$Fe(III)/Cu(II) + O_2^- \longrightarrow Fe(II)/Cu(I) + O_2$$

$$Fe(II)/Cu(I) + H_2O_2 \longrightarrow HO^{\cdot} + HO^- + Fe(III)/Cu(II)$$

Figure 1. Reaction of hydrogen peroxide with superoxide anion and ferric or cupric ions (Haber-Weiss reaction)

$$Fe(II)/Cu(I) + H_2O_2 \longrightarrow HO^{\cdot} + HO^- + Fe(III)/Cu(II)$$

Figure 2. Formation of hydroxyl radical and hydroxyl ion by Fenton reaction

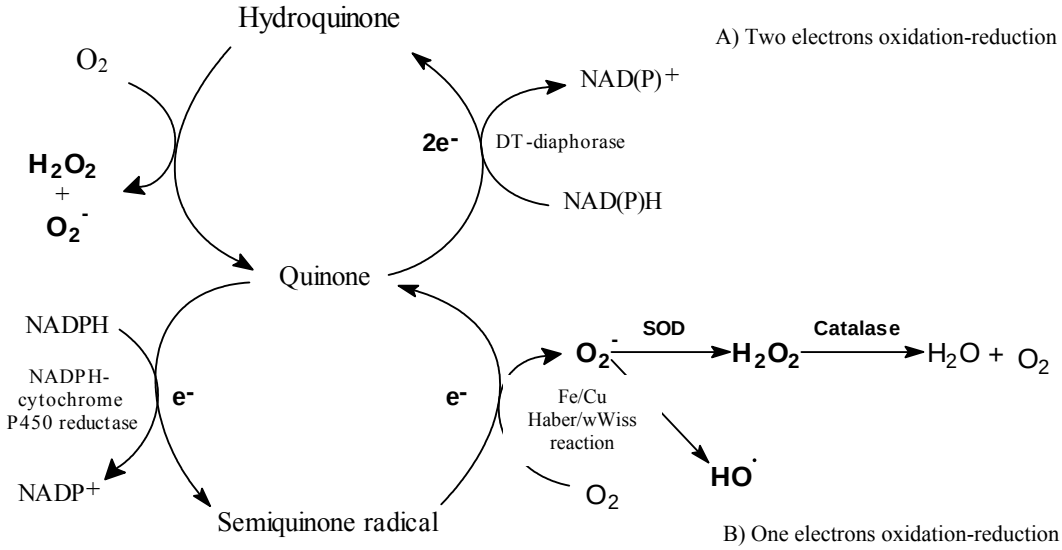

Figure 3. Formation of ROS associated with the cyclic metabolism of quinones, e.g. adriamycin (whereas SOD, GSH and GSSG are superoxide dismutase, reduced and oxidized glutathione, respectively)

$$RH + NADPH + O_2 + H^+ \xrightarrow{\text{Cyt. P450}} ROH + NADP^+ + H_2O$$

Figure 4. Oxidation of lipophilic xenobiotics (RH) by cytochrome P450

$$O_2 + 4H^+ + 4e^- \longrightarrow 2H_2O$$

Figure 5. Reduction of molecular oxygen via mitochondrial transport chain

$$\text{Monoamine} + O_2 + H_2O \xrightarrow{\text{MAO}} \text{aldehyde} + H_2O_2 + NH_3$$

Figure 6. Formation of hydrogen peroxide via oxidation of monoamines by MAO

1.4. Physiological role of ROS:

ROS and free radicals have been implicated in a number of complex biological processes such as ageing, inflammation, tissue repair and intracellular messengers (41, 42, 43). ROS generated by oxidase enzymes, mitochondria and from other cellular sources, can cause damage to cell components and initiate degradative processes (44, 45). Relatively little oxidative damage, such as that seen during prolonged exercise as a result of adrenaline catabolism, may promote the ageing process (45). Recently, it has become apparent that oxidative damage to mitochondrial DNA and antioxidant enzymes is important in age-related diseases (41, 46).

During inflammation, ROS, especially superoxide anion, can react with cell membrane components, such as arachidonic acid to produce chemotactic lipids. These attract more neutrophilis to the site of infection and amplify the inflammatory response (47). ROS also stimulate growth of epithelial cells and fibroblasts, which may be important in wound healing and normal tissue repair (43).

Control of ROS by antioxidant defense systems appears to maintain low concentrations rather than complete elimination of ROS. Hence, it has recently been reported that ROS, in very low levels, might be used as signaling molecules (48). Thus, ROS are not always a noxious challenge to viable cells. Some physiologically important chemical reactions occur *via* ROS. These include the vital functions of

macrophages and neutrophils, and the selective oxidation of polyunsaturated fatty acids to produce the eicosanoids (49).

1.5. Cellular defenses against ROS:

1.5.1. Physiological or enzymatic antioxidants

ROS are continually being formed in small amounts by normal processes of metabolism and cell respiration; consequently, all aerobic cells possess mechanisms to mitigate their harmful effects.

The structural organization of the cell is important in that it separates the pro-oxidants and catalysts (50). However, leakage of ROS can occur, resulting in damage to surrounding macromolecules. Such a leakage of ROS is assumed to occur in various oxidative diseases (51). Cells also contain several enzyme systems for removing ROS and their products as well as chemical ROS scavengers.

The function of antioxidant systems is to modify the highly reactive oxygen species to form less reactive species, which no longer pose a threat to vital cell components.

Superoxide anion is converted to molecular oxygen and hydrogen peroxide by superoxide dismutase (Figure 3 and Table 1). Hydrogen peroxide is then converted to water and molecular oxygen by catalase or to water by glutathione peroxidase (Table 1). Chemical antioxidants such vitamin C and E and glutathione can remove ROS by reduction reactions (52). Metal ion chelators are very effective antioxidants by sequestering ferric or cupric ions and inhibiting the metal catalyzed reactions, such as the Haber-Weiss type reaction.

Although, hydroxyl radical does not travel far and has very short half-life, it is the most reactive oxygen species. It is so reactive that no enzyme system exists which uses it as a substrate (53). The cell's effort is directed at preventing its formation, by removing hydrogen peroxide and transition metals.

Table 1 summarizes the main defense mechanisms within the cell. Among these defenses are enzymes including superoxide dismutase, catalase and glutathione peroxidase. Two enzymes may co-operate sequentially to detoxify ROS; for example, hydrogen peroxide produced *via* superoxide dismutase is a substrate for catalase. There are also many non-enzymatic antioxidants including, α-tocopherol, ascorbic acid and reduced glutathione.

1.5.1.1. Superoxide dismutase:

Superoxide dismutase enzymes catalyze the dismutation of superoxide anion, as shown in Table 1, at a rate constant of 2×10^9 $M^{-1}s^{-1}$. In contrast, the spontaneous dismutation of superoxide anion occurs with rate constant of 1×10^5 $M^{-1}s^{-1}$ at physiological pH (54, 55). The acceleration of this reaction ensures that no superoxide anion is available to react with hydrogen peroxide to form the hydroxyl radical through the metal-catalyzed Haber-Weiss-type reactions (Figure 1). Interestingly, cells are capable of increasing activity and synthesis of superoxide dismutase in response to oxidative stress (14, 42). Superoxide dismutase exists in several forms. One form containing manganese is found in the mitochondrial matrix and another containing copper and zinc occurs in cytoplasm (56). An iron-containing superoxide dismutase also exists in some bacteria and plants.

The zinc moiety of cytosolic superoxide dismutase is thought to stabilize the enzyme, while the copper atom (manganese and iron atoms in other superoxide dismutase forms) and histidine amino acid are required for enzymatic activity (53). During superoxide dismutase catalysis, the copper atom is reversibly oxidized and reduced by successive encounters with two superoxide anions, respectively. The reaction occurs in two stages with two molecules of superoxide anion, producing one molecule of oxygen and one molecule of hydrogen peroxide (54). In the first step one electron is transferred from superoxide anion to the copper moiety (with a concomitant addition of a hydrogen ion to histidine) and molecular oxygen is released. A second superoxide anion reacts at the catalytic site by accepting an electron from copper (with a concomitant addition of second hydrogen ion to histidine) and leaves as hydrogen peroxide, carrying with it the histidinyl hydrogen ions (Figure 7) (54).

Superoxide dismutase has been cloned and the recombinant human enzyme has been expressed in yeast (57). Recently, it has been found that both recombinant superoxide dismutase and catalase prolong the survival of amyotrophic lateral sclerosis (ALS) mice models after disease onset (58). The use of human antioxidant enzymes, obtained by gene-technology, may permit the treatment of a variety of clinical conditions associated with oxidative stress (58, 59).

1.5.1.2. Glutathione peroxidase and catalase:

Peroxidases remove hydrogen peroxide *via* reaction with a reducing agent such as GSH whereas hydrogen peroxide is decomposed directly to water and oxygen in the presence of catalase. The catalytic activity of catalase appears to be a special case of peroxidatic activity in which the electron donor is a second molecule of hydrogen peroxide. The mechanism of action for catalase is like that of superoxide dismutase, where one molecule of hydrogen peroxide is reduced to water and the other oxidized to oxygen (Figure 8).

Two enzymes systems exist which catalyze the breakdown of hydrogen peroxide. At low concentrations ($\approx 10^{-9}$ M), most hydrogen peroxide is removed by reaction with reduced glutathione (GSH, Table 1) to form oxidized glutathione (GSSG) and water, catalyzed by glutathione peroxidase (GPX) (57). Glutathione reductase catalyses the regeneration of reduced glutathione utilizing NADPH formed by the pentose phosphate pathway. On the other hand, at high concentrations of hydrogen peroxide, catalase is predominantly responsible for its removal (Table 1) (60).

Another member of the peroxidase family is phospholipids hydroperoxide glutathione peroxidase (PHGPX), which can act on hydrogen peroxide within membranes and lipoproteins. Unlike classical glutathione peroxidase, which is cytosolic, PHGPX is present in both cytosol and in the cell membrane. It has been suggested that membrane PHGPX might be involved in the cellular mechanism of adaptation of the heart to the toxic effects of adriamycin and other anthracycline anticancer drugs (61).

In addition, glutathione-S-transferase catalyses a nucleophilic addition of glutathione to electrophilic compounds. It is involved in the metabolism of many endogenous hydroperoxides and prevents any hydrogen peroxide formation from these compounds (58).

1.5.2. Non-enzymatic antioxidants:

Tissues also have a variety of non-enzymatic antioxidants for preventing damage by ROS. Vitamin E, a series of isomers of tocopherol, is a lipid phase antioxidant that partitions into all membranes and converts superoxide anion, hydroxyl and other chemical radicals to less reactive forms (62). It acts by donating a hydrogen ion to the radical and thereby confining the effect of the radical; in addition, a stable vitamin E radical is formed. Other chemical antioxidants act in a similar manner. For example, ascorbic acid, thiol-containing compounds and reduced glutathione may also be able to quench ROS by donating electrons from their oxidizable functional groups. Ascorbic acid and glutathione are aqueous phase antioxidants and are found in high concentrations in certain tissues, especially the eye (63). In addition to its direct reaction with ROS, ascorbic acid is also necessary to regenerate vitamin E (4).

The tripeptide glutathione is very important for cellular defense against ROS. It reacts directly with radicals in non-enzymatic reactions and as an electron donor in the reduction of peroxides catalyzed by GPX (64).

Melatonin, a ubiquitously distributed indole, stimulates several antioxidant enzymes, including glutathione peroxidase, glutathione reductase, catalase and superoxide dismutase to maintain intracellular ROS concentrations at normal levels (65). In addition, it reacts with ROS to produce less reactive species (66).

1.5.3. Imbalance between ROS and cellular defense systems:

Under pathological conditions or when certain drugs are present, much higher concentrations of ROS are formed than normal; these can overwhelm the cellular defenses and lead to damage and even death of the cell. As illustrated in Figure 3 redox cycling shows a typical sequence for the formation of such excess ROS. Synthetic antioxidants, e.g. ebselen, can be used to prevent lipid peroxidation and inhibit redox cycling both *in vivo* and *in vitro* and may serve as drugs or preservatives, respectively.

1.5.3.1. Antioxidant drugs and preservatives :

Numerous compounds have been synthesized and tested as antioxidants, ranging from phenols added to foodstuffs to drugs used in medicine (8). The detoxification of ROS, by chelating agents or by scavengers, is one of the important pharmacological lines of defense against ROS. Several criteria are proposed to evaluate their potential role. An effective ROS scavenger and metal chelator should, firstly, react very rapidly with ROS or with metal, respectively; secondly, be non-toxic *in vivo* conditions or not undergo toxic metabolism; thirdly, be stable under *in vivo* conditions; finally, readily cross cell membranes and distribute evenly in tissues.

Selenium displays a variety of biological effects, a prominent one being its role as selenocysteine in the active center of glutathione peroxidase (67). Ebselen, a synthetic organoselenium compound, has been found to exhibit antioxidant activity (66). This partly due to its inhibitory activity on the lipoxygenase pathway (68). Lipoxygenases are iron-containing enzymes that catalyze a direct reaction of polyunsaturated fatty acids with oxygen to give hydroperoxides. In addition to this antioxidant activity, the compound acts catalytically in the glutathione peroxidase reaction (69).

Chelating agents such as desferrioxamine have been used clinically to diminish oxidative damage caused by transition metal ions (70). Desferrioxamine, a linear molecule, chelates with ferric iron to form a stable non-toxic complex called ferrioxamine. Hence, it inhibits lipid peroxidation, and iron catalyzed reactions such as Haber-Weiss type reaction. In addition, desferrioxamine and its iron complex, ferrioxamine, are excellent hydroxyl radical scavengers (71). Penicillamine, a metabolite of penicillin that contains -SH group, is a useful chelating agent for copper. It is used to treat rheumatoid arthritis where it acts as a scavenger of ROS (72).

Cinnamophilin, a natural compound isolated from *Cinnamomum philippinense*, has a protective effect against various ROS including superoxide anion with complete inhibition at 200 μM and it also inhibited lipid peroxidation in rat brain homogenates with an IC_{50} value of 8.0 ± 0.7 μM (73). It is assumed that the two hydrogen-donating phenol groups of cinnamophilin may contribute to its lipid peroxidation chain breaking activity as well as ROS scavenging properties (74).

2. *Role of reactive species in pathological: conditions*

2.1. *Role of ROS in oxidative stress:*

Oxidative stress is a disturbance in the prooxidant-antioxidant balance in favor of the former, leading to potential damage of macromolecules (75, 76). It may result from excessive ROS formation, for instance, it can occur in the brain during increased dopamine turnover or high free iron concentrations that drive the Haber-Weiss and Fenton type reactions (Figures 1 and 2) (35).

Increased production of ROS can cause major interrelated derangements of cell metabolism, including DNA strand breakage (often an early event), rises in intracellular free calcium, damage to membrane ion transports and other specific proteins and peroxidation of lipid (79, 80, 78). Perturbation of cell metabolism by oxidative stress can be direct, e.g. the oxidation of thiol groups by hydrogen peroxide or the breakage of DNA strands *via* the hydroxyl radical, or indirect, e.g. the activation of proteases (which attack the cytoskeleton) and nucleases (which fragment DNA) by increased calcium levels (81).

Oxidative stress occurs in a cell or tissue when the concentration of ROS generated exceeds the antioxidant capability of that cell (82). ROS can be produced either endogenously or exogenously. Endogenous oxidative stress can be the result of normal cellular metabolism and oxidative phosphorylation. The metabolism of substances by the P450 enzyme system generates oxygen free radicals through normal or futile cycling mechanisms. Exogenous sources of ROS can also impact on the overall oxidative status of cell. Drugs, hormones, xenobiotic chemicals and infections can produce ROS by either direct or indirect mechanisms (83).

Alternatively, oxidative stress can also occur when there is a decrease in the antioxidant levels (vitamin E, vitamin C, glutathione, etc.) and enzymatic antioxidant levels (superoxide dismutase, glutathione peroxidase, and catalase) in the cell can be decreased through modification in gene expression, decrease in their uptake in the diet, or can be overloaded in ROS production, which creates a net increase in the amount of oxygen free radicals present in the cell (84).

Oxidants such as superoxide radicals, H_2O_2, $HO^{\cdot}$, and lipid peroxides (LOOH) are being shown to play an ever more important role in human disease (85). Several human chronic states and during many infections (bacterial, viral, etc.) oxidative hepatic stress may result through either (1) increased replication and/or pathogenesis of an infecting agent causing overproduction of free radical species and (2) failure of normal defense mechanisms, decreased antioxidant level in the target cell and tissue, leading to decreased elimination of reactive substances (74, 75). Monshoouwer *et al.* (76) have found that several biological processes including absorption, distribution, and metabolism were affected, to a great extent, in animals that were infected by a bacterial pathogen, *Actinobacillus pleuropneumonia*.

The liver is the major site of metabolism or biotransformation of drugs and other foreign compounds. Hepatic oxidative metabolism and adenosine tripolyphosphate (ATP) generating capability, which should be coupled to hepatic glucose production, have been found to be depressed in some experimental models, indicating a reduced metabolic efficiency of the liver eventually leading to organ failure (77). Also albumin production, which is the largest single protein product of the liver, is believed to be depressed by inflammatory conditions.

2.2. Mechanisms of infections mediated-hepatic oxidative stress/metabolism:

The possible mechanisms by which infections may produce hepatic oxidative stress and oxidative metabolism can be outlined to the following:

2.2.1. Effect of Lipopolysaccharide (LPS):

The endotoxin lipopolysaccharide (LPS) from the outer membrane of gram-negative bacteria is known to induce septic shock in laboratory animals (78). Free radical damage plays a key role in LPS-induced hepatic oxidative stress/metabolism, and the implication of blood phagocytes or kupffer cells as free radical sources in this pathology has been reported. LPS is known to enhance the formation of reactive oxygen species such as superoxide radicals, H_2O_2 and their secondary product malondialdehyde (MDA) through the activity of NADPH oxidase or xanthine oxidase (79, 80). The combination of these two oxygen species creates a more active and aggressive form of oxygen, $OH^{\cdot}$. LPS also generates free radicals intracellularly through the ischemia-reperfusion syndrome secondary to the decrease in tissue blood flow and by altering the activity of the major physiological sources of free radicals in liver microsomes, peroxisomes, mitochondria and even by induction of nitric oxide synthase. Free radials cause serious injury to vital tissue and organs, especially to membrane phospholipids, connective tissues and cell nucleic acids.

2.2.2. *Activation of phagocytes:*

Infection-induced activation of phagocytes is associated with oxidative stress, not only because ROS species are released but also because activated phagocytes may also release pro-oxidant cytokines, such as tumor necrosis factor (TFN), and interleukin-1, which promote iron uptake by reticuloendothelial system (81). TNF can either be released from activated phagocytes into the circulation, or in some infections it can be synthesized in infected host cells. In either case, TNF can act on host cell mitochondria, producing a pro-oxidant effect. TNF also acts to release nuclear transcription factor kappa B (TNF-κ-B) from the cytoplasmic inhibitor protein IkB. Activated monocytes also release interleukein-1, which stimulates neutrophils to release lysosomal proteins, including lactroferrin. Lactoferrin rapidly binds iron, an effect that explains the hypoferremia of acute inflammatory states; in such condition, lactroferrin-bound iron accumulates in the reticuloendothelial system. If the accumulated iron exceeds cellular iron-binding capacity, unbounded pro-oxidant iron could interact with superoxide to produce highly reactive hydroxyl radicals (82). Larrea *et al.* (83) have reported that hepatitis C virus may cause oxidative stress in infected cells. Patients with chronic hepatitis C exhibit an increased production of tumor necrosis factor-α (TNFα), a cytokine that can produce oxidative stress by stimulating the generation of ROS. The majority of membrane damage by ROS is mediated by lipid peroxidation.

2.2.3. *Respiratory Burst:*

Recently, several oxidative intermediates, including superoxide anion in the antimicrobial activity of neutrophils have been reported (84, 86). Neutrophils are highly regulated and interactive with the host's entire immunologic response. Within seconds of stimulation, neutrophils sharply increase their oxygen uptake, a phenomenon known as respiratory burst. The increased oxygen uptake leads to the formation of superoxide ions. In the presence of myeloperoxidase and a halide ion, hydrogen peroxide exhibits potent microbial activity. The myeloperoxidase utilizes hydrogen peroxide to oxidize the halide ions, yielding potent microbial agents. Reactive oxygen metabolites that escape or released into the microvascular environment can cause injury to tissues and connective tissue matrix. Reactive oxygen metabolites may lead to the peroxidation of lipids in the cell membranes resulting in the generation of fatty acid radicals that can react with other lipids, proteins, or free radicals in tissues (87). The overall effect may result in biochemical changes in the liver during the infection, including depletion of glycogen, lipid infiltration, and decrease in nucleic acid content (both DNA and RNA).

2.3. Oxidative damage to nucleic acids

Damage to DNA/chromosomes will affect their vital role in protein synthesis. In the presence of ROS, radical-mediated damage to DNA is complex and proceeds *via* peroxy radicals of the DNA bases and sugar, deoxyribose (70). The ultimate chemical agent interacting with the DNA has not been identified but one possibility is a hydroxyl radical, generated from hydrogen peroxide *via* homolytic fission (26). Base peroxidation occurs most readily intensively with thymine and guanine whereas adenine and cytosine appear to be more stable toward oxidation (8).

Alternatively, it has been hypothesized that ROS can disrupt pathways critical for the maintenance of normal, adenine and cytosine nucleotide status, such as the nucleases that repair strand breaks *via* base excision repair (26).

Damage to DNA by ROS can also lead to chromosome abnormalities (88). This is supported by the anti-chromosome-break (anticlastogenic) effect of superoxide dismutase and other antioxidants (89).

2.4. Oxidative damage to proteins and amino acids:

The non-covalent bonds that maintain the three-dimensional structure of proteins are weak, and susceptible to ROS action. Subtle changes in structure and/or in single amino acid at one site on a protein molecule may cause drastic changes in its function (90). Collagen, a component of bone and connective tissue, can be damaged by superoxide anions thereby preventing gelation (91). Collagen gelation involves the interaction of single collagen peptide chains by hydrogen bonding to form triple peptide chain helices (89). Thus, superoxide dismutase protects collagen from superoxide anion mediated inhibition of gelation.

Enzymes such as cytochrome P450 are also inactivated in similar way upon exposure to ROS (53). Histidine oxidation may operate as a marker for protein recognition by proteases, thus serving a function in protein turnover. Hence, oxidized proteins are more susceptible to proteolysis (92).

Oxidation of some amino acids in proteins can be used as an indicator for the screening of oxidative stress *in vivo*. Reversible oxidation such as oxidation of methionine to the sulfoxide and sulfone, or irreversible oxidation such as the ring cleavage in histidine or in tryptophan may be important in this respect (74).

As with DNA, the removal of oxidized proteins is an ongoing process, and oxidative cell injury will be evident only when oxidative damage exceeds the rate of protein removal or replacement with fully functional molecules (89).

2.5. Oxidative damage to carbohydrates:

Hyaluronic acid is a linear acidic polysaccharide consisting of alternating sugar molecules of glucuronic acid and *N*-acetylglucosamine. It is one of the main components of synovial fluid in joints that can be degraded by ROS (90). Superoxide dismutase was found to be capable of protecting hyaluronic acid against depolymerisation in synovial fluid (93). Proteoglycans and deoxyribose may be also subject to oxidative breakdown in a similar manner (94, 95).

2.6. Oxidative damage to lipids

Polyunsaturated fatty acids have become a central area of interest in the chemistry and biochemistry of oxidative reactions. Specific enzymatic oxidation of polyunsaturated fatty acids leads to the formation of extremely potent and biologically important compounds, e.g. prostaglandins and leukotrienes. In contrast, unspecific oxidation of polyunsaturated fatty acids can lead to lipid peroxidation, a radical mediated pathway (12). Furthermore, cholesterol is oxidized during lipid peroxidation, yielding a 5,6-epoxide in addition to a 5-hydroperoxide (12). The epoxide occurs in high concentrations in human breast milk and has been identified as a directly acting mutagen (96).

Macrophages possess receptors that recognize and bind to modified low-density lipoprotein (LDL), called scavenger receptors (97). Modified LDL bound to these scavenger receptors are rapidly engulfed by macrophages, so the intracellular cholesterol accumulates and may convert the macrophage into a foam cell, which in turn is involved in atherosclerosis development.

Recently, it has been shown that ROS are involved in LDL peroxidation (48). It was also found that modified forms of human LDL initiate the accumulation of cholesterol esters in macrophages as a result of oxidative stress (48). Vascular endothelial cells can also uptake and destroy oxidized LDL (98). However, take up of oxidized LDL by macrophages is more rapid and might be regarded as a defense mechanism to protect the vascular wall, but excess

oxidized LDL can kill macrophages, either by initiating necrosis or by apoptosis (Section 2.2). Macrophage death can release proteolytic enzymes and transition-metal ions, causing more oxidative stress to the surrounding cells that may leads to atherosclerosis (48).

2.7. Role of ROS in necrotic/apoptotic cell death:

Cell death occurs *via* an active or passive mechanism; apoptosis or necrosis, respectively. The morphological and physiological characteristics are described in table 2. Cell death induced by excessive oxidative stress has been assumed to occur by necrosis (78). ROS-mediated cellular damage (see Section 2.1) causes a destruction of membrane integrity and a loss of cellular homeostasis. This leads to an increase in osmotic pressure due to loss of ionic flux control, cell swelling and eventual lysis (62).

However, several reports indicate that ROS may also be important in apoptotic cell death (28, 80, 81). Glutathione levels are decreased during apoptosis, which may indicate that an increase in oxidative stress induces cell death (82). It has been also shown that antioxidants such as superoxide dismutase and catalase can inhibit apoptosis induced by a wide range of stimuli (83, 84). Kane *et al* has proposed that ROS may be involved in both necrosis and apoptosis (85). Consequently, it may be possible to modulate necrosis and apoptosis by antioxidants (79). However, Shimizu *et al.* have suggested that the intracellular rise in ROS seen in apoptotic systems may be caused by apoptosis, rather than being involved in its induction, thus, the detected increase in ROS could be a secondary response (86).

In agreement, apoptosis can occur in the absence of ROS and, in at least one case, is inhibited by either superoxide anion (87) or hydrogen peroxide (88) (Section 2.2.1). Therefore, there does not appear to be a consistent mechanism by which ROS functions in apoptotic cell death.

2.7.1. Mechanism of apoptosis:

While a high degree of oxidative stress can cause necrosis, lower levels will trigger apoptosis (78). Three types of proteins control apoptosis or programmed cell death. First, *bcl-2* family, which is involved in membrane protein building in mitochondria, endoplasmic reticulum and nuclear membrane. These are known as anti-apoptotic proteins (89). Secondly, ICE (interleukin-1*β*-converting enzyme) is a cysteine protease family that plays a key role during apoptosis; these are sometimes called caspases (90, 99). Caspases are present in the cell cytoplasm in an inactive form called pro-caspase, which become activated during apoptosis. The active enzyme can cleave a number of cysteine containing proteins and lead to apoptosis (90). ROS can activate the pro-caspase and lead to apoptosis (91). Kane *et al.* have suggested that *bcl-2* inhibits apoptosis by reducing the generation or effects of ROS while the activation of ICE can be prevented by antioxidants (85).

Thirdly, the tumor suppressor gene, *p53*, plays a role in the control of normal cell proliferation, partly due to its action as a transcription regulator (92). It induces apoptosis following DNA damage. Mutation of the *p53* gene and hence inactivation of the protein will shift the balance toward the cell survival (93). Protein encoded from *p53* has ten cysteine residues; oxidation of this protein by ROS inhibits its binding to DNA and lead to tumors (94).

Thompson has cited that the balance between the apoptotic proteins, the ICE members, and the anti-apoptotic proteins such as the *bcl-2* family will determine whether the cell survives or suicides (79).

$$\text{SOD-Cu(II)} + O_2^- + H^+ \longrightarrow \text{SOD-Cu(I)} + O_2 \ (1^{st}\ \text{step})$$

$$\text{SOD-Cu(I)} + O_2^- + H^+ \longrightarrow \text{SOD-Cu(II)} + H_2O_2 \ (2^{nd}\ \text{step})$$

$$O_2^- + 2H^+ \xrightarrow{\text{SOD}} H_2O_2 + O_2$$

Figure 7. Dismutation of superoxide anion catalyzed by superoxide dismutase

$$\text{lase-Fe(III)} + H_2O_2 \longrightarrow \text{catalase-Fe(IV)O} + H_2O$$

$$\text{catalase-Fe(IV)O} + H_2O_2 \longrightarrow \text{catalase-Fe(III)} + H_2O + O_2$$

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

Figure 8. Hydrogen peroxide decomposition catalyzed By catalase

Table 2: Morphological and physiological features for apoptosis and necrosis (78)

Apoptosis	Necrosis
Programmed cell death; requires energy	Random cell death; no energy required
Blobbing of the plasma membrane	Loss of the plasma membrane integrity
Cell shrinkage	Cell swelling and lysis
Nuclear and chromatin condensation	Flocculation of chromatin
Mitochondria are not perturbed	Mitochondria are perturbed
Formation of apoptotic bodies, containing cellular and nuclear components	No apoptotic bodies, complete lysis
Engulfed by neighboring cells, e.g. microglia	Engulfed by macrophages

2.8. Role of ROS within central nervous system:

There are two main mechanisms of neuronal cell death, which may act separately or cooperatively to cause neurodegeneration that is necrotic and/or apoptotic in nature. These mechanisms are excitotoxicity and oxidative stress (95). The brain differs from other organs in the generation and detoxification of ROS (see Section 2.3.2 and 2.3.3). Sun and Chen have postulated that oxidative stress generated by ROS causes the loss of neurons during the progression of neurodegenerative diseases (96, 100).

2.8.1. Detoxification of ROS in central nervous system:

Due to the selective vulnerability of neurons, the brain contains additional antioxidant defenses. The capillary endothelial cells of cerebral microvessels possess specific and unique features, forming the blood-brain barrier (BBB), which controls the entry of many types of solutes from general circulation to the cerebral parenchyma. It is formed basically by a monocellular layer of endothelial cells sealed by tight junctions, which possess high levels of enzymatic and non-enzymatic antioxidants (97). In addition, astrocytes, surrounding the BBB, contain higher antioxidant concentrations than other brain cell types (62). Although the BBB has high levels of antioxidant enzymes; the brain tissue has only low levels of these defense enzymes (4, 98).

Bayol-Denizot *et al.* found that co-cultured astrocytes protect other brain cell types against ROS whereas cultured neurons are more vulnerable to damage by ROS than astrocytes (99). *In vivo*, neurons and astrocytes are in close proximity. Evidence is indicating that an intensive metabolic exchange occurs between neuron and astrocyte cells in brain such as removal and inactivation of neurotransmitter molecules (101). Such interactions are very important regarding cerebral homeostasis and in the protection of the brain against xenobiotics and oxidative stress (62).

In addition to the contribution of astrocyte cells in defense systems, it has been reported that they increase the activity of superoxide dismutase, catalase and glutathione peroxidase in BBB endothelial cells (102). Consequently, astrocytes lower the ROS levels entering the brain and protect against degenerative diseases.

2.8.2. ROS and selective vulnerability of neurons:

Even at a cellular ratio of one astrocyte cell to twenty neurons, neurons can be damaged by ROS (99). Compared to all other tissues, the brain is particularly vulnerable to oxidative processes. Neurons of central nervous system (CNS) are almost completely dependent on the oxidative phosphorylation reactions in order to generate adenosine triphosphate (ATP) as energy source, (103). In addition, normal adult brain depends on glucose as the major nutrient and, therefore, the brain has a high glucose metabolism and respiratory turnover (104). Thus, high rate of oxygen turnover may account for the vulnerability of CNS neurons to ROS.

The brain contains high concentrations of free iron, which mediates the conversion of hydrogen peroxide to hydroxyl radicals *via* the Fenton reaction. Furthermore, neuronal membranes of the

brain contain high concentrations of polyunsaturated fatty acids, which are potential substrates for peroxidation by hydroxyl radicals (12). In addition, the loss of neurons in adult brain cannot generally be compensated by neuron re-generation (105).

Anatomically, motor neurons may be more vulnerable to ROS damage than other neurons. The large cell body and remarkable length of motor neuron axons predict that these cells have high-energy demands and a high metabolic rate, requiring a high level of mitochondrial activity in comparison to other cells (102).

The selective vulnerability of neurons may explain why neurotoxic drugs are able to damage nerve terminals and the suggested role of ROS in the pathology of several neurological diseases (46, 103, 104).

2.8.3. Role of ROS in degenerative diseases

Amyotrophic lateral sclerosis (ALS) is a progressive and fatal disease, where neurodegeneration affects primarily motor neurons of the cerebral cortex, brain stem, and spinal cord (105, 106). Since ALS is a rare disease, there are less studies in this area than more prevalent disorders such as atherosclerosis or cancer.

However, ALS is potentially a useful model for more common neurodegenerative disorders, such as Parkinson's disease, Alzheimer's disease and Huntington's chorea due to several reasons. First, a common feature of these disorders is selective neuronal death. Therefore, Doble has proposed that the proximate cause of neuronal death may differ in these diseases, but the final common pathway is likely to be similar (107). Secondly, the involvement of motor system in ALS permits simpler and more direct diagnosis than do extrapyramidal changes or dementia in Parkinson's disease and Alzheimer's disease, respectively (108). Thirdly, the relatively rapid onset (less than five years) and stereotyped nature history facilitate clinical monitoring of the disease (109).

Additionally, riluzole [2-amino-6-(trifluoro-methoxy)benzothiazole], the only drug registered for use in ALS (108, 109), is in phase III trials for Parkinson's disease (110), phase II trials for Alzheimer's disease (56) and phase I trial for Huntington's chorea (111, 112).

The causes of ALS are still unknown, but there is increasing evidence that two pathogenic mechanisms, namely excitotoxicity and oxidative stress, participate in this disorder (113, 114). However, physiological activation of motoneurons by glutamate (one of the main neurotransmitters of the nervous system) has been coupled to abnormal activity of cytoplasmic superoxide dismutase (115). It has also been reported that excitotoxicity can be increased by oxidative stress (117). Consequently, Kalra *et al.* have recently proposed that these two mechanisms may participate in a "positive feedback" manner in which one potentiates the other (118). If this is the case, then it is possible that antioxidants and glutamate antagonists could have either additive or synergistic effects in the treatment of ALS (119).

A link between ROS and ALS is supported by the selective vulnerability of motor neurons to free radical damage (Section 2.3.2) (120). Recent reports have indicated oxidative changes in proteins, lipids and DNA in the CNS of patients with ALS (44, 121). For this reason, antioxidants like recombinant SOD and procysteine (a glutathione repleting agent) are in phase I trials for ALS (122, 123).

Most patients (85-90%) present with "sporadic ALS" while the remaining 10% of ALS cases are of familial origin (124). Both sporadic and familial ALS manifest the same clinical and pathological symptoms (124). Approximately 15-20% patients with "familial ALS" pattern show mutations in the gene expressing superoxide dismutase which supports a role for ROS in ALS (121). Mutations of the superoxide dismutase gene are also found in approximately 2% of cases of sporadic ALS (126). Such mutations are dominant and the gene is located on chromosome 21q22.1 (127). A second mutation locus responsible for familial ALS has been mapped to chromosome 2q33, which is inherited in an autosomal recessive pattern (128). The aldehyde oxidase gene, which is also mapped to 2q33, has been coupled to familial ALS (129, 123, 130, 131).

The most broadly accepted hypothesis for the aetiopathology of Parkinson's disease is selective oxidative stress in the substantia nigra (132). Studies indicate that dopaminergic neurons in Parkinson's disease may be more susceptible to oxidative stress due to reduced glutathione levels and excessive free iron content (133). Dopamine generates free radicals and hydrogen peroxide by auto-oxidation or through normal enzymatic processing by MAO (125). Consequently, high levels of hydrogen peroxide are present in the substantial nigra.

It has also been suggested that the neuropathology of Huntington's chorea involves

oxidative stress, although most of the evidence is indirect (134). Post-mortem brains from patients with Huntington's chorea show an increase in oxidized DNA indicative of oxidative stress damage coupled with reduced levels of superoxide dismutase and oxidized glutathione (104).

Evidence for the role of oxidative stress in Alzheimer's disease etiology is accumulating (60, 126, 135). Various products of oxidation reactions, e.g. oxidized glutathione molecules, and mediators of oxidative stress, e.g. accumulation of free fatty acids, are found in brain of patients with Alzheimer's disease (126). Basically, most of cellular macromolecules (DNA, protein, and lipids) can be found in an oxidized form in Alzheimer's disease brain tissue (126, 127). Other studies indicate that superoxide dismutase activity is decreased in Alzheimer's disease brain although these results are not substantiated in other studies (56, 126, 128).

Recently, melatonin has been shown to be highly effective in reducing oxidative damage in Parkinson's disease, Huntington's chorea and Alzheimer's disease. This efficacy derives from its ability to function as a direct and indirect antioxidant (Section 1.5.2) (63, 64).

Conclusion

This review shows that ROS are important elements in the pathological processes. Oxidative stress appears to be an important factor in a number of human infections including the induction of cancer. The contribution of ROS in disease is one of leading hypothesizes in the etiology of cancer, ageing, inflammation, viral infections and neurodegenerative diseases. In summary, these findings may point to a deficit in ROS scavenging and/or ROS overproduction being involved in the aetiopathology of these neurodegenerative diseases. Consequently, one of the useful neuronal rescue strategies is treatment with antioxidant agents. Understanding the pathogenesis of viral, bacterial infections, the host response to them, and hepatic oxidative stress may pave the way toward important advances in the therapeutics for control of infection pathogenesis and oncogenesis.

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