ELSEVIER

Toxicology 149 (2000) 43–50

TOXICOLOGY

www.elsevier.com/locate/toxicol

The Haber–Weiss reaction and mechanisms of toxicity☆

James P. Kehrer *

Division of Pharmacology and Toxicology, College of Pharmacy, The University of Texas at Austin, Austin, TX 78712-1074, USA

Abstract

The concept that the highly reactive hydroxyl radical ($HO^{\bullet}$) could be generated from an interaction between superoxide ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2) was proposed (with Joseph Weiss) in Professor Haber's final paper published in 1934. Until it was recognized that free radicals are produced in biological systems, this finding seemed to have no relevance to biology. However, following the discovery that $O_2^{\bullet -}$ was a normal cellular metabolite, it was quickly recognized that the Haber–Weiss reaction ($O_2^{\bullet -} + H_2O_2 \rightarrow HO^{\bullet} + O_2 + HO^-$) might provide a means to generate more toxic radicals. Although the basic reaction has a second order rate constant of zero in aqueous solution and thus cannot occur in biological systems, the ability of iron salts to serve as catalysts was discussed by these authors. Because transition metal ions, particularly iron, are present at low levels in biological systems, this pathway (commonly referred to as the iron-catalyzed Haber–Weiss reaction) has been widely postulated to account for the in vivo generation of the highly reactive $HO^{\bullet}$. Recent data documenting the importance of redox regulation of various cellular signaling pathways makes it clear that free radicals are essential for normal cellular function. However, this also makes it obvious that disruptions of free radical production or defenses at many different levels can lead to adverse effects on cells. While the generation of $HO^{\bullet}$, which is by far the most reactive oxygen species, is generally indicative of an overtly toxic event, it is through studies at this level that we have reached a better understanding of free radicals as both signaling molecules and toxic species.

Keywords: Haber–Weiss reaction; Hydroxyl radical; Free radicals; Toxicology; Signal transduction

1. Introduction

Professor Fritz Haber won the Nobel prize for chemistry in 1918, primarily for his work on fixing nitrogen by a chemical reaction. Prior to this work, fixing nitrogen out of the air by making it react with hydrogen to form ammonia was a feat considered to be impossible. Professor Haber was able to devise a system whereby nitrogen and hydrogen were circulated over a catalyst at a pressure of 150–200 atmospheres and a temperature of about 500°C. Subsequent modifications of this Haber process provided ammonium sulfate for use as a fertilizer for the soil, an extremely important accomplishment at the end of World War I when natural nitrate supplies were depleted

☆ Derived from the workshop titled 'An analysis of the $C \times T$ concept and of mechanisms in free radical toxicology: the legacy of Fritz Haber' presented at the 39th Annual Meeting of the Society of Toxicology, March 21, 2000.

* Tel.: +1-512-4711107; fax: +1-512-4715002.

E-mail address: kehrerjim@mail.utexas.edu (J.P. Kehrer).

0300-483X/00/$ - see front matter
PII: S0300-483X(00)00231-6

as a consequence of making explosives. The principle used for this process also led to the synthesis of methyl alcohol, the hydrogenation of coal, and the production of nitric acid.

Importantly, Professor Haber's contributions did not end with his work on nitrogen fixation. In terms of free radical chemistry, perhaps his most significant contribution was the concept found in his final paper (Haber and Weiss, 1934) that the highly reactive hydroxyl radical ($HO^{\bullet}$) could be generated from an interaction between superoxide ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2).

Chemical studies involving free radicals were ongoing for many years before it was recognized that such reactive species are produced in biological systems. At that point, it was quickly realized that the Haber–Weiss reaction (Eq. (3)) might provide a means to generate more toxic radicals from the less reactive superoxide and hydrogen peroxide that could be generated enzymatically. Early biochemical work that occurred after the recognition that free radicals were important toxicologic species focused on the Haber–Weiss reaction. Shortly, however, it was recognized that this reaction is thermodynamically unfavorable in biologic systems, having a second order rate constant of zero in aqueous solution, and would require some sort of catalyst to proceed. Interestingly, the original paper by Haber and Weiss discussed the need for a metal ion catalyst and illustrated that the net reaction creating the hydroxyl radical (Eq. (3)) can be broken down into two chemical reactions (Eqs. (1) and (2)). Although other transition metal ions are capable of catalyzing this reaction, the iron-catalyzed Haber–Weiss reaction, which makes use of Fenton chemistry, is now considered to be the major mechanism by which the highly reactive hydroxyl radical is generated in biological systems (Liochev, 1999).

$$Fe^{3+} + O_2^{\bullet -} \rightarrow Fe^{2+} + O_2 \quad (1)$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\bullet} \quad \text{(Fenton reaction)} \quad (2)$$

The net reaction:

$$O_2^{\bullet -} + H_2O_2 \rightarrow O_2 + OH^- + OH^{\bullet} \quad (3)$$

2. Oxidative stress

Oxygen is the most abundant molecule in a biological system. Although only minimally reactive due to spin restrictions (Kehrer, 1987), it exists as a diradical and thus reacts extremely rapidly with other radicals. Oxygen itself is often the source of such radicals as partially reduced species are generated through normal metabolic processes, and some of these reactive species can escape. As a result, reactive oxygen species (ROS) are prominent toxicologic intermediates, and are commonly involved in 'oxidative stress'. This term is defined as a disruption of the prooxidant–antioxidant balance in favor of the former, leading to potential damage (Sies, 1991). Although potentially arising from either an increased production of oxidizing species or a decreased protective capacity, it is the former mechanism that has received the greatest attention. Since redox balance is inviolable, oxidative stress is necessarily a compartmentalized phenomenon and is intimately related to 'reductive stress' which can also lead to potential damage (Kehrer, 2000).

Research over the past 30 years has demonstrated that there are a number of reactive species present in biological systems that have the potential to induce damage. These include hydrogen peroxide, organic hydroperoxides and hypohalous acids with half lives in the range of minutes, the peroxyl radical and nitric oxide with half lives of seconds, peroxynitrite with a half-life of a millisecond, superoxide anion, singlet oxygen and the alkoxyl radical with half lives of about a microsecond, and the hydroxyl radical with a half life that is diffusion limited at about a nanosecond. The exact contribution of these different species to various pathologic conditions remains unclear. This is most likely due to their being both an initiator and product of tissue damage, making assigning cause and effect difficult. In addition, quantitative considerations have suggested that overt physical damage may require high local concentrations that may or may not be achievable in vivo. Recent work demonstrating significant effects on signaling pathways have, however, revealed alternate pathways to injury that can occur at much lower oxidant concentrations.

3. Potential sources of ROS

ROS can be derived from numerous sources in vivo. These include autooxidation, photochemical and enzymatic reactions, and may involve both endogenous compounds and various xenobiotics. The number of different enzymes shown to be capable of generating ROS is extensive, and includes the cytochromes P450, various oxidases, peroxidases, lipoxygenases and dehydrogenases. The involvement of xenobiotics can be particularly important in determining the extent of ROS generated by these enzymes. For example, various quinones can undergo redox cycling, generating large amounts of ROS without themselves being degraded (Fig. 1). In addition, NADPH oxidase is well known to generate ROS as part of its antibacterial function on phagocytic cells. However, this enzyme also appears to be present on numerous other cells and may have important signal transduction activities (Finkel, 1999).

Perhaps the most important in vivo source of ROS is the mitochondrion (Loschen et al., 1971, 1974; Boveris and Cadenas, 1975; Chance et al., 1979). Although there is no doubt this organelle can generate ROS, particularly hydrogen peroxide and the superoxide anion (Boveris et al., 1972; Boveris and Cadenas, 1975; Turrens and Boveris, 1980), the quantity produced under both normal and pathologic conditions is unknown. The figure given in most literature is that up to 2% of total mitochondrial oxygen consumption 'normally' goes toward the production of ROS. However, this number was derived using isolated mitochondria respiring under state 4 conditions (without added ADP, but with all other components present in saturating amounts). Under conditions of state 3 respiration (where all components are present in saturating amounts), ROS production falls close to zero (Boveris et al., 1972). Since the process of isolating mitochondria unavoidably causes some damage, and respiration states are determined in an artificial in vitro system, the actual production of radicals by intact mitochondria in healthy tissues is likely to account for far less than 2% of total oxygen consumption. However, substantially more may be produced under damaging conditions or in the presence of various xenobiotics.

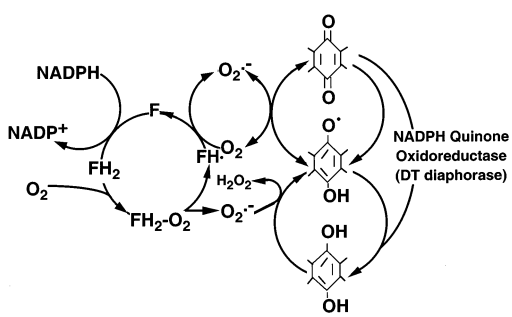

Fig. 1. Quinone redox cycling as a mechanism to generate ROS. Quinones can be reduced by a flavin-containing enzyme (F) that obtains electrons from NADPH. The one electron reduction product, a semiquinone, can give up its electron to oxygen forming the superoxide anion, or can receive a second electron yielding a hydroquinone product. A single two-electron reduction step catalyzed by NADPH quinone oxidoreductase, without a semiquinone intermediate, can generate the hydroquinone, which is relatively stable.

The source of mitochondrial ROS appears to involve a non-heme iron protein that transfers electrons to oxygen. This occurs primarily at Complex I (NADH-coenzyme Q) and, to a lesser extent, following the autoxidation of coenzyme Q from the Complex II (succinate-coenzyme Q) and/or Complex III (coenzyme QH_2-cytochrome c reductases) sites (Fig. 2). Once again, the precise contribution of each site to total mitochondrial ROS production is probably determined by local conditions including chemical or physical damage to the mitochondria, oxygen availability and the presence of xenobiotics.

4. ROS and DNA damage

The presence of oxidized DNA bases is often used as a marker for ROS-mediated DNA damage (Helbock et al., 1999). The guanine base is particularly sensitive to oxidation making this a reasonable biomarker for oxidative injury. As analytical methodology has improved, it has become possible to detect as little as 25 fmol of 8-hydroxy-

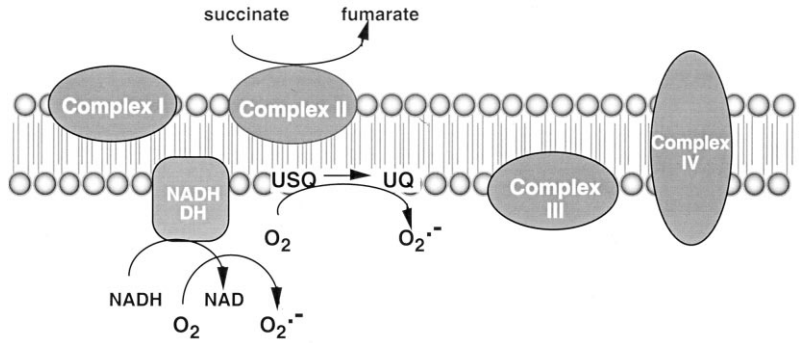

Fig. 2. Sites of mitochondrial ROS production. Superoxide can be formed by the donation of an electron to oxygen from NADH dehydrogenase at complex I. Superoxide and/or hydrogen peroxide may also be formed following the autoxidation of reduced ubiquinone at Complex II (succinate-coenzyme Q) and/or Complex III (coenzyme QH_2-cytochrome c reductases) sites.

deoxyguanosine. Further, initial problems with spurious oxidation during sample preparation appear to have been overcome (Helbock et al., 1998) and current estimates of steady-state levels are less than 100–1000 8-hydroxydeoxyguanosine residues in normal cells (Klungland et al., 1999). It is important to remember, however, that while oxidized DNA bases will impair DNA function, such bases always exist at some basal level, and cells have numerous repair systems to remove such species (Lindahl and Wood, 1999). However, if they occur at critical sites, or are not quickly repaired, oxidized purines or pyrmidines can cause functional problems. As a result, oxidized DNA bases are considered an important event in chemical carcinogenesis (Klaunig et al., 1998).

In general, oxidized DNA exhibits an increased propensity for genetic mutations and alterations in transcription. There are several mechanisms by which these effects may occur. These include effects on hydrogen bonding, a decreased fidelity of DNA and/or RNA polymerase, and conformational changes in the DNA template. By damaging DNA directly, interfering with DNA repair, affecting cell division or the promotion process, or mediating the activation of carcinogens, ROS have the potential to modulate the development of cancer at several levels as well as to disrupt cell functions during non-carcinogenic toxicity events. The extreme reactivity of the hydroxyl radical makes it a particularly important player in free radical-mediated toxic processes.

5. ROS and lipid damage

Lipids have a critical structural and functional role in membranes. Any disruption of this role can lead to cell death. The double bonds found in polyunsaturated fatty acids are ready targets for free radical attack. The abstraction of a hydrogen atom from one of these double bonds, which can be mediated by free radicals, yields a new radical species that can readily interact with molecular oxygen, which is a diradical. The resultant lipid peroxyl radical can abstract a hydrogen atom from another fatty acid yielding yet another radical and a lipid hydroperoxide thereby establishing a chain reaction. The lipid hydroperoxide formed is unstable and can decompose to various species including malondialdehyde, or it can be reduced to the more stable alcohol form. As these reactions progress, ionic channels may be affected, membrane transport proteins or enzymes may be inactivated, or the lipid bilayer itself may become more permeable thereby disrupting ion homeostasis. In addition, some of the oxidized fatty acid species that are formed such as the isoprostanes or the hydroperoxides have biologic activity in terms of an ability to affect signaling pathways including those that regulate the apoptotic form of cell death.

Apoptosis involves a series of well organized events that require active cell participation. It is the basis for normal tissue remodeling as well as the end result of certain toxic insults (Cummings

et al., 1997). It differs morphologically from oncotic cell death (Uren and Vaux, 1996) and several genes have been identified as potentially controlling apoptosis in different species (Nagata, 1997). A relationship between ROS and several effectors of apoptosis has been reported (Sandstrom and Buttke, 1994; Sarafian and Bredesen, 1994; Payne et al., 1995; Slater et al., 1996). However, the mechanism by which oxidants induce apoptosis is unknown. It is likely some signaling factor is generated. Since polyunsaturated fatty acids are highly susceptible to oxidation, lipid messengers (Maccarrone et al., 1997) or lipid peroxides (Sandstrom et al., 1994, 1995; Ramakrishnan et al., 1996) that can induce apoptosis are reasonable candidates.

6. ROS and protein damage

The oxidation of proteins by ROS can generate a range of stable as well as reactive products. Among the reactive products are protein hydroperoxides that can generate additional radicals particularly upon interactions with transition-metal ions (Dean et al., 1997). Oxidized protein and amino acid species found in biologic systems are listed in Table 1. The formation of such species can alter protein functions, but this is not often quantitatively significant because of the sheer number of each type of protein. For similar reasons, except for enzymatic repair of oxidized thiols from *S*-alkyl derivatives (which may control activities and/or signaling functions) protein repair systems do not appear to exist since it is apparently more efficient to either prevent the formation of oxidized proteins through the action of various cellular antioxidant systems, or to simply destroy the modified species and start over. This latter process is evident by the fact that oxidized proteins tend to denature making them more readily recognized and susceptible to proteolysis and subsequent removal from the system (Grune et al., 1997). Although most oxidized proteins that are functionally inactive are rapidly removed, some can gradually accumulate with time and thereby contribute to the damage associated with aging as well as various diseases including diabetes, atherosclerosis and neurodegenerative diseases. Oxidized proteins may also help regulate cellular remodeling and cell growth.

Table 1
Oxidized protein and amino acids found in biologic systems[a]

2-Oxohistidine
3-Chlorotyrosine
3-Nitrotyrosine
5-Hydroxy-2-aminovaleric acid
Aminomalonic acid
Dimers of hydroxylated aromatic amino acids
Dopa
Hydro(pero)xyleucine
Hydro(pero)xyvalines
N-Formylkynurenine; kynurenine
o- and *m*-tyrosine
p-Hydroxyphenylacetaldehyde
Protein carbonyls

[a] Dean et al., 1997.

7. ROS and signal transduction

In recent years, there has been increasing interest in the role of ROS in cell signaling processes. This activity may be mediated directly by various ROS acting on signal transduction pathways, or indirectly through the generation of bioactive mediators (Fig. 3). The number of transcription factors whose activities are modulated by ROS is substantial, and more continue to be identified (Table 2). ROS also appear capable of affecting the activity of calcium signaling, antioxidant enzymes, ion transporters, various cell growth related genes and, importantly, various kinases including c-jun N terminal kinase (JNK), extracellular regulated kinase (ERK) and the mitogen activated protein kinase (MAP kinase) family (Sen, 1998; Adler et al., 1999).

The effects of ROS on signal transduction pathways can be both positive and negative. The repression of gene expression by oxidative stress was recently reviewed (Morel and Barouki, 1999). These authors documented that the sensitivity of various transcription factors to oxidation is highly variable and suggested that a redox switch may exist allowing one transcription factor to replace another based on their redox responsiveness.

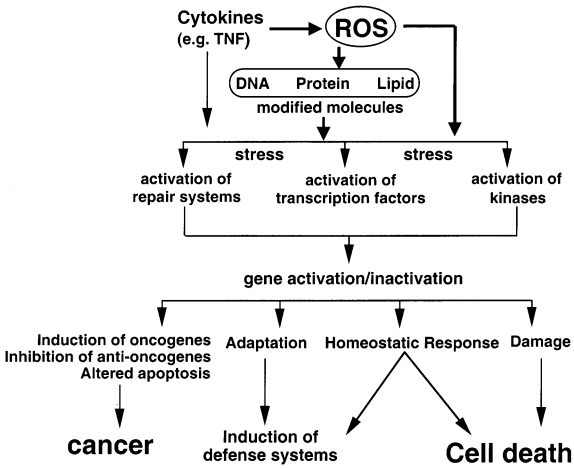

Fig. 3. The role of reactive species in cell signaling processes. Reactive species, particularly superoxide and NO, can interact with DNA, proteins or lipids modifying their structure or function and leading to the activation or inactivation of various genes. These changes can be part of adaptation or homeostatic responses, but may also lead to abnormalities that affect cell or tissue functions leading to cell dysfunction, transformation or death.

Table 2
Redox sensitive transcription factors

AP-1
Egr
Elk-1
GABP
HIF-1
HSF
NF-AT
NF-Y
NF-κB
PAX-8
PEBP2
Sp-1
TTF-1

Overall, it is apparent that ROS affect many steps in the complex signaling mechanisms that regulate cell division and differentiation.

There are multiple mechanisms by which ROS alter signaling pathways. Although not all are known, it appears that controlling the thiol status of a cell is critical (Sen, 1998). ROS can catalyze the formation of disulfide linkages between proteins, the formation of protein-glutathione mixed disulfides, or perhaps more directly alter protein–protein interactions (Fig. 4). Changes in signaling pathways may modify the activity of growth factors, protein kinases, protein phosphatases and transcription factors, which in turn modulate gene expression or cause the cell to recognize that it has been damaged and thus to activate that apoptotic machinery (Fig. 5).

8. Conclusions

A mechanism for the formation of the hydroxyl radical involving hydrogen peroxide and the superoxide anion as described by Professor Haber has proven to be a cornerstone of free radical biochemistry. Extensive research has shown that such radicals are formed in vivo, and are capable of explaining the damage associated with various xenobiotics and disease processes. More recently, the subtle effects of low levels of free radical species on signal transduction pathways have begun to be appreciated. The role of hydroxyl radicals in these signaling processes is almost certainly indirect since this species is so reactive. However, the generation of secondary signaling species is attracting increasing interest. As described above, this is particularly true in terms of lipid signaling molecules. It seems likely that as research in this area progresses, we will improve our understanding of the pathways involved while increasing our appreciation of the elegant ways that cells have

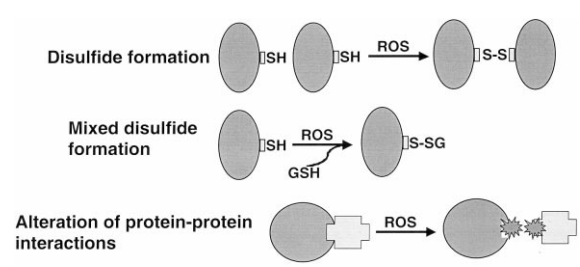

Fig. 4. Mechanisms by which ROS may alter signaling pathways. Cellular thiols appear to be an important regulatory target. ROS can oxidize a sulfhydryl group resulting in the formation of disulfide linkages between proteins or the formation of protein-glutathione mixed disulfides, or perhaps more directly alter protein–protein interactions.

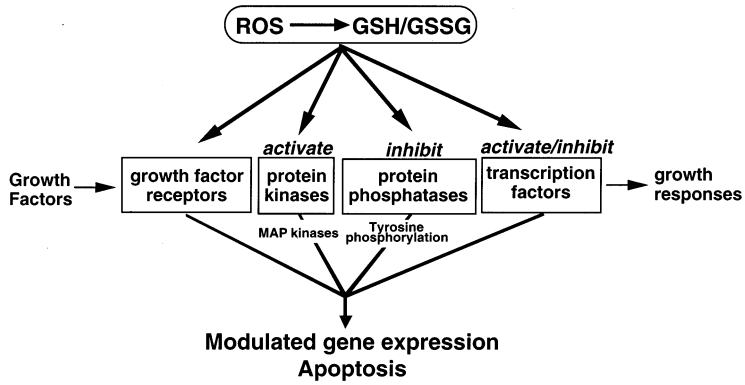

Fig. 5. Cellular targets for ROS that may affect function. ROS, either directly or through their action on thiols such as glutathione, can affect the activity of growth factors, protein kinases, protein phosphatases or transcription factors. Changes in the activities of one or more of these items may, in turn, modulate gene expression or induce apoptosis.

made beneficial use of potentially toxic species. We owe a debt to Professor Haber for his early insights into mechanisms of free radical formation and for laying the foundation for today's research into free radical biochemistry.

Acknowledgements

This work was supported by NIH RO1 grants ES09791, HL51005, HL48035 and Center grant ES07784. Kehrer is the Gustavus and Louise Pfeiffer Professor of Toxicology.

References

Adler, V., Yin, Z., Tew, K.D., Ronai, Z., 1999. Role of redox potential and reactive oxygen species in stress signaling. Oncogene 18, 6104–6111.

Boveris, A., Cadenas, E., 1975. Mitochondrial production of superoxide anions and its relationship to the antimycin insenstive respiration. FEBS Lett. 54, 311–314.

Boveris, A., Oshino, N., Chance, B., 1972. The cellular production of hydrogen peroxide. Biochem. J. 128, 617–630.

Chance, B., Sies, H., Boveris, A., 1979. Hydroperoxide metabolism in mammalian organs. Physiol. Rev. 59, 527–605.

Cummings, M.C., Winterford, C.M., Walker, N.I., 1997. Apoptosis. Am. J. Surg. Pathol. 21, 88–101.

Dean, R.T., Fu, S., Stocker, R., Davies, M.J., 1997. Biochemistry and pathology of radical-mediated protein oxidation. Biochem. J. 324, 1–18.

Finkel, T., 1999. Signal transduction by reactive oxygen species in non-phagocytic cells. J. Leukocyte Biol. 65, 337–340.

Grune, T., Reinheckel, T., Davies, K.J., 1997. Degradation of oxidized proteins in mammalian cells. FASEB J. 11, 526–534.

Haber, F., Weiss, J., 1934. The catalytic decomposition of hydrogen peroxide by iron salts. Proc. R. Soc. London, Ser. A. 147, 332–351.

Helbock, H.J., Beckman, K.B., Shigenaga, M.K., Walter, P.B., Woodall, A.A., Helen, C., Yeo, H.C., Ames, B.N., 1998. DNA oxidation matters: the HPLC-electrochemical detection assay of 8-oxo-deoxyguanosine and 8-oxo-guanine. Proc. Natl. Acad. Sci. U.S.A. 95, 288–293.

Helbock, H.J., Beckman, K.B., Ames, B.N., 1999. 8-Hydroxydeoxyguanosine and 8-hydroxyguanine as biomarkers of oxidative DNA damage. Methods Enzymol. 300, 156–166.

Kehrer, J.P., 1987. Oxygen. In: Seiler, H.G., Sigel, H. (Eds.), Handbook on Toxicity of Inorganic Compounds. Marcel Dekker, New York, pp. 505–515.

Kehrer, J.P., 2000. Reductive stress. In: Fink, G. (Ed.), Encyclopedia of Stress, vol. 3. Academic Press, San Diego, CA, pp. 327–333.

Klaunig, J.E., Xu, Y., Isenberg, J.S., Bachowski, S., Kolaja, K.L., Jiang, J., Stevenson, D.E., Walborg, E.F. Jr, 1998. The role of oxidative stress in chemical carcinogenesis. Environ. Health Perspect. 106 (Suppl. 1), 289–295.

Klungland, A., Rosewell, I., Hollenbach, S., Larsen, E., Daly, G., Epe, B., Seeberg, E., Lindahl, T., Barnes, D.E., 1999. Accumulation of premutagenic DNA lesions in mice defective in removal of oxidative base damage. Proc. Natl. Acad. Sci. U.S.A. 96, 13300–13305.

Lindahl, T., Wood, R.D., 1999. Quality control by DNA repair. Science 286, 1897–1905.

Liochev, S.I., 1999. The mechanism of 'Fenton-like' reactions and their importance for biological systems. A biologist's view. Metal Ions Biol. Syst. 36, 1–39.

Loschen, G., Flohé, L., Chance, B., 1971. Respiratory chain linked H_2O_2 production in pigeon heart mitochondria. FEBS Lett. 18, 261–264.

Loschen, G., Azzi, A., Richter, C., Flohé, L., 1974. Superoxide radicals as precursors of mitochondrial hydrogen peroxide. FEBS Lett. 42, 68–72.

Maccarrone, M., Catani, M.V., Agro, A.F., Melino, G., 1997. Involvement of 5-lipoxygenase in programmed cell death of cancer cells. Cell Death Differentiation 4, 396–402.

Morel, Y., Barouki, R., 1999. Repression of gene expression by oxidative stress. Biochem. J. 342, 481–496.

Nagata, S., 1997. Apoptosis by death factor. Cell 88, 355–365.

Payne, C.M., Bernstein, C., Bernstein, H., 1995. Apoptosis overview emphasizing the role of oxidative stress, DNA damage and signal-transduction pathways. Leuk. Lymphoma 19, 43–93.

Ramakrishnan, N., Kalinich, J.F., McClain, D.E., 1996. Ebselen inhibition of apoptosis by reduction of peroxides. Biochem. Pharmacol. 51, 1443–1451.

Sandstrom, P.A., Buttke, T.M., 1994. Inhibition of activation-induced death in T cell hybridomas by thiol antioxidants: oxidative stress as a mediator of apoptosis. J. Leukocyte Biol. 55, 221–226.

Sandstrom, P.A., Tebbey, P.W., Van Cleave, S., Buttke, T.M., 1994. Lipid hydroperoxides induce apoptosis in T cells displaying an HIV-associated glutathione peroxidase deficiency. J. Biol. Chem. 269, 798–801.

Sandstrom, P.A., Pardi, D., Tebbey, P.W., Dudek, R.W., Terrian, D.M., Folks, T.M., Buttke, T.M., 1995. Lipid hydroperoxide-induced apoptosis: lack of inhibition by Bcl-2 over-expression. FEBS Lett. 365, 66–70.

Sarafian, T.A., Bredesen, D.E., 1994. Is apoptosis mediated by reactive oxygen species? Free Radic. Res. 21, 1–8.

Sen, C.K., 1998. Redox signaling and the emerging therapeutic potential of thiol antioxidants. Biochem. Pharmacol. 55, 1747–1758.

Sies, H., 1991. Oxidative stress: introduction. In: Sies, H. (Ed.), Oxidative Stress, Oxidants and Antioxidants. Academic Press, San Diego, CA, pp. XV–XXII.

Slater, A.F.G., Stefan, C., Nobel, I., van den Dobbelsteen, D.J., Orrenius, S., 1996. Intracellular redox changes during apoptosis. Cell Death Differentiation 3, 57–62.

Turrens, J.F., Boveris, A., 1980. Generation of superoxide anion by the NADH dehydrogenase of bovine heart mitochondria. Biochem. J. 191, 421–427.

Uren, A.G., Vaux, D.L., 1996. Molecular and clinical aspects of apoptosis. Pharmacol. Ther. 72, 37–50.