ELSEVIER

Clinical Biochemistry, Vol. 32, No. 8, 595–603, 1999

0009-9120/99/$–see front matter

PII S0009-9120(99)00075-2

Antioxidant Enzymes and Human Diseases

JOSÉ M. MATÉS, CRISTINA PÉREZ-GÓMEZ, and IGNACIO NÚÑEZ DE CASTRO

Department of Molecular Biology and Biochemistry, Faculty of Sciences, University of Málaga, Campus de Teatinos, s/n 29071 Málaga, Spain

Objectives: To describe the importance of the antioxidant enzymes superoxide dismutase, glutathione peroxidase, and catalase working together in human cells against toxic reactive oxygen species, their relationship with several pathophysiologic processes and their possible therapeutic implications.
Conclusions: Reactive oxygen species (ROS) are involved in the cell growth, differentiation, progression, and death. Low concentrations of ROS may be beneficial or even indispensable in processes such as intracellular signaling and defense against micro-organisms. Nevertheless, higher amounts of ROS play a role in the aging process as well as in a number of human disease states, including cancer, ischemia, and failures in immunity and endocrine functions. As a safeguard against the accumulation of ROS, several non-enzymatic and enzymatic antioxidant activities exist. Therefore, when oxidative stress arises as a consequence of a pathologic event, a defense system promotes the regulation and expression of these enzymes. Copyright © 1999 The Canadian Society of Clinical Chemists

KEY WORDS: antioxidants; catalase; glutathione peroxidase; human diseases; oxidative damage; oxidative stress; reactive oxygen species; superoxide dismutase; therapy.

Introduction

Aerobic organisms possess antioxidant defense systems that deal with reactive oxygen species (ROS) produced as a consequence of aerobic respiration and substrate oxidation (Figure 1). Small amounts of ROS, including hydroxyl radicals ($^{\bullet}OH$), superoxide anions ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2), are constantly generated in aerobic organisms in response to both external and internal stimuli (1–3). Low levels of ROS are indispensable in many biochemical processes, including intracellular messaging in the cell differentiation and cell progression or the arrest of growth, apoptosis (4), immunity (5), and defense against micro-organisms (6,7). In contrast, high doses and/or inadequate removal of ROS result in oxidative stress, which may cause severe metabolic malfunctions and damage to biological macromolecules (8–10).

The naturally occurring antioxidants in low-density lipoproteins (LDLs) and plasma protect cells from oxidation (11–14). The prevention of lipid peroxidation is an essential process in aerobic organisms, as lipid peroxidation products can cause DNA damage and directly inhibit proteins such as Na^+/K^+ ATPases and glutamate transporters (1,11, 12,14). Increased lipid peroxidation and decreased antioxidant protection generate epoxides that may spontaneously react with nucleophilic centers in the cell and thereby covalently bind to DNA, RNA, and protein (5,15). Such a reaction may lead to cytotoxicity, allergy, mutagenicity, and/or carcinogenicity, depending on the properties of the epoxide in question (16). In addition, oxidative events may play an important role in the mechanism of action of ether lipids, and ability to oxidize may contribute to cellular drug sensitivity (17).

Lipid peroxidation can be evaluated by the thiobarbituric acid reactive substances method (TBARS). This method evaluates the oxidative stress assayed for malondialdehyde, the last product of lipid breakdown caused by oxidative stress (18–21).

The enzymatic and non-enzymatic antioxidant defenses include superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT), ascorbic acid (vitamin C), α-tocopherol (vitamin E), glutathione (GSH), β-carotene, and vitamin A, which can be evaluated using easy photometric assays (22–26). There is a balance between both the activities and the intracellular levels of these antioxidants that are essential for the survival of organisms and their health (27–33).

Antioxidant enzymes: properties and biological implications

Superoxide dismutase

Superoxide dismutase (EC 1.15.1.1) is the antioxidant enzyme that catalyses the dismutation of the highly reactive superoxide anion to O_2 and to the

Correspondence: José M. Matés, Department of Molecular Biology and Biochemistry, Faculty of Sciences, University of Málaga, Campus de Teatinos, s/n 29071 Málaga, Spain. E-mail: jmates @uma.es

Manuscript received April 30, 1999, revised August 11, 1999; accepted August 31, 1999.

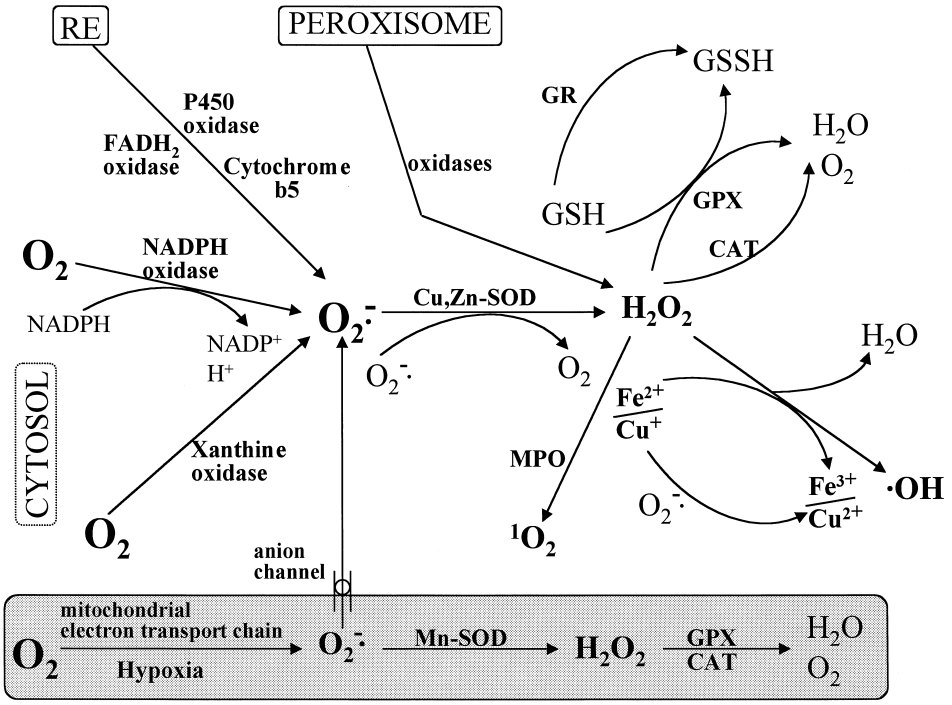

Figure 1 — Generation of reactive oxygen species and the defense mechanisms against damage by active oxygen. During hypoxia superoxide generated may be degraded into the mitochondria by Mn-SOD or, if it reaches the cytosol, by Cu, Zn-SOD. In the endoplasmic reticulum, NADPH-cytochrome P450 reductase can leak electrons onto O_2 generating $O_2^{\bullet -}$. $FADH_2$ and cytochrome b_5 can also contribute to this system. Within peroxisomes, there are enzymes localized that produce H_2O_2 without intermediation of $O_2^{\bullet -}$. Contrarily to $O_2^{\bullet -}$, H_2O_2 is able to cross cell membranes and within the cells it can react with Fe^{2+} or Cu^+ to form hydroxyl radicals via Fenton reaction. GR = glutathione reductase; MPO = myeloperoxidase; RE = endoplasmic reticulum; $^1O_2$: singlet oxygen.

less reactive species H_2O_2. Peroxide can be destroyed by CAT or GPX reactions (34–36).

$$O_2^{\bullet -} + O_2^{\bullet -} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2 \qquad [1]$$

In humans, there are three forms of SOD: cytosolic Cu/Zn-SOD, mitochondrial Mn-SOD, and extracellular SOD (EC-SOD) (37,38). SOD destroys $O_2^{\bullet -}$ by successive oxidation and reduction of the transition metal ion at the active site in a Ping Pong type mechanism with remarkably high reaction rates (39). All types of SOD bind single charged anions such as azide and fluoride, but distinct differences have been noted in the susceptibilities of Fe-, Mn^- or Cu/Zn-SODs. Cu/Zn-SOD is competitively inhibited by N_3^-, CN^- (40), and by F^- (41).

Mn-SOD is a homotetramer (96 kDa) containing one manganese atom per subunit that cycles from Mn (III) to Mn (II) and back to Mn (III) during the two step dismutation of superoxide (42). The respiratory chain in mitochondria is a major source of oxygen radicals. Mn-SOD has been shown to be greatly induced and depressed by cytokines, but is only moderately influenced by oxidants (43). Inactivation of recombinant human mitochondrial Mn-SOD by peroxynitrite is caused by nitration of a specific tyrosine residue (42,44).

The biological importance of Mn-SOD is demonstrated among others by the following observations: (a) inactivation of Mn-SOD genes in *Escherichia coli* increases mutation frequency when grown under aerobic conditions (45); (b) elimination of the gene in *Saccharomyces cerevisiae* increases its sensitivity to oxygen (46); (c) lack of expression in Mn-SOD knockout mice results in dilated cardiomyopathy and neonatal lethality (47); (d) tumor necrosis factor (TNF) selectively induces Mn-SOD, but not Cu/Zn-SOD, CAT or GPX mRNA in various mouse tissues and cultured cells (48,49); (e) transfection of Mn-SOD cDNA into cultured cells rendered the cells resistant to paraquat, TNF and Adriamycin-induced cytotoxicity, and radiation induced-neoplastic transformation (50); 6) expression of human Mn-SOD genes in transgenic mice protects against oxygen-induced pulmonary injury and Adriamycin-induced cardiac toxicity (51).

Cu/Zn-SOD (SOD-1) is another type of enzymes that has been conserved throughout evolution. These enzymes have two identical subunits of about 32 kDa, although a monomeric structure can be found in a high protein concentration from *E. coli* (52). Each subunit contains a metal cluster, the active site, constituted by a copper and a zinc atom bridged by a histamine residue (53–55).

Cu/Zn-SOD is believed to play a major role in the

first line of antioxidant defense. Calves that were fed milk supplemented with 25 ppm Cu and 100 ppm Zn showed a stronger immune response and a higher SOD activity (56). Other recent reports involving SOD knock-outs have revealed that Mn-SOD is essential for life whereas Cu/Zn-SOD is not. Cu/Zn-SOD knock-out mice appear normal and exhibit differences only after traumatic injury, whereas Mn-SOD knockouts do not survive past 3 weeks of age (47). Among various human tissues Mn-SOD contents were roughly one-half as large as the Cu/Zn-SOD contents (57).

Extracellular superoxide dismutase (EC-SOD) is a secretory, tetrameric, copper and zinc containing glycoprotein; with a high affinity for certain glycosaminoglycans such as heparin and heparan sulfate. EC-SOD was found in the interstitial spaces of tissues and also in extracellular fluids, accounting for the majority of the SOD activity in plasma, lymph, and synovial fluid (37,57). EC-SOD is not induced by its substrate or by other oxidants and its regulation in mammalian tissues primarily occurs in a manner coordinated by cytokines, rather than as a response of individual cells to oxidants (58).

Catalase

Catalase (EC 1.11.1.6) is a tetrameric enzyme consisting of four identical tetrahedrally arranged subunits of 60 kDa that contains a single ferriprotoporphyrin group per subunit, and has a molecular mass of about 240 kDa (59). CAT reacts very efficiently with H_2O_2 to form water and molecular oxygen; and with H donors (methanol, ethanol, formic acid, or phenols) with peroxidase activity:

$$2\ H_2O_2 \xrightarrow{CAT} 2\ H_2O + O_2$$

$$ROOH + AH_2 \xrightarrow{CAT} H_2O + ROH + A$$

In animals, hydrogen peroxide is detoxified by CAT and by GPX. Catalase protects cells from hydrogen peroxide generated within them. Even though CAT is not essential for some cell types under normal conditions, it plays an important role in the acquisition of tolerance to oxidative stress in the adaptive response of cells. Survival of rats exposed to 100% oxygen was increased when liposomes containing SOD and CAT were injected intravenously before and during the exposure (60). The increased sensitivity of transfected CAT-enriched cells to some drugs and oxidants is attributed to the property of CAT in cells to prevent the drug-induced consumption of O_2 either for destroying H_2O_2 to oxygen or for direct interaction with the drug (61).

Glutathione peroxidase

The selenium-containing peroxidase glutathione peroxidase (EC 1.11.1.19) contains a single selenocysteine (Sec) residue in each of the four identical subunits, which is essential for enzyme activity (62). GPX (80 kDa) catalyses the reduction of hydroperoxides using GSH, thereby protecting mammalian cells against oxidative damage. In fact, glutathione metabolism is one of the most essential antioxidative defense mechanisms (12,15,29,32).

$$ROOH + 2GSH \xrightarrow{GPX} ROH + GSSG + H_2O$$

There are five GPX isoenzymes found in mammals. Although their expression is ubiquitous, the levels of each isoform vary depending on the tissue type. Cytosolic and mitochondrial glutathione peroxidase (cGPX or GPX1) reduces fatty acid hydroperoxides and H_2O_2 at the expense of glutathione. GPX1 and the phospholipid hydroperoxide glutathione peroxidase (PHGPX or GPX4) are found in most tissues. GPX4 is located in both the cytosol and the membrane fraction. PHGPX can directly reduce the phospholipid hydroperoxides, fatty acid hydroperoxides, and cholesterol hydroperoxides that are produced in peroxidized membranes and oxidized lipoproteins (63). GPX1 is predominantly present in erythrocytes, kidney, and liver, and GPX4 is highly expressed in renal epithelial cells and testes. Cytosolic GPX2 or GPX-G1, and extracellular GPX3 or GPX-P are poorly detected in most tissues except for the gastrointestinal tract and kidney, respectively. Recently, a new member, GPX5, expressed specifically in mouse epididymis, is interestingly selenium-independent (64).

Although GPX shares the substrate, H_2O_2, with CAT, it alone can react effectively with lipid and other organic hydroperoxides, being the major source of protection against low levels of oxidant stress.

Antioxidant enzymes: toward an active oxygen balance

Small deviations from the physiological activity of antioxidant enzymes may have a dramatic effect on the resistance of cells to oxidant-induced damage to the genome and cell killing (65–67). Cellular oxygen radical homeostasis is linked to three different classes of messenger molecules: growth factors, prostaglandins, and nitric oxide. The ability of platelet-derived growth factor (PDGF) to induce prostaglandin E2 (PGE2) release in fibroblasts is abolished when Cu/Zn-SOD or GPX activity is increased by cell transfection. Besides, the increase of nitric oxide synthase induced by PDGF is mediated in part by production of superoxide (68).

Addition of H_2O_2 causes a dose-dependent increase in CAT mRNA in both exponentially growing and confluent cells. Enhancement in the steady-state mRNA levels of GPX and SOD is equally found. In addition, cells of the respiratory tract respond to different oxidant insults by selective induction of the different antioxidant enzymes (69).

Transfection of a human SOD expression vector into murine fibroblast resulted in stable clones producing increased amounts of Cu/Zn-SOD. A significant increase in endogenous GPX activity and a smaller increase in glutathione transferase activity also occurred. Mn-SOD activity was decreased in all clones, while CAT and NADPH reductase activities were not affected (70). Although it seems clear that the Fenton reaction is responsible for DNA damage produced under oxidative stress, superoxide anion behaves as an iron reducing species in the production of 8-oxo-2′-deoxyguanosine, a DNA lesion produced by $^{\bullet}OH$ (31). Thus, it is interesting to notice that the need for Fe(II) is dismissed as it is recycled by $O_2^{\bullet -}$. It probably happens when H_2O_2-induced damage is reduced in cells overexpressing SOD (71).

$$Fe^{2+} + H_2O \rightarrow Fe^{3+} + {}^{\bullet}OH + {}^{-}OH$$

(Fenton reaction)

$$O_2^{\bullet -} + H_2O_2 \rightarrow O_2 + {}^{\bullet}OH + {}^{-}OH$$

(Haber-Weiss reaction)

The cellular regulation of free radicals using antioxidant enzymes was proved by several experiments: (a) a SOD-deficient strain and a CAT-deficient strain produced elevated levels of $O_2^{\bullet -}$ in Drosophila (72). (b) Expression of catalase-peroxidase of *Cyanobacterium synechococcus* in animal cells caused the transfected cells to become more resistant to H_2O_2 or paraquat (agents which reduce dioxygen) than the parental cells (73). (c) Overexpression of Cu/Zn-SOD and CAT caused a decrease in the accumulation of 8-hydroxydeoxyguanosine during aging (33). (d) Under conditions of overexpression of SOD and low $O_2^{\bullet -}$ levels, down-regulation of this system lead to decreases in antioxidant enzymes (70).

Reactive oxygen species and human diseases

Reactive oxygen species generated during metabolism can enter into reactions that, when uncontrolled, can affect certain processes leading to clinical manifestations (74,75). Direct effects include peroxidative changes in membranes and other cellular components, including oxidative DNA damage (76). SOD, GPX, and CAT within cells remove superoxide and peroxides before they react with metal catalysis to form more reactive species. Finally, peroxidative chain reactions initiated by reactive species that escaped enzymatic degradation are terminated by chain-breaking antioxidants, including among others water-soluble ascorbate, lipid-soluble vitamin E, and ubiquinone. To optimize performance, oxidative stress must be controlled by supplying known antioxidant nutrients and by minimizing effects of substances that stimulate ROS (77).

An imbalanced production of ROS plays a role in the pathogenesis of a number of human diseases such as ischemia/reperfusion injury, atherosclerosis, neurodegenerative diseases, cancer, and allergy. When antioxidant, free radical scavenging systems are overwhelmed, inflammation, hypersensitivity, and autoimmune conditions may result. Inflammatory cells may also increase DNA damage by activating pro-carcinogens to DNA-damaging species, *e.g.,* neutrophils can activate aromatic amines, aflatoxins, estrogens, phenols and polycyclic aromatic hydrocarbons by ROS-dependent mechanisms. Much cancer can be considered as a degenerative disease of old age, related to the effects of continuous damage over a life span by toxic oxygen. Thus, tumor promotion can be inhibited in animal models

TABLE 1
Reactive Oxygen Species, Antioxidant Enzymes Imbalance and Human Diseases

Human Disease	Key References
Allergy	
Bronchial asthma	(79–83)
Intolerance to aspirin	(84)
Intolerance to foods	(85)
Response to mercury	(86)
Response to other drugs	(87)
Response to other oxidants	(68)
Cancer	
Bladder	(78)
Bowel	(78, 88)
Breast	(7, 78)
Colorectal	(78, 89)
Esophageal	(90)
Kidney	(78, 91)
Leukemia	(58)
Liver	(78, 92)
Lung	(78)
Prostate	(93)
Skin	(94)
Cardiac and vessels injuries	
Atherosclerosis	(30, 95–101)
Ischemia	(31, 102–106)
Genetic and metabolic disorders	
Chronic granulomatous disease	(107)
Diabetes	(108–112)
Down's syndrome	(113, 114)
Infectious diseases	
Helicobacter pylori	(115)
Hepatitis	(116)
HIV	(117)
Influenza virus	(118)
Pneumonia	(119)
Rheumatoid arthritis	(120–122)
Neurodegenerative diseases	
Allergic encephalomyelitis	(123)
Alzheimer's disease	(103, 124–126)
Amyotrophic lateral sclerosis	(54, 127–131)
Huntington's disease	(124)
Parkinson's disease	(125, 126, 132)
Prion disease	(124, 133)
Ophthalmologic problems	
Cataract	(134)
Glaucoma	(135)

by the use of agents that can inhibit the phagocyte respiratory burst (78).

ROS have been also implicated in many lung diseases, including acute respiratory syndrome associated with exposure to oxidants, *e.g.*, asbestos, nitrogen dioxide, ozone, paraquat, hyperoxia, carbon tetrachloride, and the anticancer drugs bleomycin and Adriamycin. In addition, oxidative stress, superoxide production and an imbalance in antioxidant enzymes has been related with many other specific pathologies as chronic granulomatous disease, Downs syndrome, diabetic complications, hepatitis, rheumatoid arthritis, *Influenza* virus, ulcer, pneumonia, HIV infection, cataract and glaucoma (Table 1).

Conclusion

Oxygen species are key participants in damage caused by virus infections (that cause airway epithelial inflammation), progression to cancer (tumor invasion, and metastasis injuring local tissues), neurodegenerative processes (including cell death, motor neuron diseases and axonal injury), and both infarction and brain edema. Therefore, tissues must be protected from this oxidative injury by expression of stress-response genes and genes encoding antioxidant enzymes and activation of other related transcriptional regulatory proteins.

Those abnormalities appeared in the cellular regulation and expression of antioxidant enzymes play a main role in cell division cycle and in the balance of life. This fact shows us the importance of the ROS scavenging and the antioxidant defense system in maintaining normal cellular physiology, facing diseases and promoting immunity. In fact, the regulation of gene expression by means of oxidants, antioxidants and the redox state, has emerged as a novel target that promises therapeutic implications (136).

Thus, further efforts are necessary to fully elucidate the importance of antioxidant enzymes in the therapy of several human disease states.

Acknowledgements

This work was supported by project SAF98-0150 (Ministry of Education, Spain). We wish to thank Dr. M.A. Medina for critically reading the manuscript and to postgraduate students L. Olalla, J.M. Segura, and R. Rosado for their help in the bibliographic search.

References

1. Hurst R, Bao Y, Jemth P, Mannervik B, Williamson G. Phospholipid hydroperoxide glutathione peroxidase activity of rat class Theta glutathione transferase T2-2. *Biochem Soc Trans* 1997; **25**: S559.
2. Jornot L, Petersen H, Junod AF. Hydrogen peroxide-induced DNA damage is independent of nuclear calcium but dependent on redox-active ions. *Biochem J* 1998; **335**: 85–94.
3. Mills EM, Takeda K, Yu ZX, *et al.* Nerve growth factor treatment prevents the increase in superoxide produced by epidermal growth factor in PC12 cells. *J Biol Chem* 1998; **273**: 22165–8.
4. Ghosh J, Myers CE. Inhibition of arachidonate 5-lipoxygenase triggers massive apoptosis in human prostate cancer cells. *Proc Natl Acad Sci* 1998; **95**: 13182–7.
5. Yin GY, Yin YF, He XF. Effect of zhuchun pill on immunity and endocrine function of elderly with kidney-yang deficiency. *Chung Kuo Chung Hsi I Chieh Ho Tsa Chih* 1995; **15**: 601–3.
6. Bae YS, Kang SW, Seo MS, *et al*. Epidermal growth factor (EGF)-induced generation of hydrogen peroxide. *J Biol Chem* 1997; **272**: 217–21.
7. Lee YJ, Galoforo SS, Berns CM, *et al*. Glucose deprivation-induced cytotoxicity and alterations in mitogen-activated protein kinase activation are mediated by oxidative stress in multidrug-resistant human breast carcinoma cells. *J Biol Chem* 1998; **273**: 5294–9.
8. Chopra S, Wallace HM. Induction of spermidine/spermine N1-acetyltransferase in human cancer cells in response to increased production of reactive oxygen species. *Biochem Pharmacol* 1998; **55**: 1119–23.
9. Czene S, Tibäck M, Harms-Ringdahl M. pH-dependent DNA cleavage in permeabilized human fibroblasts. *Biochem J* 1997; **323**: 337–41.
10. Wojtaszek P. Oxidative burst: an early plant response to pathogen infection. *Biochem J* 1997; **322**: 681–92.
11. Da Silva EL, Piskula MK, Yamamoto N, Moon JH, Terao J. Quercetin metabolites inhibit copper ion-induced lipid peroxidation in rat plasma. *FEBS Lett* 1998; **430**: 405–8.
12. Esterbauer H, Gebicki J, Puhl H, Jügens G. The role of lipid peroxidation and antioxidants in oxidative modification of LDL. *Free Radic Biol Med* 1992; **13**: 341–90.
13. Meyer DF, Nealis AS, Macphee CH, *et al*. Time-course studies by synchrotron X-ray solution scattering of the structure of human low-density lipoprotein during Cu^{2+}-induced oxidation in relation to changes in lipid composition. *Biochem J* 1996; **319**: 217–27.
14. Patterson RA, Leake DS. Human serum, cysteine and histidine inhibit the oxidation of low density lipoprotein less at acidic pH. *FEBS Lett* 1998; **434**: 317–21.
15. Rikans LE, Hornbrook KR. Lipid peroxidation, antioxidant protection and aging. *Biochim Biophys Acta* 1997; **1362**: 116–27.
16. Oesch F. Metabolism of carcinogens, possibilities for modulation. *Acta Pharmacol Toxicol (Copenh)* 1984; **55**: 15–33.
17. Wagner BA, Buettner GR, Oberley LW, Burns CP. Sensitivity of K562 and HL-60 cells to edelfosine, an ether lipid drug, correlates with production of reactive oxygen species. *Cancer Res* 1998; **58**: 2809–16.
18. Kowaltowski AJ, Castilho RF, Grijalba MT, Bechara EJ, Vercesi AE. Effect of inorganic phosphate concentration on the nature of inner mitochondrial membrane alterations mediated by Ca^{2+} ions. A proposed model for phosphate-stimulated lipid peroxidation. *J Biol Chem* 1996; **271**: 2929–34.
19. Omodeo-Salè F, Basilico N, Folini M, Olliaro P, Taramelli D. Macrophage populations of different origins have distinct susceptibilities to lipid peroxidation induced by β-haematin (malaria pigment). *FEBS Lett* 1998; **433**: 215–18.

20. Requena JR, Fu MX, Ahmed MU, *et al*. Quantification of malondialdehyde and 4-hydroxynonenal adducts to lysine residues in native and oxidized human low-density lipoprotein. *Biochem J* 1997; **322**: 317–25.
21. Scott MD, Eaton JW, Kuypers FA, Chiu DT, Lubin BH. Enhancement of erythrocyte superoxide dismutase activity: effects on cellular oxidant defense. *Blood* 1989; **74**: 2542–9.
22. Beaudeux JL, Gardes-Albert M, Delattre J, Legrand A, Rousselet F, Peynet J. Resistance of lipoprotein(a) to lipid peroxidation induced by oxygenated free radicals produced by gamma radiolysis: a comparison with low-density lipoprotein. *Biochem J* 1996; **314**: 277–84.
23. Hall L, Williams K, Perry ACF, Frayne J, Jury JA. The majority of human glutathione peroxidase type 5 (GPX5) transcripts are incorrectly spliced: implications for the role of GPX5 in the male reproductive tract. *Biochem J* 1998; **333**: 5–9.
24. Jankowska R, Passowicz-Muszynska E, Banas T, Marcinkowska A, Medrala W. The influence of vitamin A on production of oxygen free radicals and activity of granulocyte catalase in patients with chronic bronchitis. *Pneumonol Alergol Pol* 1994; **62**: 628–33.
25. Stahl W, Junghans A, de Boer B, Driomina ES, Brivida K, Sies H. Carotenoid mixtures protect multilamellar liposomes against oxidative damage: synergistic effects of lycopene and lutein. *FEBS Lett* 1998; **427**: 305–8.
26. Stait SE, Leake DS. The effects of ascorbate and dehydroascorbate on the oxidation of low-density lipoprotein. *Biochem J* 1996; **320**: 373–81.
27. Aleryani S, Milo E, Rose Y, Kostka P. Superoxide-mediated decomposition of biological S-nitrosothiols. *J Biol Chem* 1998; **273**: 6041–5.
28. Brouwer M, Brouwer TH. Biochemical defense mechanisms against copper-induced oxidative damage in the blue crab, Callinectes sapidus. *Arch Biochem Biophys* 1998; **351**: 257–64.
29. Grazioli V, Schiavo R, Casari E, *et al*. Antioxidant enzymatic activities and lipid peroxidation in cultured human chondrocytes from vertebral plate cartilage. *FEBS Lett* 1998; **431**: 149–53.
30. Lapenna D, Gioia S, Ciofani G, *et al*. Antioxidant properties of ticlopidine on human low density lipoprotein oxidation. *FEBS Lett* 1998; **436**: 357–60.
31. Mao GD, Thomas PD, Lopaschuk GD, Poznansky MJ. Superoxide dismutase (SOD)-catalase conjugates. Role of hydrogen peroxide and the Fenton reaction in SOD toxicity. *J Biol Chem* 1993; **268**: 416–20.
32. Sigalov AB, Stern LJ. Enzymatic repair of oxidative damage to human apolipoprotein A-I. *FEBS Lett* 1998; **433**: 196–200.
33. Sohal RS, Agarwal A, Agarwal S, Orr WC. Simultaneous overexpression of copper- and zinc-containing superoxide dismutase and catalase retards age-related oxidative damage and increases metabolic potential in Drosophila melanogaster. *J Biol Chem* 1995; **270**: 15671–4.
34. Fridovich I. Superoxide radical and superoxide dismutases. *Annu Rev Biochem* 1995; **64**: 97–112.
35. Sandalio LM, López-Huertas E, Bueno P, Del Río LA. Immunocytochemical localization of copper, zinc superoxide dismutase in peroxisomes from watermelon (Citrullus vulgaris Schrad.) cotyledons. *Free Radic Res* 1997; **26**: 187–94.
36. Teixeira HD, Schumacher RI, Meneghini R. Lower intracellular hydrogen peroxide levels in cells overexpressing CuZn-superoxide dismutase. *Proc Natl Acad Sci* 1998; **95**: 7872–5.
37. Sandström J, Nilsson P, Karlsson K, Marklund SL. 10-fold increase in human plasma extracellular superoxide dismutase content caused by a mutation in heparin-binding domain. *J Biol Chem* 1994; **269**: 19163–6.
38. Sun E, Xu H, Liu Q, Zhou J, Zuo P, Wang J. The mechanism for the effect of selenium supplementation on immunity. *Biol Trace Elem Res* 1995; **48**: 231–8.
39. Meier B, Scherk C, Schmidt M, Parak F. pH-dependent inhibition by azide and fluoride of the iron superoxide dismutase from *Propionibacterium shermanii*. *Biochem J* 1998; **331**: 403–7.
40. Leone M, Cupane A, Militello V, Stroppolo ME, Desideri A. Fourier Transform infrared analysis of the interaction of azide with the active site of oxidized and reduced bovine Cu,Zn superoxide dismutase. *Biochemistry* 1998; 37: 4459–64.
41. Vance CK, Miller AF. Spectroscopic comparisons of the pH dependence of Fe-substituted (Mn)superoxide dismutase and Fe-superoxide dismutase. *Biochemistry* 1998; **37**: 5518–27.
42. MacMillan-Crow LA, Crow JP, Thompson JA. Peroxynitrite-mediated inactivation of manganese superoxide dismutase involves nitration and oxidation of critical tyrosine residues. *Biochemistry* 1998; **37**: 1613–22.
43. Stralin P, Marklund SL. Effects of oxidative stress on expression of extracellular superoxide dismutase, CuZn-superoxide dismutase and Mn-superoxide dismutase in human dermal fibroblasts. *Biochem J* 1994; **298**: 347–52.
44. Yamakura F, Taka H, Fujimura T, Murayama K. Inactivation of human manganese-superoxide dismutase by peroxynitrite is caused by exclusive nitration of tyrosine 34 to 3-nitrotyrosine. *J Biol Chem* 1998; **273**: 14085–9.
45. Farr SB, D'ari R, Touati D. Oxygen-dependent mutagenesis in *Escherichia coli* lacking superoxide dismutase. *Proc Natl Acad Sci* 1986; **83**: 8268–72.
46. van Loon APGM, Pesold-Hurt B, Schatz G. A yeast mutant lacking mitochondrial manganese-superoxide dismutase is hypersensitive to oxygen. *Proc Natl Acad Sci* 1986; **83**: 3820–4.
47. Li Y, Huang TT, Carlson EJ, *et al*. Dilated cardiomyopathy and neonatal lethality in mutant mice lacking manganese superoxide dismutase. *Nat Genet* 1995; **11**: 376–81.
48. Hachiya M, Shimizu S, Osawa Y, Akashi M. Endogenous production of tumour necrosis factor is required for manganese superoxide dismutase expression by irradiation in the human monocytic cell line THP-1. *Biochem J* 1997; **328**: 615–23.
49. Kizaki M, Sakashita A, Karmakar A, Lin CW, Koeffler HP. Regulation of manganese superoxide dismutase and other antioxidant genes in normal and leukemic hematopoietic cells and their relationship to cytotoxicity by tumor necrosis factor. *Blood* 1993; **82**: 1142–50.
50. St. Clair DK, Oberley TD, Ho YS. Overproduction of human Mn-superoxide dismutase modulates para-

quat-mediated toxicity in mammalian cells. *FEBS Lett* 1991; **293**: 199–203.
51. Wispé JR, Warner BB, Clark JC, *et al*. Human Mn-superoxide dismutase in pulmonary epithelial cells of transgenic mice confers protection from oxygen injury. *J Biol Chem* 1992; **267**: 23937–41.
52. Battistoni A, Folcarelli S, Gabbianelli R, Capo C, Rotilio G. The Cu,Zn superoxide dismutase from Escherichia coli retains monomeric structure at high protein concentration. Evidence for altered subunit interaction in all the bacteriocupreins. *Biochem J* 1996; **320**: 713–16.
53. Battistoni A, Folcarelli S, Cervoni L, *et al*. Role of the dimeric structure in Cu,Zn superoxide dismutase. pH-Dependent, reversible denaturation of the monomeric enzyme from Escherichia coli. *J Biol Chem* 1998; **273**: 5655–61.
54. Leah RB, Casareno DW, Gitlin JD. The copper chaperone CCS directly interacts with copper/zinc superoxide dismutase. *J Biol Chem* 1998; **273**: 23625–8.
55. Stroppolo ME, Sette M, O'Neill P, Polizio F, Cambria MT. Cu,Zn superoxide dismutase from *Photobacterium leignathi* is an hyperefficient enzyme. *Biochemistry* 1998; **37**: 12287–92.
56. Prasad T, Kundu MS. Serum IgG and IgM responses to sheep red blood cells (SRBC) in weaned calves fed milk supplemented with Zn and Cu. *Nutrition* 1995; **11**: 712–15.
57. Marklund S. Distribution of Cu/Zn superoxide dismutase and Mn superoxide dismutase in human tissues and extracellular fluids. *Acta Physiol Scand Suppl* 1980; **492**: 19–23.
58. Buschfort C, Müller MR, Seeber S, Rajewsky MF, Thomale J. DNA excision repair profiles of normal and leukemic human lymphocytes: functional analysis at the single-cell level. *Cancer Res* 1997; **57**: 651–8.
59. Aebi HE. Enzymes 1: oxidoreductases, transferases. In: Bergmeyer H, Ed. *Methods of enzymatic analysis*, vol. III. Pp. 273–82. Deerfield Beach, FL: Verlag Chemie, 1980.
60. Turrens JF, Crapo JD, Freeman BA. Protection against oxygen toxicity by intravenous injection of liposome-entrapped catalase and superoxide dismutase. *J Clin Invest* 1984; **73**: 87–95.
61. Speranza MJ, Bagley AC, Lynch RE. Cells enriched for catalase are sensitized to the toxicities of bleomycin, adriamycin, and paraquat. *J Biol Chem* 1993; **268**: 19039–43.
62. Tappel AL. Glutathione peroxidase and hydroperoxides. *Methods Enzymol* 1978; **52**: 506–13.
63. Imai H, Narashima K, Arai M, Sakamoto H, Chiba N, Nakagawa Y. Suppression of leukotriene formation in RBL-2H3 cells that overexpressed phospholipid hydroperoxide glutathione peroxidase. *J Biol Chem* 1998; **273**: 1990–7.
64. De Haan JD, Bladier C, Griffiths P, *et al*. Mice with a homozygous null mutation for the most abundant glutathione peroxidase, Gpx1, show increased susceptibility to the oxidative stress-inducing agents paraquat and hydrogen peroxide. *J Biol Chem* 1998; **273**: 22528–36.
65. Amstad P, Moret R, Cerutti P. Glutathione peroxidase compensates for the hypersensitivity of Cu,Zn-superoxide dismutase overproducers to oxidant stress. *J Biol Chem* 1994; **269**: 1606–9.
66. Dedon PC, Plastaras JP, Rouzer CA, Marnett LJ. Indirect mutagenesis by oxidative damage: formation of the pyrimidopurinone adduct of deoxyguanosine by base propenal. *Proc Natl Acad Sci* 1998; **95**: 11113–16.
67. Limoli CL, Hartmann A, Shephard L, *et al*. Apoptosis, reproductive failure, and oxidative stress in chinese hamster ovary cells with compromised genomic integrity. *Cancer Res* 1998; **58**: 3712–8.
68. Kelner MJ, Uglik SF. Superoxide dismutase abolishes the platelet-derived growth factor-induced release of prostaglandin E2 by blocking induction of nitric oxide synthase: role of superoxide. *Arch Biochem Biophys* 1995; **322**: 31–8.
69. Shull S, Heintz NH, Periasamy M, *et al*. Differential regulation of antioxidant enzymes in response to oxidants. *J Biol Chem* 1991; **266**: 24398–403.
70. Kelner MJ, Bagnell R. Alteration of endogenous glutathione peroxidase, manganese superoxide dismutase, and glutathione transferase activity in cells transfected with a copper-zinc superoxide dismutase expression vector. Explanation for variations in paraquat resistance. *J Biol Chem* 1990; **265**: 10872–5.
71. Teixeira HD, Meneghini R. Chinese hamster fibroblasts overexpressing CuZn-superoxide dismutase undergo a global reduction in antioxidants and an increasing sensitivity of DNA to oxidative damage. *Biochem J* 1996; **315**: 821–5.
72. Nappi AJ, Vass E, Frey F, Carton Y. Superoxide anion generation in Drosophila during melanotic encapsulation of parasites. *Eur J Cell Biol* 1995; **68**: 450–6.
73. Ishikawa T, Ohta Y, Takeda T, Shigeoka S, Nishikimi M. Increased cellular resistence to oxidative stress by expression of cyanobacterium catalase-peroxidase in animal cells. *FEBS Lett* 1998; **426**: 221–4.
74. Rhoden EL, Mauri M, Petteffi L, Belló-Klein A, Zettler CG, Rhoden CR. Protective effect of colchicine on tissue damage caused by free radical in hepatic cirrhosis: an experimental study in rats. *Arq Gastroenterol* 1997; **34**: 91–6.
75. Tsai KJ, Hung IJ, Chow CK, Stern A, Chao SS, Chiu DTY. Impaired production of nitric oxide, superoxide, and hydrogen peroxide in glucose 6-phosphate-dehydrogenase-deficient granulocytes. *FEBS Lett* 1998; **436**: 411–14.
76. Ramotar D, Belanger E, Brodeur I, Masson JY, Drobetsky EA. A yeast homologue of the human phosphotyrosyl phosphatase activator PTPA is implicated in protection against oxidative damage induced by the model carcinogen 4-nitroquinoline 1-oxide. *J Biol Chem* 1998; **272**: 21489–96.
77. Miller JK, Brzezinska-Slebodzinska E, Madsen FC. Oxidative stress, antioxidants, and animal function. *J Dairy Sci* 1993; **76**: 2812–23.
78. Wiseman H, Halliwell B. Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer. *Biochem J* 1996; **313**: 17–29.
79. Beasley R, Thomson C, Pearce N. Selenium, glutathione peroxidase and asthma. *Clin Exp Allergy* 1991; **21**: 157–9.
80. Bibi H, Schlesinger M, Tabachnik E, Schwartz Y, Iscovitz H, Iaina A. Erythrocyte glutathione peroxidase activity in asthmatic children. *Ann Allergy* 1988; **61**: 339–40.
81. Kato M, Morikawa A, Kimura H, Shimizu T, Nakano M, Kuroume T. Effects of antiasthma drugs on su-

peroxide anion generation from human polymorphonuclear leukocytes or hypoxanthine-xanthine oxidase system. *Int Arch Allergy Appl Immunol* 1991; **96**: 128–33.
82. Kurosawa M, Kobayashi H, Nakano M. Cu-Zn superoxide dismutase activities in platelets from stable bronchial asthmatic patients. *Int Arch Allergy Immunol* 1993; **101**: 61–5.
83. Misso NL, Powers KA, Gillon RL, Stewart GA, Thompson PJ. Reduced platelet glutathione peroxidase activity and serum selenium concentration in atopic asthmatic patients. *Clin Exp Allergy* 1996; **26**: 838–47.
84. Pearson DJ, Suarez-Mendez VJ, Day JP, Miller PF. Selenium status in relation to reduced glutathione peroxidase activity in aspirin-sensitive asthma. *Clin Exp Allergy* 1991; **21**: 203–8.
85. Buchanan BB, Adamidi C, Lozano RM, *et al*. Thioredoxin-linked mitigation of allergic responses to wheat. *Proc Natl Acad Sci* 1997; **94**: 5372–7.
86. Björkman L, Langworth S, Lind B, Elinder CG, Nordberg M. Activity of antioxidative enzymes in erythrocytes and concentration of selenium in plasma related to mercury exposure. *J Trace Elem Electrolytes Health Dis* 1993; **7**: 157–64.
87. Niwa Y, Ishimoto K, Kanoh T. Induction of superoxide dismutase in leukocytes by paraquat: correlation with age and possible predictor of longevity. *Blood* 1990; **76**: 835–41.
88. Tanaka T, Kawabata K, Kakumoto M, *et al*. Citrus auraptene exerts dose-dependent chemopreventive activity in rat large bowel tumorigenesis: the inhibition correlates with suppression of cell proliferation and lipid peroxidation and with induction of phase II drug-metabolizing enzymes. *Cancer Res* 1998; **58**: 2550–6.
89. Chinery R, Beauchamp RD, Shyr Y, Kirkland SC, Coffey RJ, Morrow JD. Antioxidants reduce cyclooxygenase-2 expression, prostaglandin production, and proliferation in colorectal cancer cells. *Cancer Res* 1998; **58**: 2323–7.
90. Torzewski M, Sarbia M, Heep H, Dutkowski P, Willers R, Gabbert HE. Expression of Bcl-X(L), an antiapoptotic member of the Bcl-2 family, in esophageal squamous cell carcinoma. *Clin Cancer Res* 1998; **4**: 577–83.
91. Durak I, Beduk Y, Kavutcu M, Ozturk S, Canbolat O, Ulutepe S. Activities of superoxide dismutase and glutathione peroxidase enzymes in cancerous and non-cancerous human kidney tissues. *Int Urol Nephrol* 1997; **29**: 5–11.
92. Kennedy CH, Cueto R, Belinsky SA, Lechner JF, Pryor WA. Overexpression of hMTH1 mRNA: a molecular marker of oxidative stress in lung cancer cells. *FEBS Lett* 1998; 4**29**: 17–20.
93. Kiningham RB. Physical activity and the primary prevention of cancer. *Prim Care* 1998; **25**: 515–36.
94. Shisler JL, Senkevich TG, Berry MJ, Moss B. Ultraviolet-induced cell death blocked by a selenoprotein from a human dermatotropic poxvirus. *Science* 1998; **279**: 40–1.
95. Cantwell H, Devery R, Stanton C, Lawless F. The effect of a conjugated linoleic acid on a superoxide dismutase, catalase and glutathione peroxidase in oxidatively-challenged liver cells. *Biochem Soc Trans* 1998; **26**: S62.
96. Caspar-Bauguil S, Saadawi M, Negre-Salvayre A, Thomsen M, Salvayre R, Benoist H. Mildly oxidized low-density lipoproteins suppress the proliferation of activated CD4+ T-lymphocytes and their interleukin 2 receptor expression in vitro. *Biochem J* 1998; **330**: 659–66.
97. Fu S, Davies MJ, Stocker R, Dean RT. Evidence for roles of radicals in protein oxidation in advanced human atherosclerotic plaque. *Biochem J* 1998; **333**: 519–25.
98. Napoli G. Low density lipoprotein oxidation and atherogenesis: from experimental models to clinical studies. *G Ital Cardiol* 1997; **27**: 1302–14.
99. Schmidt AM, Hori O, Brett J, Yan SD, Wautier JL, Stern D. Cellular receptors for advanced glycation end products. Implications for induction of oxidant stress and cellular dysfunction in the pathogenesis of vascular lesions. *Arterioscler Thromb* 1994; **14**: 1521–8.
100. Stroes E, Hijmering M, van Zandvoort M, Wever R, Rabelink TJ, van Faassen EE. Origin of superoxide production by endothelial nitric oxide synthase. *FEBS Lett* 1998; **438**: 161–4.
101. Sundaresan M, Yu ZX, Ferrans VJ, Irani K, Finkel T. Requirement for generation of H_2O_2 for platelet-derived-growth factor signal transduction. *Science* 1995; **270**: 296–9.
102. Duranteau J, Chandel NS, Kulisz A, Shao Z, Schumacker PT. Intracellular signaling by reactive oxygen species during hypoxia in cardiomyocytes. *J Biol Chem* 1998; **273**: 11619–24.
103. Mukhopadhyay CK, Fox PL. Ceruloplasmin copper induces oxidant damage by a redox process utilizing cell-derived superoxide as reductant. *Biochemistry* 1998; **37**: 14222–9.
104. Vanden Hoek TL, Becker LB, Shao Z, Li C, Schumacker PT. Reactive oxygen species released from mitochondria during brief hypoxia induce preconditioning in cardiomyocytes. *J Biol Chem* 1998; **273**: 18092–8.
105. Weisbrot-Lefkowitz M, Reuhl K, Perry B, Chan PH, Inouye M, Mirochnitchenko O. Overexpression of human glutathione peroxidase protects transgenic mice against focal cerebral ischemia/reperfusion damage. *Brain Res Mol Brain Res* 1998; **53**: 333–8.
106. Zager RA, Burkhart K. Decreased expression of mitochondrial-derived H_2O_2 and hydroxyl radical in cytoresistant proximal tubules. *Kidney Int* 1997; **52**: 942–52.
107. Chang YC, Segal BH, Holland SM, Miller GF, Kwon-Chung KJ. Virulence of catalase-deficient aspergillus nidulans in p47(phox)−/− mice. Implications for fungal pathogenicity and host defense in chronic granulomatous disease. *J Clin Invest* 1998; **101**: 1843–50.
108. Aguirre F, Martin I, Grinspon D, *et al*. Oxidative damage, plasma antioxidant capacity, and glucemic control in elderly NIDDM patients. *Free Radic Biol Med* 1998; **24**: 580–5.
109. Hannon MPA, Hughes C, O'Kane MJ, Moles KW, Barnett CR, Barnett YA. Antioxidant status and DNA damage in patients with insulin-dependent Diabetes Mellitus. *Biochem Soc Trans* 1998; **26**: S57.
110. Leinonen J, Lehtimäki T, Toyokuni S, *et al*. New biomarker evidence of oxidative DNA damage in patients with non-insulin-dependent diabetes mellitus. *FEBS Lett* 1997; **417**: 150–2.
111. Wautier JL, Wautier MP, Schmidt AM, *et al.* Advanced glycation end products (AGEs) on the surface of diabetic erythrocytes bind to the vessel wall via a

specific receptor inducing oxidant stress in the vasculature: a link between surface-associated AGEs and diabetic complications. *Proc Natl Acad Sci* 1994; **91**: 7742–6.
112. Yan SD, Schmidt AM, Anderson GM, *et al*. Enhanced cellular oxidant stress by the interaction of advanced glycation end products with their receptors/binding proteins. *J Biol Chem* 1994; **269**: 9889–97.
113. Odetti P, Angelini G, Dapino D, *et al*. Early glycoxidation damage in brains from Down's syndrome. *Biochem Biophys Res Commun* 1998; **243**: 849–51.
114. Pastor MC, Sierra C, Dolade M, *et al*. Antioxidant enzymes and fatty acid status in erythrocytes of Down's syndrome patients. *Clin Chem* 1998; **44**: 924–9.
115. Nagata K, Yu H, Nishikawa M, *et al*. Helicobacter pylori generates superoxide radicals and modulates nitric oxide metabolism. *J Biol Chem* 1998; **273**: 14071–3.
116. Cuzzocrea S, Zingarelli B, Villari D, Caputi AP, Longo G. Evidence for in vivo peroxynitrite production in human chronic hepatitis. *Life Sci* 1998; **63**: 25–30.
117. Chen C, Zhou J, Xu H, Jiang Y, Zhu G. Effect of selenium supplementation on mice infected with LP-BM5 MuLV, a murine AIDS model. *Biol Trace Elem Res* 1997; **59**: 187–93.
118. Peterhans E. Reactive oxygen species and nitric oxide in viral diseases. *Biol Trace Elem Res* 1997; **56**: 107–16.
119. Dandyshev S. Risk factors and the molecular cellular mechanisms of a protracted course in pnemonia. *Ter Arkh* 1998; **70**: 41–4.
120. Aaseth J, Haugen M, Forre O. Rheumatoid arthritis and metal compounds: perspectives on the role of oxygen radical detoxification. *Analyst* 1998; **123**: 3–6.
121. Szabo C, Virag L, Cuzzocrea S, *et al.* Protection against peroxynitrite-induced fibroblast injury and arthritis development by inhibition of poly(ADP-ribose) synthase. *Proc Natl Acad Sci* 1998; **95**: 3867–72.
122. Taraza C, Mohora M, Vargolici B, Dinu V. Importance of reactive oxygen species in rheumatoid arthritis. *Rom J Intern Med* 1997; **35**: 89–98.
123. Cross AH, Manning PT, Stern MK, Misko TP. Evidence for the production of peroxynitrite in inflammatory CNS demyelination. *J Neuroimmunol* 1997; **80**: 121–30.
124. Price DL, Sangram SS, Borchelt DR. Genetic neurodegenerative diseases: the human illness and transgenic models. *Science* 1998; **286**: 1079–83.
125. Yan SD, Chen X, Schmidt AM, *et al*. Glycated tau protein in Alzheimer disease: a mechanism for induction of oxidant stress. *Proc Natl Acad Sci* 1994; **91**: 7787–91.
126. Guttmann RP, Johnson GV. Oxidative stress inhibits calpain activity in situ. *J Biol Chem* 1998; **273**: 13331–8.
127. Couillard-Després S, Zhu Q, Wong PC, Price DL, Cleveland DW, Julien JP. Protective effect of neurofilament heavy gene overexpression in motor neuron disease induced by mutant superoxide dismutase. *Proc Natl Acad Sci* 1998; **95**: 9626–30.
128. Hardy J, Gwinn-Hardy K. Genetic classification of primary neurodegenerative disease. *Science* 1998; **282**: 1075–8.
129. Rosen DR, Siddique T, Patterson D, *et al*. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature* 1993; **362**: 59–62.
130. Singh RJ, Karoui H, Gunther MR, Beckman JS, Mason RP, Kalyanaraman B. Reexamination of the mechanism of hydroxyl radical adducts formed from the reaction between familial amyotrophic lateral sclerosis-associated Cu,Zn superoxide dismutase mutants and H_2O_2. *Proc Natl Acad Sci* 1998; **95**: 6675–80.
131. Williamson TL, Bruijn LI, Zhu Q, *et al.* Absence of neurofilaments reduces the selective vulnerability of motor neurons and slows disease caused by a familial amyotrophic lateral sclerosis-linked superoxide dismutase 1 mutant. *Proc Natl Acad Sci* 1998; **95**: 9631–6.
132. Ziv I, Melamed E, Nardi N, *et al*. Dopamine induces apoptosis-like cell death in cultured chick sympathetic neurons a possible novel pathogenetic mechanism in Parkinson's disease. *Neurosci Lett* 1994; **170**: 136–40.
133. Brown DR, Besinger A. Prion protein expression and superoxide dismutase activity. *Biochem J* 1998; **334**: 423–9.
134. Spector A, Ma W, Wang RR, Kleiman NJ. Microperoxidases catalytically degrade reactive oxygen species and may be anti-cataract agents. *Exp Eye Res* 1997; **65**: 457–70.
135. Kurysheva NI, Deeva IB, Deev AI, Erichev VP. Comparative study of antiradical effects of several antiglaucoma drugs. *Vestn Oftalmol* 1998; **114**: 6–9.
136. Vang O, Rasmussen BF, Andersen O. Combined effects of complex mixtures of potentially anti-carcinogenic compounds on antioxidant enzymes and carcinogen metabolizing enzymes in the rat. *Cancer Lett* 1997; **114**: 283–6.