Biochemistry of Hydrogen Peroxide

Subjects: Neurosciences
Contributor: Nitesh Sanghai

Hydrogen Peroxide (H2O2) is the nonionized 2-electron reduction product of unstable molecular oxygen (O2), which plays a central role in maintaining the redox cycle of living cells.

Keywords: Chemistry ; H2O2 ; oxidative ; Free radical ; Fenton Reaction ; Neurodegenerative diseases ; Iron ; Hydroxyl radical

1. Introduction

Hydrogen Peroxide (H_2O_2) is a diverse potent oxidizing and inexpensive chemical molecule, which has chemical applications [1], biological functions [2], and therapeutic applications which include antimicrobial and oxidizing agents [3]. Over the last decade, H_2O_2 has been used as a green oxidant and alternate source of oxygen (O_2) to convert biomass to chemical synthesis [1]. It also acts as an environmentally friendly oxidizing catalyst in many oxidizing chemical processes because the end product of its decomposition is only a water (H_2O) molecule [4]. H_2O_2 is one of the closest cousins of water and is a non-planar molecule having an open book structure. It is also regarded as the smallest chiral molecule, which can undergo a disproportionation reaction to act both as oxidizing and reducing agent [5][6].

H_2O_2 is a strong oxidant, having a reduction potential of 1.76 V at pH 7.0, 25 °C. Therefore, it is more oxidizing than hypochlorous acid (HOCl) or peroxynitrite ($ONOO^-$), for which the reduction potentials are 1.48 and 1.4 V, respectively. However, relative to these two reactive species, the reactivity of H_2O_2 is relatively low with various biological molecules like nucleic acid, proteins, and lipids. Further, this can be explained by its higher activation energy barrier, which must be overcome to release its oxidizing power. In other words, the chemical reactions of hydrogen peroxide are kinetically controlled rather than thermodynamically driven [7]. The H_2O_2 produced during cellular metabolism is found to be stable, compared to (HOCl) having a half-life in minutes and ($ONOO^-$) having a half-life of 10^{-3} s [8][9]. It is a major oxidant produced by the activated neutrophils at the site of inflammation from H_2O_2 and chloride, catalyzed by the enzyme myeloperoxidase, a leukocyte-derived enzyme. HOCl exerts its oxidizing power through the oxidation and chlorination of biomolecules like nucleic acids, lipids, and cholesterol [10]. It confers its oxidizing power via chlorination of amino acids like tyrosine to form 3-chlorotyrosine and damage the collagen [11]. HOCl is more reactive than H_2O_2 (rate constants of 3×10^7 M^{-1} s^{-1} and 0.9 M^{-1} s^{-1}, respectively), however, the redox potential for the 2-electron reduction is larger for the H_2O_2 in forming H_2O, than for the former in releasing chloride [12]. $ONOO^-$ is formed by the reaction between superoxide radical anion ($O_2^{\bullet -}$) and nitric oxide radical ($NO^{\bullet}$). It is highly toxic to biomolecules, oxidizes lipids, Met, and tyrosine residues in proteins [13]. The nitrotyrosine residues are considered as a marker of $ONOO^-$ induced cellular damage, potentially serving as a “peroxynitrite footprint” in the biological oxidation process. The main biological target of $ONOO^-$ is deoxyribonucleic acid (DNA), where it oxidizes the base pairs, causing nitrative and oxidative DNA lesions such as 8-nitroguanine and 8-oxodeoxyguanosine respectively [14][15]. Recent evidence has shown that the protein sulphenic acid (SH) significantly reacts faster with $ONOO^-$ compared to H_2O_2 [16][17].

H_2O_2 is a weak one-electron oxidant, although the one-electron reduction product, the hydroxyl radical ($HO^{\bullet}$) is one of nature’s most vulnerable bio-reactive species, which can create an oxidizing environment in the living cell and ultimately lead to its death [18][19][20]. The redox property of H_2O_2 is dependent upon the pH of the solutions, as the pKa of H_2O_2 is 11.6, so it is mostly unionized at physiological pH. The strong nucleophilicity of hydroperoxide nucleophile (^-OOH) is limited due to its high pKa. H_2O_2 also acts as electrophile due to the polarization of the peroxide bond (O−O). The homolytic bond dissociation enthalpies of peroxide in H_2O_2 at 298 K is 50 kcal/mol, whereas the heterolytic bond dissociation enthalpies of H_2O_2 is 290 kcal/mol. The unexpected chemical reactivity of hydrogen peroxide is generally attributed to the weakness of (O-O) and therefore, it is homolytically cleaved in presence of heating, radiolysis, photolysis, or transition metals [21]. Prof. John O. Edwards, a pioneer in the field of peroxide chemistry, has demonstrated the mechanism of peroxide chemistry exquisitely. According to him, thiolates ($-S^-$) are more reactive than (SH). Hence, ($-S^-$), being nucleophilic, react with H_2O_2, an electrophile, by bimolecular nucleophilic substitution (SN2) reaction mechanism

displacing ^{-}OH as leaving group [22]. However, recent developments on this mechanism suggest that the by-product of the reaction is H_2O instead of hydroxyl anion (HO^{-}) [23][24]. The nucleophilic reaction of a protein ($-S^{-}$) on electrophilic H_2O_2 forms H_2O as a by-product and results in the formation of cysteine sulphenic acid (CysSOH), the process is also known as S-sulfenylation. Depending upon the protein microenvironment where the thiolate is located, the reaction is exclusive to ($-S^{-}$), and at physiological pH, dependent upon the pKa values of the sulfur-containing amino acids. In addition, hydrogen bonding also plays an important part in the ionization of (SH) to ($-S^{-}$) [25][26][27]. Further, the SN2 paradigm was also challenged and proved by hybrid quantum-classical (QM-MM) molecular dynamics simulations [26]. The nucleophilicity of H_2O_2 could be explained by the reaction of ^{-}OOH with organohalides [28], and oxidation of boron compounds [29]. H_2O_2, a green redox molecule that has properties of both oxidant and reductant, also can be a nucleophile and electrophile in chemical reactions. Having all these characteristics it reacts poorly with most biological molecules because of the high activation energy barrier and the reaction rate is kinetically controlled. Thus, the vigorous oxidizing power of H_2O_2 comes indirectly from its transition metal catalysis into $HO^{\bullet}$ by Fenton and Haber-Weiss reactions [7]. In addition, Copper/zinc superoxide dismutase (Cu/Zn SOD1), which is a ubiquitous natural antioxidant enzyme, is mainly involved in dismutation of ionizable toxic $O_2^{\bullet-}$ radical in vivo to produce a non-ionizable less toxic redox molecule H_2O_2, which has the ability to generate $HO^{\bullet}$, thereby acting as pro-oxidant in certain disease conditions [30][31].

2. Hydrogen Peroxide as Double Edge Sword in Living Cells

H_2O_2acts both as a redox signaling molecule and an oxidative stress molecule. As a signal transduction molecule H_2O_2 has a role in controlling various key cellular processes like cell shape changes, initiating proliferation, recruitment of immune cells, calcium ion (Ca^{+2}) signaling in the lumen of endoplasmic reticulum and mitochondria-associated membranes [32][33]. It acts as a secondary messenger in insulin signaling and several growth factor-induced signaling cascades [32]. Also, H_2O_2 is involved in the chemical modifications of specific Cys amino acids, which are expressed in some cellular proteins [34]. H_2O_2 generated during physiological oxidative stress conditions in the concentration of around (1–10 nM) acts as a redox signaling molecule in various cellular processes creating oxidative eustress, although, the higher or pathological concentration of H_2O_2 of around (>100 nM) is known to cause deleterious effects to cellular biomolecules, this effect is called oxidative distress [2]. According to many reports, the higher pathological concentration of H_2O_2 in oxidative stress conditions can go up to 150µM [35][36][37].

Various studies have been conducted to examine the concentration at which the H_2O_2 acts as a cytotoxic and neurotoxic agent. Further, various studies have investigated the mode of cell death caused by H_2O_2, mainly due to apoptosis or necrosis [38][39][40]. It was found that the effects of H_2O_2 are largely dependent upon the mode of cell death induced (apoptosis or necrosis) depending on the cell type used, its physiological state, length of exposure to H_2O_2, the H_2O_2 concentration used, and the cell culture media employed [38][41]. Yoshiro and colleagues (2006) [42], investigated that 50µM of H_2O_2 exhibited caspase-9 and caspase-3 activation, finally leading to apoptotic cell death in human T-lymphoma Jurkat cells, whereas a higher concentration of 500 µM caused necrotic death. Teramoto and the group (1999) [43], demonstrated that a lower concentration of 10–100 µM predominantly caused apoptosis, however, a higher concentration of 1–10 mM induced necrosis in human lung fibroblasts cells. Troyano and associates (2003) [44], demonstrated caspase-9 and caspase-3 activation and death by apoptosis in U-937 human promonocytic cells when treated with 200 µM H_2O_2. Although, treatment with 2 mM H_2O_2 caused necrosis. Gulden and the group (2010) [35], investigated in detail how exposure time and cell concentration affect the cytotoxic potency of H_2O_2 in vitro. They investigated those median cytotoxic concentrations decreased from 500 to 30 µM with increasing incubation time from 1 to 24 h. The cytotoxic effects of H_2O_2 were also evaluated in neuroblastoma × spinal cord motor neuron cell line (NSC34). A short (30 min) exposure of H_2O_2 caused delayed cell death with the median effective concentration (EC_{50}) of ~1 mM [45]. Also, treatment of 500 µM H_2O_2 for 24 h in a hippocampal neuronal cell line (HT-22) induces around 50% of cell death [46]. Further, investigation of exposure to 1 mM H_2O_2 for 2 h on human embryonic kidney 293 cells (HEK293) in vitro, cells displayed the extent of programmed cell death, with Condensed chromatin and apoptotic nuclei [47]. The above-reported studies and various other studies [48][49][50][51][52] elucidated H_2O_2 concentration-dependent change in cell signaling and death. Very low concentration of H_2O_2 cause cell signaling and hence, cell growth, a mid-higher concentration of around (120 µM to 150 µM) induce a temporary growth arrest, the intermediate concentration of (250 µM–400 µM) causes permanent growth arrest and a higher concentration of (≥1 mM) causes cell damage by necrosis and hence death.

3. Metabolic Sources and Sinks of Hydrogen Peroxide

The superoxide anion radical is the precursor of all radicals, and it is generated during the respiratory electron transport chain process complexed with cytochrome I, II, and III or by NAD(P)H oxidases (NOXs) enzymes in the mitochondria [53]. A total of 31 H_2O_2 generating oxidases have been reported [54]. Superoxide dismutases (SODs) like SOD1, SOD2, SOD3,

with their presence in cytosolic, mitochondrial matrix, and extracellular locations, respectively are the major sources of H_2O_2. Besides that, the endoplasmic reticulum and peroxisomes are also responsible for the production of H_2O_2 [2]. There exist an H_2O_2 gradient, which is largely associated with their generation in association with respiratory cytochromes like complex III and is associated with the generation of H_2O_2 within mitochondrial cristae, whereas, complex I and II contribute H_2O_2 within the mitochondrial matrix [55]. The H_2O_2 removal from cells is mainly carried out by natural antioxidant enzymes like dismutation of catalases, peroxidases like glutathione peroxidases (GPxs), and peroxiredoxins in the form of H_2O and O_2 [2][56][57]. GPxs are a family of selenoenzymes homologous to selenocysteine, containing mammalian GPx-1, and have a high degree of affinity for H_2O_2 in humans. GPx-1 is one of the most expressed and abundant members of the GPx family of enzymes that include an epithelial-specific enzyme that is highly expressed in the intestine (GPx-2); a secreted subtype (GPx-3); and GPx-4, which is widely expressed and differs in its substrate specificity compared to the other family members. Accordingly, GPx-1 is a key selenoenzyme, an enzyme involved in alleviating the detrimental effects of H_2O_2 [58][59]. It is present in all cells; found in cytosolic, mitochondrial, and, in some cells, in peroxisomal compartments, GPx-1 can also reduce lipid hydroperoxides and other soluble hydroperoxides. GPx-4 [60] and GPx-7 [61] are also known to scavenge H_2O_2 in humans but are not globally expressed compared to GPx-1 [58].

4. Generation of Highly Unstable and Reactive Free Radical Species, the $HO^{\bullet}$ from H_2O_2

Iron (Fe) is a component of various metalloproteins in living systems and is involved in several critical biochemical processes like oxygen transport through hemoglobin, electron transport during respiration in mitochondria, synthesis, and repair of nucleic acids, metabolism of xenobiotics, essential for oxidation-reduction catalysis and bioenergetics though heme of cytochrome enzymes [62]. Three major classes of Fe-containing proteins facilitate oxygen-based chemistry in living cells: iron–sulfur cluster-containing proteins; heme-containing proteins; and iron-containing enzymes that are devoid of iron–sulfur clusters or heme. The redox ability of Fe makes it an indispensable metal of life, making it a key element in numerous biochemical processes of life [63]. However, the ability to take part in (oxidation, reduction cycle) renders Fe to act as a catalyst in a free state to generate toxic free radicals in oxygen-consuming organisms. For this reason, the circulating Fe is protected in a tightly bound state in form of Fe carrier transferrin, which keeps Fe in its inactive redox state. On the other hand, physiological cells also have some free Fe called labile Fe, which acts to generate free radical H_2O_2, for redox signaling. However, any alteration in the normal pool of either bound or labile Fe in a state of redox disbalance will give stimulus to start Fenton chemistry to form one of the most toxic radical $HO^{\bullet}$. Over the last few decades, the role of Fe in neurodegenerative diseases has grabbed everybody's attention [64][65]. The requirement of Fe in the brain is high because of the high demand for energy [66][67]. Fe is an important component for the synthesis of neurotransmitters and myelin sheath of the neuron [68]. Fe toxicity due to Fe deposition and Fe-related oxidative damage is implicated in various neuropathology such as AD, PD, and ALS [69]. A large number of evidence suggests that Fe is involved in the onset and progression of ALS. Fe load was evident in the spinal cord of ALS patients [70], the motor cortex of ALS patients [71], gray matter from the frontal cortex of ALS patients [72], in the serum of ALS patients [73], and the CSF of ALS patients [74]. Recent evidence has shown that oxidative burst due to Fenton chemistry is implicated in the pathology of ALS [65][75][76][77].

Fe and H_2O_2 are involved in creating oxidizing environments inside living systems, causing the oxidation of biomolecules and hence cell damage. Being a transition metal, it has the capability to undergo oxidation and reduction inside living systems. Fe can react catalytically with H_2O_2 to form highly reactive and toxic species. Higher concentrations of H_2O_2 in the range of >100 nM cause disruptive redox signaling, causing oxidative distress and therefore, the oxidation of biomolecules. A higher concentration of H_2O_2 undergoes Fenton's reaction, which is a kind of disproportionation redox chemical reaction in the presence of ferrous ion (Fe^{+2}) or Copper (Cu). The toxicity of H_2O_2 is mainly due to the generation of an $OH^{\bullet}$ via the Fenton reaction in the presence of transition metal ion Fe or Cu, or via Haber–Weiss reaction in the presence of $O_2^{\bullet-}$ [78].

The $OH^{\bullet}$ is the most powerful oxidant among the reactive oxygen species (ROS), with a potential of $E^0(HO^{\bullet}/H_2O) = 2.34$ V. At very low pH, $HO^{\bullet}$ converts into its conjugate base $O^{\bullet-}$ ($pKa(HO^{\bullet};O^{\bullet-}) = 11.9$), the oxide radical, which is less reactive but not relevant at physiological pH [79]. The $HO^{\bullet}$ radical is electrophilic in nature and has a strong affinity towards aromatic and sulfur-containing biomolecules. There are three modes of action for the $HO^{\bullet}$ radical: electron abstraction, hydrogen abstraction, and double bond addition. The addition of $HO^{\bullet}$ radical causes oxidation of biomolecules, like 8-oxoguanine from guanine [80] and 2-oxo-histidine from histidine [81]. Whereas oxidation of sulfur-containing amino acid methionine (Met) forms Met sulphoxide and sulphone [82]. Electron abstraction is also observed with inorganic substrates like (Fe^{+2}) [83]. The hydride anion (H^-) abstraction mechanism is involved in reactions with various biomolecules, such as polyunsaturated fatty acids like linoleate and arachidonate [84], also from (SH) or hydroxyl (OH) functional groups from different proteins and peptides [85]. This $HO^{\bullet}$ initiates lipid peroxidation to form lipid peroxides ultimately leading to the

formation of malonaldehyde or 4-Hydroxy-2-Nonenal (4-HNE), causing alteration in gene expression and promoting cell death [86].

H_2O_2 can also be converted into a $HO^{\bullet}$ in the presence of a superoxide radical anion ion called Heber-Weiss reaction [79] [87]. Intriguingly, ascorbic acid, which is one of the antioxidants present in the lining of lungs and prevents the harmful effects of pollution, can also generate cytotoxic $OH^{\bullet}$ when it is oxidized in the presence of hydrogen peroxide and transition metal catalyst like Fe and Cu in vitro [18]. This led to the development of acellular assays to measure particle-bound ROS and aerosol oxidative potential (OP) of the particulate matter present in air pollution [88]. Furthermore, it is important to note that, ascorbic acid acts as a pro-oxidant and recycles the ferric ion (Fe^{+3}) to (Fe^{+2}), hence, facilitating and enhancing the generation of ROS, through successive Fenton cycles [89].

References

1. Teong, S.P.; Li, X.; Zhang, Y. Hydrogen peroxide as an oxidant in biomass-to-chemical processes of industrial interest. Green Chem. 2019, 21, 5753–5780.
2. Sies, H. Hydrogen peroxide as a central redox signaling molecule in physiological oxidative stress: Oxidative eustress. Redox Biol. 2017, 11, 613–619.
3. Boateng, M.K.; Price, S.L.; Huddersman, K.D.; Walsh, S.E. Antimicrobial activities of hydrogen peroxide and its activati on by a novel heterogeneous Fenton's-like modified PAN catalyst. J. Appl. Microbiol. 2011, 111, 1533–1543.
4. Goti, A.; Cardona, F. (Eds.) Hydrogen Peroxide in Green Oxidation Reactions: Recent Catalytic Processes; Springer: D ordrecht, The Netherlands, 2008.
5. Elango, M.; Parthasarathi, R.; Subramanian, V.; Ramachandran, C.N.; Sathyamurthy, N. Hydrogen peroxide clusters: T he role of open book motif in cage and helical structures. J. Phys. Chem. A 2006, 110, 6294–6300.
6. Engdahl, A.; Nelander, B.; Karlström, G. A matrix isolation and ab initio study of the hydrogen peroxide dimer. J. Phys. Chem. A 2001, 105, 8393–8398.
7. Winterbourn, C.C. The biological chemistry of hydrogen peroxide. Methods Enzymol. 2013, 528, 3–25.
8. Phaniendra, A.; Jestadi, D.B.; Periyasamy, L. Free radicals: Properties, sources, targets, and their implication in various diseases. Indian J. Clin. Biochem. 2015, 30, 11–26.
9. Halliwell, B. Free radicals and antioxidants: Updating a personal view. Nutr. Rev. 2012, 70, 257–265.
10. Winterbourn, C.C. Comparative reactivities of various biological compounds with myeloperoxidase-hydrogen peroxide-chloride, and similarity of the oxidant to hypochlorite. Biochim. Biophys. Acta 1985, 840, 204–210.
11. Prütz, W.A. Hypochlorous acid interactions with thiols, nucleotides, DNA, and other biological substrates. Arch. Bioche m. Biophys. 1996, 332, 110–120.
12. Winterbourn, C.C. Reconciling the chemistry and biology of reactive oxygen species. Nat. Chem. Biol. 2008, 4, 278–28 6.
13. Beckman, J.S.; Koppenol, W.H. Nitric oxide, superoxide, and peroxynitrite: The good, the bad, and ugly. Am. J. Physiol. 1996, 271, C1424–C1437.
14. Ischiropoulos, H.; Zhu, L.; Chen, J.; Tsai, M.; Martin, J.C.; Smith, C.D.; Beckman, J.S. Peroxynitrite-mediated tyrosine n itration catalyzed by superoxide dismutase. Arch. Biochem. Biophys. 1992, 298, 431–437.
15. Douki, T.; Cadet, J. Peroxynitrite mediated oxidation of purine bases of nucleosides and isolated DNA. Free Radic. Re s. 1996, 24, 369–380.
16. Radi, R. Oxygen radicals, nitric oxide, and peroxynitrite: Redox pathways in molecular medicine. Proc. Natl. Acad. Sci. USA 2018, 115, 5839–5848.
17. Schöpfer, F.; Riobó, N.; Carreras, M.C.; Alvarez, B.; Radi, R.; Boveris, A.; Radi, R.; Boveris, A.; Cadenas, E.; Poderoso, J.J. Oxidation of ubiquinol by peroxynitrite: Implications for protection of mitochondria against nitrosative damage. Bioc hem. J. 2000, 349 Pt 1, 35–42.
18. Lipinski, B. Hydroxyl radical and its scavengers in health and disease. Oxid. Med. Cell. Longev. 2011, 2011, 809696.
19. Pinto, E.; Sigaud-kutner, T.C.; Leitao, M.A.; Okamoto, O.K.; Morse, D.; Colepicolo, P. Heavy metal–induced oxidative st ress in algae 1. J. Phycol. 2003, 39, 1008–1018.
20. Rojanasakul, Y.; Wang, L.; Hoffman, A.H.; Shi, X.; Dalal, N.S.; Banks, D.E.; Ma, J.K.H. Mechanisms of hydroxyl free ra dical-induced cellular injury and calcium overloading in alveolar macrophages. Am. J. Respir. Cell Mol. Biol. 1993, 8, 37 7–383.

21. Bach, R.D.; Ayala, P.Y.; Schlegel, H. A reassessment of the bond dissociation energies of peroxides. An ab initio study. J. Am. Chem. Soc. 1996, 118, 12758–12765.

22. Behrman, E.J.; John, O.; Edwards, A. Half-Century of Peroxide Chemistry: A translation from R. Curci, L. D'Accolti, & C. Fusco. Chim. E L'Industria 2020, 88, 32–39.

23. Chu, J.W.; Trout, B.L. On the mechanisms of oxidation of organic sulfides by H2O2 in aqueous solutions. J. Am. Chem. Soc. 2004, 126, 900–908.

24. Cardey, B.; Enescu, M. Selenocysteine versus cysteine reactivity: A theoretical study of their oxidation by hydrogen per oxide. J. Phys. Chem. A 2007, 111, 673–678.

25. Gupta, V.; Carroll, K.S. Profiling the Reactivity of Cyclic C-Nucleophiles towards Electrophilic Sulfur in Cysteine Sulfeni c Acid. Chem. Sci. 2016, 7, 400–415.

26. Zeida, A.; Babbush, R.; Lebrero, M.C.; Trujillo, M.; Radi, R.; Estrin, D.A. Molecular basis of the mechanism of thiol oxid ation by hydrogen peroxide in aqueous solution: Challenging the SN2 paradigm. Chem. Res. Toxicol. 2012, 25, 741–74 6.

27. Roos, G.; Foloppe, N.; Messens, J. Understanding the pK(a) of redox cysteines: The key role of hydrogen bonding. Anti oxid. Redox Signal. 2013, 18, 94–127.

28. Pearson, R.G.; Edgington, D.N. Nucleophilic reactivity of the hydrogen peroxide anion: Distinction between SN2 and S N1 CB mechanisms. J. Am. Chem. Soc. 1962, 84, 4607–4608.

29. Brown, H.; Rao, B. Communications-selective conversion of olefins into organoboranes through competitive hydroborat ion, isomerization and displacement reactions. J. Org. Chem. 1957, 22, 1137–1138.

30. Yim, M.B.; Chock, P.B.; Stadtman, E.R. Copper, zinc superoxide dismutase catalyzes hydroxyl radical production from hydrogen peroxide. Proc. Natl. Acad. Sci. USA 1990, 87, 5006–5010.

31. Yim, M.B.; Yim, H.S.; Boon Chock, P.; Stadtman, E.R. Pro-oxidant activity of Cu,Zn-superoxide dismutase. Age 1998, 2 1, 91–93.

32. Sies, H. Role of metabolic H2O2 generation: Redox signaling and oxidative stress. J. Biol. Chem. 2014, 289, 8735–874 1.

33. Krols, M.; Bultynck, G.; Janssens, S. ER-Mitochondria contact sites: A new regulator of cellular calcium flux comes into play. J. Cell Biol. 2016, 214, 367–370.

34. Marino, S.M.; Gladyshev, V.N. Cysteine function governs its conservation and degeneration and restricts its utilization o n protein surfaces. J. Mol. Biol. 2010, 404, 902–916.

35. Gülden, M.; Jess, A.; Kammann, J.; Maser, E.; Seibert, H. Cytotoxic potency of H2O2 in cell cultures: Impact of cell con centration and exposure time. Free Radic. Biol. Med. 2010, 49, 1298–1305.

36. Juarez, J.C.; Manuia, M.; Burnett, M.E.; Betancourt, O.; Boivin, B.; Shaw, D.E.; Tonks, N.K.; Mazar, A.P.; Donate, F. Su peroxide dismutase 1 (SOD1) is essential for H2O2-mediated oxidation and inactivation of phosphatases in growth fact or signaling. Proc. Natl. Acad. Sci. USA 2008, 105, 7147–7152.

37. Ezzi, S.A.; Urushitani, M.; Julien, J.P. Wild-type superoxide dismutase acquires binding and toxic properties of ALS-link ed mutant forms through oxidation. J. Neurochem. 2007, 102, 170–178.

38. Clément, M.V.; Ponton, A.; Pervaiz, S. Apoptosis induced by hydrogen peroxide is mediated by decreased superoxide anion concentration and reduction of intracellular milieu. FEBS Lett. 1998, 440, 13–18.

39. Gutiérrez-Venegas, G.; Guadarrama-Solís, A.; Muñoz-Seca, C.; Arreguín-Cano, J.A. Hydrogen peroxide-induced apopt osis in human gingival fibroblasts. Int. J. Clin. Exp. Pathol. 2015, 8, 15563–15572.

40. Zhao, L.; Lin, H.; Chen, S.; Chen, S.; Cui, M.; Shi, D.; Wang, B.; Ma, K.; Shao, Z. Hydrogen peroxide induces program med necrosis in rat nucleus pulposus cells through the RIP1/RIP3-PARP-AIF pathway. J. Orthop. Res. 2018, 36, 1269–1282.

41. Halliwell, B.; Gutteridge, J.M. Free Radicals in Biology and Medicine; Oxford University Press: Oxford, UK, 2015.

42. Saito, Y.; Nishio, K.; Ogawa, Y.; Kimata, J.; Kinumi, T.; Yoshida, Y.; Noguchi, N.; Niki, E. Turning point in apoptosis/necr osis induced by hydrogen peroxide. Free Radic. Res. 2006, 40, 619–630.

43. Teramoto, S.; Tomita, T.; Matsui, H.; Ohga, E.; Matsuse, T.; Ouchi, Y. Hydrogen peroxide-induced apoptosis and necros is in human lung fibroblasts: Protective roles of glutathione. Jpn. J. Pharmacol. 1999, 79, 33–40.

44. Troyano, A.; Sancho, P.; Fernández, C.; de Blas, E.; Bernardi, P.; Aller, P. The selection between apoptosis and necrosi s is differentially regulated in hydrogen peroxide-treated and glutathione-depleted human promonocytic cells. Cell Deat h Differ. 2003, 10, 889–898.

45. Cookson, M.R.; Ince, P.G.; Shaw, P.J. Peroxynitrite and hydrogen peroxide induced cell death in the NSC34 neuroblast oma x spinal cord cell line: Role of poly (ADP-ribose) polymerase. J. Neurochem. 1998, 70, 501–508.

46. Zhao, Z.Y.; Luan, P.; Huang, S.X.; Xiao, S.H.; Zhao, J.; Zhang, B.; Gu, B.-B.; Pi, R.-B.; Liu, J. Edaravone protects HT22 neurons from H2O2-induced apoptosis by inhibiting the MAPK signaling pathway. CNS Neurosci. Ther. 2013, 19, 163–169.

47. Bian, Y.Y.; Guo, J.; Majeed, H.; Zhu, K.X.; Guo, X.N.; Peng, W.; Zhou, H.-M. Ferulic acid renders protection to HEK293 cells against oxidative damage and apoptosis induced by hydrogen peroxide. Vitr. Cell. Dev. Biol. Anim. 2015, 51, 722–729.

48. Xiang, J.; Wan, C.; Guo, R.; Guo, D. Is Hydrogen Peroxide a Suitable Apoptosis Inducer for All Cell Types? Biomed. Re s. Int. 2016, 2016, 7343965.

49. Ali, M.A.; Kandasamy, A.D.; Fan, X.; Schulz, R. Hydrogen peroxide-induced necrotic cell death in cardiomyocytes is ind ependent of matrix metalloproteinase-2. Toxicol. In Vitro 2013, 27, 1686–1692.

50. Ohguro, N.; Fukuda, M.; Sasabe, T.; Tano, Y. Concentration dependent effects of hydrogen peroxide on lens epithelial c ells. Br. J. Ophthalmol. 1999, 83, 1064–1068.

51. Okuda, S.; Nishiyama, N.; Saito, H.; Katsuki, H. Hydrogen peroxide-mediated neuronal cell death induced by an endog enous neurotoxin, 3-hydroxykynurenine. Proc. Natl. Acad. Sci. USA 1996, 93, 12553–12558.

52. Huang, B.K.; Sikes, H.D. Quantifying intracellular hydrogen peroxide perturbations in terms of concentration. Redox Bio l. 2014, 2, 955–962.

53. Lennicke, C.; Rahn, J.; Lichtenfels, R.; Wessjohann, L.A.; Seliger, B. Hydrogen peroxide-production, fate and role in re dox signaling of tumor cells. Cell Commun. Signal. 2015, 13, 39.

54. Go, Y.M.; Chandler, J.D.; Jones, D.P. The cysteine proteome. Free Radic. Biol. Med. 2015, 84, 227–245.

55. Bleier, L.; Wittig, I.; Heide, H.; Steger, M.; Brandt, U.; Dröse, S. Generator-specific targets of mitochondrial reactive oxy gen species. Free Radic. Biol. Med. 2015, 78, 1–10.

56. Chang, C.; Worley, B.L.; Phaëton, R.; Hempel, N. Extracellular Glutathione Peroxidase GPx3 and Its Role in Cancer. C ancers 2020, 12, 2197.

57. West, J.D.; Roston, T.J.; David, J.B.; Allan, K.M.; Loberg, M.A. Piecing Together How Peroxiredoxins Maintain Genomic Stability. Antioxidants 2018, 7, 177.

58. Lubos, E.; Loscalzo, J.; Handy, D.E. Glutathione peroxidase-1 in health and disease: From molecular mechanisms to th erapeutic opportunities. Antioxid. Redox Signal. 2011, 15, 1957–1997.

59. Day, B.J. Catalase and glutathione peroxidase mimics. Biochem. Pharmacol. 2009, 77, 285–296.

60. Fisher, A.B. Peroxiredoxin 6: A bifunctional enzyme with glutathione peroxidase and phospholipase A_2 activities. Antioxi d. Redox Signal. 2011, 15, 831–844.

61. Wang, L.; Zhang, L.; Niu, Y.; Sitia, R.; Wang, C.C. Glutathione peroxidase 7 utilizes hydrogen peroxide generated by Er o1α to promote oxidative protein folding. Antioxid. Redox Signal. 2014, 20, 545–556.

62. Galaris, D.; Barbouti, A.; Pantopoulos, K. Iron homeostasis and oxidative stress: An intimate relationship. Biochim. Biop hys. Acta Mol. Cell Res. 2019, 1866, 118535.

63. Kaplan, J.; Ward, D.M. The essential nature of iron usage and regulation. Curr. Biol. 2013, 23, 2325.

64. Liu, D.; Wen, J.; Liu, J.; Li, L. The roles of free radicals in amyotrophic lateral sclerosis: Reactive oxygen species and el evated oxidation of protein, DNA, and membrane phospholipids. FASEB J. 1999, 13, 2318–2328.

65. Bu, X.L.; Xiang, Y.; Guo, Y. The Role of Iron in Amyotrophic Lateral Sclerosis. Adv. Exp. Med. Biol. 2019, 1173, 145–15 2.

66. Piñero, D.J.; Connor, J.R. Iron in the brain: An important contributor in normal and diseased states. Neuroscientist 200 0, 6, 435–453.

67. McCann, S.; Perapoch Amadó, M.; Moore, S.E. The Role of Iron in Brain Development: A Systematic Review. Nutrients 2020, 12, 2001.

68. Ward, R.J.; Zucca, F.A.; Duyn, J.H.; Crichton, R.R.; Zecca, L. The role of iron in brain ageing and neurodegenerative di sorders. Lancet Neurol. 2014, 13, 1045–1060.

69. Ndayisaba, A.; Kaindlstorfer, C.; Wenning, G.K. Iron in Neurodegeneration-Cause or Consequence? Front. Neurosci. 2 019, 13, 180.

70. Ince, P.G.; Shaw, P.J.; Candy, J.M.; Mantle, D.; Tandon, L.; Ehmann, W.D.; Markesbery, W. Iron, selenium and glutathione peroxidase activity are elevated in sporadic motor neuron disease. Neurosci. Lett. 1994, 182, 87–90.
71. Ignjatović, A.; Stević, Z.; Lavrnić, S.; Daković, M.; Bačić, G. Brain iron MRI: A biomarker for amyotrophic lateral sclerosis. J. Magn. Reson. Imaging 2013, 38, 1472–1479.
72. Yasui, M.; Ota, K.; Garruto, R.M. Concentrations of zinc and iron in the brains of Guamanian patients with amyotrophic lateral sclerosis and parkinsonism-dementia. Neurotoxicology 1993, 14, 445–450.
73. Veyrat-Durebex, C.; Corcia, P.; Mucha, A.; Benzimra, S.; Mallet, C.; Gendrot, C.; Moreau, C.; Devos, D.; Piver, E.; Pages, J.-C.; et al. Iron metabolism disturbance in a French cohort of ALS patients. Biomed. Res. Int. 2014, 2014, 485723.
74. Zheng, Y.; Gao, L.; Wang, D.; Zang, D. Elevated levels of ferritin in the cerebrospinal fluid of amyotrophic lateral sclerosis patients. Acta Neurol. Scand. 2017, 136, 145–150.
75. Popović-Bijelić, A.; Mojović, M.; Stamenković, S.; Jovanović, M.; Selaković, V.; Andjus, P.; Bačić, G. Iron-sulfur cluster damage by the superoxide radical in neural tissues of the SOD1(G93A) ALS rat model. Free Radic. Biol. Med. 2016, 96, 313–322.
76. Sheykhansari, S.; Kozielski, K.; Bill, J.; Sitti, M.; Gemmati, D.; Zamboni, P.; Singh, A.V. Redox metals homeostasis in multiple sclerosis and amyotrophic lateral sclerosis: A review. Cell Death Dis. 2018, 9, 348.
77. Bozzo, F.; Mirra, A.; Carrì, M.T. Oxidative stress and mitochondrial damage in the pathogenesis of ALS: New perspectives. Neurosci. Lett. 2017, 636, 3–8.
78. Winterbourn, C.C. Toxicity of iron and hydrogen peroxide: The Fenton reaction. Toxicol. Lett. 1995, 82–83, 969–974.
79. Collin, F. Chemical Basis of Reactive Oxygen Species Reactivity and Involvement in Neurodegenerative Diseases. Int. J. Mol. Sci. 2019, 20, 2407.
80. Cadet, J.; Delatour, T.; Douki, T.; Gasparutto, D.; Pouget, J.P.; Ravanat, J.L.; Sauvaigo, S. Hydroxyl radicals and DNA base damage. Mutat. Res. 1999, 424, 9–21.
81. Uchida, K.; Kawakishi, S. 2-Oxo-histidine as a novel biological marker for oxidatively modified proteins. FEBS Lett. 1993, 332, 208–210.
82. Schöneich, C.; Pogocki, D.; Hug, G.L.; Bobrowski, K. Free radical reactions of methionine in peptides: Mechanisms relevant to beta-amyloid oxidation and Alzheimer’s disease. J. Am. Chem. Soc. 2003, 125, 13700–13713.
83. Masuda, T.; Shinohara, H.; Eda, M.; Kondo, M. Reactivity of nucleotides and polynucleotides toward hydroxyl radical in aqueous solution. J. Radiat. Res. 1980, 21, 173–179.
84. Gardner, H.W. Oxygen radical chemistry of polyunsaturated fatty acids. Free Radic. Biol. Med. 1989, 7, 65–86.
85. Stadtman, E.R. Protein oxidation in aging and age-related diseases. Ann. N. Y. Acad. Sci. 2001, 928, 22–38.
86. Ramana, K.V.; Srivastava, S.; Singhal, S.S. Lipid peroxidation products in human health and disease 2014. Oxid. Med. Cell. Longev. 2014, 2014, 162414.
87. Kehrer, J.P. The Haber-Weiss reaction and mechanisms of toxicity. Toxicology 2000, 149, 43–50.
88. Shen, J.; Griffiths, P.T.; Campbell, S.J.; Utinger, B.; Kalberer, M.; Paulson, S.E. Ascorbate oxidation by iron, copper and reactive oxygen species: Review, model development, and derivation of key rate constants. Sci. Rep. 2021, 11, 7417.
89. Timoshnikov, V.A.; Kobzeva, T.V.; Polyakov, N.E.; Kontoghiorghes, G.J. Redox Interactions of Vitamin C and Iron: Inhibition of the Pro-Oxidant Activity by Deferiprone. Int. J. Mol. Sci. 2020, 21, 3967.