Peroxynitrite Mediated Oxidation Damage and Cytotoxicity in Biological Systems

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Abstract: Peroxynitrite is the product of the diffusion-controlled termination reaction between two radicals, nitric oxide and superoxide and is a strong oxidant and nitrating regeant. Critical biomolecules like proteins, lipids and DNA react with peroxynitrite via direct or radical-mediated mechanisms, resulting in alterations in enzyme activities and signaling pathways. The biological consequences of peroxynitrite-mediated oxidative modifications depend on the levels of oxidant achieved *in vivo* and its cellular site of production. In this article we overview multiple biological toxicity of peroxynitrite including the biological reactivity of peroxynitrite, peroxynitrite mediated oxidation damage of biomacromolecules such as proteins, lipids and DNA and the cytotoxic effects (apoptosis and necrosis) of peroxynitrite. [Life Science Journal. 2006;3(3):41-44] (ISSN: 1097-8135).

Keywords: peroxynitrite; toxicity; cytotoxicity; oxidation damage

Abbreviations: NOS: nitric oxide synthase; PARP-1: poly(ADP-ribose) polymerase-1

1 Production of peroxynitrite in biological systems

$ONOO^-$, formed by the reaction of nitric oxide with superoxide (O_2^-) which is a byproduct of cellular respiration at near diffusion controlled rates[1], is very likely to occur even in the presence of physiological concentrations of SOD. Nitric oxide is enzymatically produced from L-arginine by nitric oxide synthase (NOS). Three isoforms of this enzyme have been described: nNOS (neuronal), eNOS (endothelial) and iNOS (induced, inflammatory). On the other hand, superoxide can be catalytically produced (for example, by xanthine oxidase or NADPH oxidase), and also formed by partial reduction of oxygen in the mitochondrial membrane or non-enzymatic monoelectron reduction of oxygen (for example, hemoglobin autoxidation). Peroxynitrite has a short biological half-life (10 - 20 ms) but can cross biological membranes and diffuse one to two cell diameters[2]. *In vivo* formation of peroxynitrite is supported by the growing experimental evidence[3].

2 Reactivity of Peroxynitrite

Peroxynitrite mediated oxidation damage by a decomposition intermediate with the biological activity of hydroxyl radical[4]. The decomposition of peroxynitrite to nitrate is intimately coupled with the oxidation chemistry of this species, and both reactions have been the subject of recent investigations. $ONOO^-$ is a relatively stable at alkaline pH, but at physiological pH it is capable of effecting one and two electron reactions akin to those of HO^+, NO_2, and nitrosonium cation. Oxidations of ascorbate[5], transition metal complexes, halide ions, thiols, sulfides[6], olefins, benzenes, phenols[7] and other aromatics by peroxynitrite have been described. Peroxynitrite is a particularly effective oxidant of aromatic molecules and organosulfur compounds that include free amino acids and peptide residues. Cysteine and glutathione, which are significant components of antioxidant reservoirs, are converted to disulfides. Methionine is converted to sulfoxide or is fragmented to ethylene and dimethyldisulfide. Tyrosine and tryptophan undergo one-electron oxidations to radical cations, which are competitively hydroxylated, nitrated and dimerized[8]. Purine nucleotides are vulnerable to oxidation and to adduct formation[9]. Other reports have more detailed reviews on the chemistry, decomposition and reactivity of peroxynitrite, peroxynitrous acid and its activated isomer[10]. The various reactions of peroxynitrite when occurring during the reaction of peroxynitrite with enzymes, macromolecules and lipids, have been shown to influence cellular functions.

3 Peroxynitrite Mediated Oxidation of Biomacro-Molecules

3.1 Protein oxidation

Peroxynitrite-induced protein modifications include protein oxidation (on methionine, cysteine, tryptophane or tyrosine residues) and nitration (of tyrosine or tryptophane residues). However, enzymes containing a redox active transition metal center are the prime targets of the oxidant[11]. Reactions of peroxynitrite are affected by the local pH and the microenvironment with hydrophobic membrane compartments favoring nitration and aqueous environments favoring oxidation. Moreover, carbon dioxide reacts with peroxynitrite resulting in the formation of nitroso-peroxocarbonates[1]. The ubiquitous presence of CO_2 at high concentration may favor this reaction route. As nitroso-peroxocarbonates divert peroxynitriteinduced protein modifications toward nitration, CO_2 is now considered as key determinant of peroxynitrite chemistry.

As just mentioned above, peroxynitrite can directly oxidize the prosthetic group of a protein, for example, hemoglobin, or directly react with the peptide chain leading to conformational and functional changes with potential severe biological consequences. Enzymes with critical cysteine residues can be inactivated by peroxynitrite[12]. In contrast, oxidation of a critical cysteine has been shown to activate an enzyme, that is the case of matrix metalloproteinases where the cysteine residue is in the autoinhibitory domain of the proenzyme[13].

In some cases, the oxidation of a cysteine residue to disulfide (via sulfenic acid) is part of the catalytic cycle, as is the case of peroxiredoxins, thiol-dependent peroxidases[14]. Critical methionine residues can be oxidized by peroxynitrite to yield methionine sulfoxide with loss of protein function as the case α1-antiproteinase[15] which lose its ability to inhibit proteases, in particular, elastase. The oxidation of methionine is readily reversed by methionine sulfoxide reductase at the expenses of thioredoxin. Peroxynitrite does not directly react with tyrosine residues[16] but can oxidize and nitrate them. Nitration (i. e. addition of a NO_2 group) of protein tyrosines to 3-nitrotyrosine has been interpreted as a footprint of peroxynitrite *in vivo* and which can inactivate the enzymes[17] or the proteins loss function after nitration[18].

3.2 DNA oxidation

Peroxynitrite can mediate DNA damage such as the oxidative modification of nitrogen bases and the sugar moiety as well as strand breaks[19]. The most reactive nitrogen base is guanine to yield 8-oxoguanine and 8-nitroguanine. The formation of strand breaks have been shown to activate poly-ADP ribose synthase (PARS) which catalyze the poly-ADP ribosylation of histones, topoisomerases, DNA ligase Ⅱ, triggering signaling towards cell cycle arrest[20]. Excessive PARS activation may lead to NAD consumption and energy depletion[21].

3.3 Lipid peroxidation

Peroxynitrite can initiate oxidation of lipids (membranes, liposomes and lipoproteins) yielding lipid hydroperoxides, conjugated dienes, aldehydes, and even nitrated lipids have been detected[22]. In contrast to the well-known oxygen radical dependent lipid peroxidation that requires transition metal ion catalysis, no iron is required to initiate lipid peroxidation by peroxynitrite[1]. Oxidation of polyunsaturated fatty acids and cholesterol in the process of lipoperoxidation causes membrane permeability and fluidity changes with biological consequences. In addition, the intermediate products of lipoperoxidation (lipid hydroperoxides, malondialdehyde, 4-hydroxynonenal, isoprostanes) are not inert and can initiate secondary oxidative events. A significant correlation has been found between these products plasma concentration and several disorders like Alzheimer[23] or diabetes. The reactivity and functions of novel nitrated derivatives found after peroxynitrite-mediated lipoperoxidation are under study and their participation in cell signaling has been suggested[24].

4 Cytotoxicity of Peroxynitrite

4.1 Peroxynitrite-induced apoptosis

When peroxynitrite-induced cellular damage reaches a level that cannot be handled by the repair mechanisms, cells will undergo one of the basic cell death pathways, apoptosis or necrosis. Apoptosis is the "default" death pathway characterized, among other parameters, by a compact morphology, maintenance of plasma membrane integrity, mitochondrial depolarization, secondary oxidant production, activation of caspases (cysteinyl aspartate specific proteases) and oligonucleosomal DNA fragmentation[25]. Pryor had the first report that peroxynitrite can trigger apoptotic death. They have detected DNA fragmentation in peroxynitrite treated thymocytes[26]. Later, activation of caspase-3, a key player in the caspase cascade has also been detected in thymocytes and HL-60 cells[27]. Prototypical apoptosis models utilize apoptosis inducers such as tumor necrosis factor acting upon cell surface death receptors. Channeling the death signal from these receptors to apoptotic effector machineries is well

described[25]. However, it is not quite clear, how peroxynitrite triggers the apoptotic machinery. Mitochondria are likely sites for peroxynitrite induced apoptosis initiation. Mitochondria are now recognized as central organizators of apoptosis[25]. A characteristic sequence of events including opening of mitochondrial permeability transition pore, mitochondrial depolarization, secondary superoxide production, release of apoptotic mediators from the intermembrane space to the cytoplasm, takes place in apoptosing cells[25]. Furthermore, adenosine nucleotide translocator, a member of the permeability pore is also targeted by peroxynitrite[28]. The role of mitochondria in peroxynitrite-induced apoptosis is also supported by findings that bcl-2, a mitochondrial antiapoptotic protein inhibits peroxynitrite-induced apoptosis[29]. The cellular energetics may become compromised by peroxynitrite also via alternative mechanisms (e. g. inactivation of creatine kinase in cardiomyocytes) which may also contribute to peroxynitrite cytotoxicity[30].

4.2 Peroxynitrite-induced necrosis

It has been found that low concentrations of peroxynitrite trigger apoptosis, higher concentrations of the oxidant compromise the apoptotic machinery forcing the cells to die by necrosis[30]. Recently, a new method has emerged identifying an active element in oxidative stress-induced necrosis. According to method, degree of the activation of poly(ADP-ribose) polymerase-1 (PARP-1) determines the fate of the oxidatively-injured cells[31]. PARP-1 is activated by DNA strand breakage. Activated PARP-1 cleaves NAD^+ into nicotinamide and ADP-ribose and polymerizes the latter on nuclear acceptor proteins. Peroxynitrite-induced over activation of PARP consumes NAD^+ and consequently ATP culminating in cell dysfunction, apoptosis or necrosis. These findings indicate that PARP-1 activation diverts the default apoptotic process toward necrosis[31].

Moreover, peroxynitrite-induced DNA breakage activates PARP leading to NAD^+ and ATP depletion and consequently to necrosis. The concerted action of PARP-1 and PARG maintains a highly accelerated ADP-ribose turnover in peroxynitrite treated cells. As a result, NAD^+ becomes depleted in the cells leading to malfunctioning glycolysis, Krebs cycle, mitochondrial electron transport and eventually to ATP depletion[32]. The deterioration of cellular energetic status may play a central role in the "cell death switch" of PARP-1[33].

5 Conclusions

Peroxynitrite formed *in vivo* from superoxide and nitric oxide can mediate selective oxidation and nitration of biomolecules via direct or radical-dependent pathways. Depending on the levels and cellular sites of peroxynitrite produced, these oxidative modifications can lead to conformational changes, impaired functions, enzyme inactivation, or signaling pathways alterations, apoptotic or necrotic cell death and result in various diseases. Pharmacological approaches to ameliorate peroxynitrite-mediated drug toxicity could be focused on diminishing the flux of precursor radicals (nitric oxide and superoxide) or on scavenging the peroxynitrite formed.

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References

1. Radi R, Peluffo G, Alvarez MN, Naviliat M, Cayota A. Unraveling peroxynitrite formation in biological system. Free Radic Biol Med 2001; 30: 463-88.
2. Denicola A, Souza JM, Radi R. Diffusion of peroxynitrite across erythrocyte membranes. Proc Natl Acad Sci 1998; 95: 3566-71.
3. Fries DM, Paxinou E, Themistocleous M, Swanberg E. Expression of inducible nitricoxide synthase and intracellular protein tyrosine nitration in vascular smooth muscle cells: role of reactive oxygen species. J Biol Chem 2003; 278: 22901-7.
4. Pryor WA, Squadrito GL. The chemistry of peroxynitrite: a product from the reaction of nitric oxide with superoxide. Am J Physiol 1995; 268: L699-L722.
5. Barlett D, Church DF, Bounds, PL, Koppenol WH. The kinetics of the oxidation of L-ascorbic acid by peroxynitrite. Free Radic Biol Med 1995; 18: 85-90.
6. Padmaja S, Squadrito GL, Lemercier JN, Cueto R, Pryor WA. Rapid oxidation of DL-selenomethionine by peroxynitrite. Free Radic Biol Med 1996; 21: 317-24.
7. Ischiropoulos H, Zhu L, Chen J, Tsai M, Martin J C, Smith CD, Beckman JS. Peroxynitrite-mediated tyrosine nitration catalyzed by superoxide dismutase. Arch Biochem Biophys 1992; 298:431-7.
8. Ramezanian MS, Padmaja S, Koppenol WH. Nitration and hydroxylation of phenolic compounds by peroxynitrite. Chem Res Toxicol 1996; 9: 232-7.
9. Douki T, Cadet J. Peroxynitrite mediated oxidation of purine bases of nucleosides and isolated DNA. Free Radic Res 1996; 24: 369-80.
10. Groves JT. Peroxynitrite: reactive, invasive and enigmatic. Curr Opin Chem Biol 1999; 3: 226-35.
11. Beckman JS, Koppenol WH. Nitric oxide, superoxide,

and peroxynitrite: the good, the bad, and ugly. Am J Physiol 1996; 271: C1424 – 37.
12. Takakura K, Beckman JS, MacMillan-Crow LA, Crow JP. Rapid and irreversible inactivation of protein tyrosine phosphatases PTP1B, CD45, and LAR by peroxynitrite. Arch Biochem Biophys 1999; 369: 197 – 207.
13. Okamoto T, Akaike T, Nagano T, Miyajima S, Suga M, Ando M, Ichimori K, Maeda H. Activation of human neutrophil procollagenase by nitrogen dioxide and peroxynitrite: a novel mechanism for procollagenase activation involving nitric oxide. Arch Biochem Biophys 1997; 342: 261 – 74.
14. Bryk R, Griffin P, Nathan C. Peroxynitrite reductase activity of bacterial peroxiredoxins. Nature 2000; 407: 211 – 5.
15. Moreno JJ, Pryor WA. Inactivation of alpha 1-proteinase inhibitor by peroxynitrite. Chem Res Toxicol 1992; 5: 425 – 31.
16. Alvarez B, Radi R. Peroxynitrite reactivity with amino acids and proteins. Amino Acids 2003;25, 295 – 311.
17. Greenacre SA, Ischiropoulos H. Tyrosine nitration: localisation, quantification, consequences for protein function and signal transduction. Free Radic Res 2001; 34: 541 – 81.
18. Crow JP, Ye YZ, Strong M, Kirk M, Barnes S, Beckman JS. Superoxide dismutase catalyzes nitration of tyrosines by peroxynitrite in the rod and head domains of neurofilament-L. J Neurochem 1997; 69: 1945 – 53.
19. Burney S, Caulfield JL, Niles JC, Wishnok JS, Tannenbaum SR. The chemistry of DNA damage from nitric oxide and peroxynitrite. Mutat Res 1999; 424: 37 – 49.
20. Satoh MS, Lindahl T. Role of poly(ADP-ribose) formation in DNA repair. Nature 1992; 356: 356 – 8.
21. Szabo C, Zingarelli B, O'Connor M, Salzman AL. DNA strand breakage, activation of poly (ADP-ribose) synthetase, and cellular energy depletion are involved in the cytotoxicity of macrophages and smooth muscle cells exposed to peroxynitrite. Proc Natl Acad Sci 1996; 93: 1753 – 8.
22. O'Donnell VB, Eiserich JP, Chumley PH, Jablonsky MJ, Krishna NR, Kirk M, Barnes S, Darley-Usmar VM, Freeman BA. Nitration of unsaturated fatty acids by nitric oxide-derived reactive nitrogen species peroxynitrite, nitrous acid, nitrogen dioxide, and nitronium ion. Chem Res Toxicol 1999; 12: 83 – 92.
23. Cecchi C, Fiorillo C, Sorbi S, Latorraca S, Nacmias B, Bagnoli S, Nassi P, Liguri G. Oxidative stress and reduced antioxidant defenses in peripheral cells from familial Alzheimer's patients. Free Radic Biol Med 2002; 33: 1372 – 9.
24. Lim DG, Sweeney S, Bloodsworth A, White CR, Chumley PH, Krishna NR, Schopfer F, O'Donnell VB, Eiserich JP, Freeman BA. Nitrolinoleate, a nitric oxide-derived mediator of cell function: synthesis, characterization, and vasomotor activity. Proc Natl Acad Sci 2002; 99: 15941 – 6.
25. Green D, Kroemer G. The central executioners of apoptosis: caspases or mitochondria? Trends Cell Biol 1998; 8: 267 – 71.
26. Salgo MG, Squadrito GL, Pryor WA. Peroxynitrite causes apoptosis in rat thymocytes. Biochem Biophys Res Commun 1995; 215: 1111 – 8.
27. Lin KT, Xue JY, Lin MC, Spokas EG, Sun FF, Wong PY. Peroxynitrite induces apoptosis of HL-60 cells by activation of a caspase-3 family protease. Am J Physiol 1998; 274: C855 – 60.
28. Vieira HL, Belzacq AS, Haouzi D, Bernassola F, Cohen I, Jacotot E, Ferri KF, El Hamel C, Bartle L M, Melino G, Brenner C, Goldmacher V, Kroemer G. The adenine nucleotide translocator: a target of nitric oxide, peroxynitrite, and 4-hydroxynonenal. Oncogene 2001; 20: 4305 – 16.
29. Virag L, Szabo C. Bcl-2 protects peroxynitrite-treated thymocytes from poly(ADP-ribose) synthase (PARS) independent apoptotic but not from PARS-mediated necrotic cell death. Free Radical Biol Med 2000; 29: 704 – 13.
30. Mihm MJ, Coyle CM, Schanbacher BL, Weinstein, DM, Bauer, JA. Peroxynitrite induced nitration and inactivation of myofibrillar creatine kinase in experimental heart failure. Cardiovasc Res 2001a; 49: 798 – 807.
31. Virag L, Szabo C. The therapeutic potential of poly (ADP-ribose) polymerase inhibitors. Pharm Rev 2002; 54: 375 – 429.
30. Virag L, Salzman AL, Szabo C. Poly(ADP-ribose) synthetase activation mediates mitochondrial injury during oxidant-induced cell death. J Immunol 1998; 161: 3753 – 9.
32. Berger SJ, Sudar DC, Berger NA. Metabolic consequences of DNA damage: DNA damage induces alterations in glucose metabolism by activation of poly (ADP-Ribose) polymerase. Biochem Biophys Res Commun 1986; 134: 227 – 32.
33. Ying W, Chen Y, Alano CC, Swanson RA. Tricarboxylic acid cycle substrates prevent PARP-mediated death of neurons and astrocytes. J Cereb Blood Flow Metab 2002; 22: 774 – 9.

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