



Review

Multiple pathways of peroxynitrite cytotoxicity

Csaba Szabó *

Inotek Pharmaceuticals Corporation, 100 Cummings Center, Suite #419E, Beverly, MA 01915, USA

Received 15 September 2002; accepted 12 December 2002

Abstract

Peroxynitrite is a reactive oxidant produced from nitric oxide (NO) and superoxide, which reacts with a variety of biomolecules including proteins, lipids and DNA. Peroxynitrite is produced by the body in response to a variety of toxicologically relevant molecules including environmental toxins. It is also produced by the body in response to environmental toxins, as well as in reperfusion injury and inflammation. Here we overview the multiple pathways of peroxynitrite cytotoxicity. Initiation of lipid peroxidation, direct inhibition of mitochondrial respiratory chain enzymes, inactivation of glyceraldehyde-3-phosphate dehydrogenase, inhibition of membrane Na^+/K^+ ATP-ase activity, inactivation of membrane sodium channels, and other oxidative protein modifications contribute to the cytotoxic effect of peroxynitrite. In addition, peroxynitrite is a potent trigger of DNA strand breakage, with subsequent activation of the nuclear enzyme poly-ADP ribosyl synthetase or polymerase (PARP), with eventual severe energy depletion and necrosis of the cells. Studies conducted with peroxynitrite decomposition catalysts suggest that neutralization of peroxynitrite is of significant therapeutic benefit after exposure to various environmental toxins as well as in a variety of inflammatory and reperfusion disease conditions.

© 2003 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Nitric oxide; Superoxide; Mitochondria; Inflammation; Free radicals; Environmental toxins

1. The reactivity of peroxynitrite

Simultaneous generation of nitric oxide (NO) and superoxide favors the production of a toxic reaction product, peroxynitrite anion (ONOO^-) (Beckman et al., 1990; see for reviews Pryor and Squadrito, 1996; Szabó, 1996). In *in vitro* systems, the ratio of superoxide and NO determines the reactivity of peroxynitrite: excess of NO reduces

the oxidation elicited by peroxynitrite (Rubbo et al., 1994; Miles et al., 1996). The end-products of specific oxidative processes triggered by peroxynitrite can be detected *in vivo*, suggesting *in vivo* formation of peroxynitrite. The oxidant reactivity of peroxynitrite is mediated by an intermediate with the biological activity of hydroxyl radical (Pryor and Squadrito, 1996).

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 and intense debate. Peroxynitrite and its conjugate acid are strong

* Tel.: +1-978-232-9660; fax: +1-978-232-8975.

E-mail address: szabocsaba@aol.com (C. Szabó).

oxidants, capable of effecting one- and two-electron reactions akin to those of HO^\bullet , nitrogen dioxide (NO_2), and nitrosonium cation. Oxidations of thiols (Radi et al., 1991), sulfides (Padmaja et al., 1996), transition metal complexes (Goldstein and Czapski, 1995), halide ions (Goldstein and Czapski, 1995), ascorbate (Barlett et al., 1995), olefins, benzenes, phenols (Halfpenny and Robinson, 1992; Ischiropoulos et al., 1992), 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. Dimethyl sulfoxide is oxidized to formaldehyde. Tyrosine and tryptophan undergo one-electron oxidations to radical cations, which are competitively hydroxylated, nitrated, and dimerized (Ischiropoulos et al., 1992; Ramezani et al., 1996). The formation of nitrotyrosine is particularly favorable, and the appearance of this product in biological samples is taken as diagnostic of exposure to peroxynitrite. Purine nucleotides are vulnerable to oxidation and to adduct formation (Douki and Cadet, 1996). For a more detailed review on the chemistry, decomposition and reactivity of peroxynitrite, peroxynitrous acid and its activated isomer (see Pryor and Squadrito, 1996; Groves, 1999).

In *in vitro* systems, peroxynitrite is highly reactive. Its reported activities include a rapid oxidation of sulfhydryl groups and thioethers, as well as nitration and hydroxylation of aromatic compounds, including tyrosine, tryptophan and guanine. While the reaction with the sulfhydryl groups is likely to represent a direct reaction of peroxynitrite, the tyrosine nitration probably occurs through a NO_2^+ -like intermediate. The detection of 3-nitrotyrosine by analytical and immunological techniques has established that a marked increase in tyrosine nitration occurs in a wide variety of disease states (Greenacre and Ischiropoulos, 2001). Other reactions such as a reaction catalyzed by myeloperoxidase may also

contribute to the net tyrosine nitration seen in pathophysiological states (Halliwell, 1997; Eiserich et al., 1998).

The various reactions of peroxynitrite when occurring during the reaction of peroxynitrite with enzymes, macromolecules and lipids, have been shown to influence cellular functions. For instance, tyrosine nitration may lead to dysfunction of nitrated proteins, as has been shown or suggested in the case of superoxide dismutase, cytoskeletal actin, neuronal tyrosine hydroxylase, cytochrome P450 and prostacyclin synthase (over-viewed in Greenacre and Ischiropoulos, 2001). Oxidation of critical sulfhydryl groups is responsible for the inhibition of mitochondrial and cytosolic aconitase and other critical enzymes in the mitochondrial respiratory chain (Hausladen and Fridovich, 1994). There is also evidence that peroxynitrite can cause covalent modification of an active site thiol of glyceraldehyde-3-phosphate dehydrogenase (Mohr et al., 1994) and in creatine kinase (Konorev et al., 1998). Peroxynitrite-mediated nitration of myofibrillar creatine kinase activity may lead to contractile dysfunction of the heart (Mihm et al., 2001). Peroxynitrite-modified cellular proteins are subject to accelerated degradation via the proteasome (Grune et al., 1998).

Peroxynitrite has been shown to inhibit a variety of ion pumps including calcium pumps (Klebl et al., 1998), calcium-activated potassium channels and also membrane Na^+/K^+ ATP-ase activity (Muriel and Sandoval, 2000). These effects are likely to contribute to a global dysregulation of ion balance and a variety of related cellular functions in peroxynitrite-challenged cells.

The reaction of peroxynitrite with lipids leads to peroxidation (malondialdehyde and conjugated diene formation) and formation of nitrito-, nitro-, nitrosoperoxo- and/or nitrated lipid oxidation adducts (Rubbo et al., 1994).

It has been recently discovered that peroxynitrite potently oxidizes various biomolecules. Peroxynitrite-mediated oxidation of tetrahydrobiopterin (BH_4) to quinonoid 5,6-dihydrobiopterin has been demonstrated *in vitro*. A large proportion of the quinonoid isomer readily loses its side chain to form 7,8-dihydropterin which is not a cofactor for NO synthase. Thus, in endothelial

cells and other cell types, pathophysiologically low levels of BH₄ can promote a cycle of its own destruction mediated by NO synthase-dependent formation of peroxynitrite (Milstien and Katusic, 1999). This mechanism might contribute to vascular endothelial dysfunction induced by oxidative stress in various diseases. In vitro it has been reported that reaction of NADH with authentic peroxynitrite resulted in the formation of NAD⁺ and superoxide and, thus, of hydrogen peroxide (Goldstein and Czapski, 2000). This reaction can both induce an imbalance in cellular pyrimidine nucleotide levels, as well as a positive feedback cycle of cytotoxic oxidant generation. Peroxynitrite mediated oxidation of catecholamines (Kerry and Rice-Evans, 1998) has also been described and may contribute to a variety of CNS and cardiovascular pathologies.

It is important to note that peroxynitrite can inhibit superoxide dismutase (Ischiropoulos et al., 1992; MacMillan-Crow et al., 1998; Yamakura et al., 1998), glutaredoxin (Aykac-Toker et al., 2001) and other antioxidant molecules and systems. Peroxynitrite-mediated depletion of one of the key cellular antioxidants, glutathione (Cuzzocrea et al., 1998) can lead to positive feedback cycles of intracellular oxidant generation and exacerbation of the oxidative cellular injury.

Oxidative stress may cause tissue injury through activation of the precursors of matrix metalloproteinase (proMMPs). Recent work suggests that the activation of proMMPs is triggered by peroxynitrite generation, via an extensive S-glutathiolation reaction (Okamoto et al., 2001). By inhibiting this reaction, peroxynitrite decomposition catalysts may reduce MMP activation, an important mechanism of tissue injury in inflammation and reperfusion.

An important interaction of peroxynitrite occurs with nucleic acids, with the production of 8-hydroxydeoxyguanosine or 8-nitroguanine. Peroxynitrite can also cause DNA cleavage in solutions of end-labelled DNA restriction fragments and can initiate DNA nicking in the supercoiled plasmid pBR322 (overviewed in Szabó and Ohshima, 1997). Peroxynitrite-induced DNA single strand breakage can activate the nuclear enzyme poly(ADP-ribose) polymerase, which can trigger

a cellular suicide pathway (Szabó et al., 1996, 1997; overviewed in Virág and Szabó, 2002).

2. The cytotoxicity of peroxynitrite

Peroxynitrite is more cytotoxic than NO or superoxide in a variety of experimental systems. In fact, recent studies suggest that, peroxynitrite, and not NO, may be the ultimately cytotoxic species in many conditions. In cells exposed to authentic peroxynitrite or to compounds that simultaneously generate NO and superoxide, marked changes in the level of cellular energetics and DNA integrity occur (overviewed in Szabó, 1996).

In addition to being a terminal mediator of cell injury—also enhances and triggers a variety of pro-inflammatory processes. For example, peroxynitrite enhances the expression of ICAM-1 and P-selectin in human endothelial cells (Zingarelli et al., 1998), and it mediates the cytokine-induced IL-8 expression in human leukocytes (Zouki et al., 2001). In human neutrophils, peroxynitrite triggers the down-regulation of L-selectin expression, and up-regulation of CD11b/CD18 expression (Zouki et al., 2001). These effects are likely to be mediated, at least in part, by the ability of peroxynitrite to trigger and enhance nuclear factor kappa B (NF- κ B) mediated pro-inflammatory signal transduction pathways (Matata and Galinanes, 2001). These alterations can culminate in a global dysregulation of cellular signal transduction pathways.

In pathophysiologically relevant situations—e.g. in macrophages that produce NO and superoxide, and thus peroxynitrite from endogenous sources—DNA strand breakage also occurs, and the time course of the strand breakage parallels the time course of NO and peroxynitrite production (Zingarelli et al., 1996). Moreover, in immunostimulated macrophages, (5-hydroxymethyl)uracil; 2,6-diamino-4-hydroxy-5-formamidopyrimidine and 8-oxoguanine formation have been reported, indicating both oxidative and deaminative DNA injury (Rojas-Walker et al., 1995). In motor neurons, both axotomy and peroxynitrite exposure

leads to a time-dependent accumulation of DNA single strand breaks (Liu and Martin, 2001).

DNA single strand breakage, initiated by endogenous or exogenous peroxynitrite, is a potent trigger of PARP activation (overviewed in Virág and Szabó, 2002), which is a major contributor to cell necrosis under conditions of severe oxidative stress.

While exposure to high concentrations of peroxynitrite leads to rapid cell death, associated with rapid energetic derangements, lower concentrations of peroxynitrite, after several hours, can lead to apoptotic cell death which is dependent on cytochrome *c* release from the mitochondria and activation of caspases 3, 2, 8 and 9 (Virág et al., 1998, Zhuang and Simon, 2000).

In various tissues and organs, peroxynitrite elicits a variety of alterations. Table 1 overviews some of the peroxynitrite-mediated deleterious molecular, subcellular and cellular pathophysiological alterations. Peroxynitrite has been implicated in the pathogenesis of a wide variety of diseases and toxicologically relevant conditions. In the following sections, we will restrict our overview to a few selected conditions induced by environmental toxins (Chapter 3), ischemia–reperfusion (Chapter 4) and inflammatory conditions (Chapter 5).

3. The toxicological relevance of peroxynitrite

Recent work demonstrates the formation of peroxynitrite, and its potential pathogenetic relevance in cells or animals exposed to various environmental toxins. For example, in vitro studies by Liu et al. concluded that peroxynitrite formation contributes to the in vitro cytotoxicity induced by peroxyacetyl nitrate, an ubiquitous air pollutant (Liu et al., 1999; Lin et al., 2000). Similarly, benzene-induced cytotoxicity may involve peroxynitrite (Tuo et al., 1998). It has been suggested that the increased production of peroxynitrite during chronic inflammation combined with benzene exposure may increase the carcinogenicity of benzene by a mechanism that includes the formation of metabolites from the chemical reaction between benzene and peroxynitrite.

Table 1
Selected cytotoxic processes initiated by peroxynitrite

Action	Mechanism
<i>On the molecular level</i>	
Cytosolic enzyme inhibition	Oxidation, nitration
Membrane pump inhibition	Oxidation, nitration
Antioxidant enzyme inhibition	Oxidation, nitration
Signal transduction pathway disturbances	Oxidation, nitration
DNA injury	Oxidation, nitration, deamination, adduct formation
Surfactant protein damage	Nitration
Metalloproteinase activation	S-glutoxidation of pro-metalloproteinases
Antioxidant enzyme depletion	Glutathione, cysteine oxidation
Inhibition of BH4-dependent enzymes	BH4 oxidation
Inhibition of NAD-dependent enzymes	NAD oxidation
Lipid peroxidation	Peroxidation, lipid peroxide chain reactions
<i>On the subcellular level</i>	
Mitochondrial dysfunction	Inhibition of cytochromes, NADH-COQ1, etc.
NAD depletion	PARP activation, direct NAD oxidation
Upregulation of adhesion receptors	NF-κB activation
DNA fragmentation	DNA injury, caspase activation
Calcium dysregulation	Dysfunctional calcium pumps and cell energetics
<i>On the cellular level</i>	
Necrosis	Mitochondrial injury, energetic collapse, oxidation, nitration, antioxidant depletion, calcium dysregulation
Apoptosis	Mitochondrial injury, DNA injury, caspase activation, signal transduction disturbances, calcium dysregulation

Peroxynitrite formation has been implicated in various forms of pulmonary injury induced by respirable mineral dusts or asbestos fibers (Choe et al., 1998; Zhu et al., 1998; Tanaka et al., 1998; Morio et al., 2001), diesel exhaust particles (Bai et al., 2001) and ozone, bleomycin (Yamazaki et al., 1998). With respect to asbestos, studies in Chinese

hamster cells have demonstrated a link between peroxynitrite formation and mutagenicity (Park and Aust, 1998).

The toxicity of nitroarene 1,3-dinitrobenzene (1,3-DNB), a cerebellar neurotoxin in rats, is an interesting example of how peroxynitrite formation can mediate cytotoxicity in response to neurotoxins. 1,3-DNB is metabolized by the NADPH-cytochrome P450 reductase in liver. In a manner similar to NADPH-cytochrome P450 reductase, the neuronal NO synthase can interact with 1,3-DNB and generate superoxide anion radical. Therefore, NO, L-citrulline, and superoxide are simultaneously produced by the neuronal NO synthase in the presence of 1,3-DNB and other nitroarenes. The simultaneous production of NO and superoxide leads to peroxynitrite, and the resulting nitrosative stress plays a role in the cerebellar neurotoxicity of 1,3-DNB (Miller, 2002). There is also evidence that peroxynitrite formation in the hepatocytes may contribute to the hepatotoxic effects of various drugs and xenobiotics (Jaeschke et al., 2002).

4. The pathophysiological relevance of peroxynitrite: reperfusion injury

There is experimental evidence demonstrating the protective effect of peroxynitrite decomposition catalysts in various models of reperfusion injury. For example, Cuzzocrea et al. studied the protective effect of the peroxynitrite decomposition catalyst 5,10,15,20-tetrakis(2,4,6-trimethyl-3,5-disulfonatophenyl)-porphyrinato iron (III) (FeTMPS) in a model of splanchnic artery occlusion (SAO) shock (Cuzzocrea et al., 2000). Administration of FeTMPS significantly reduced ischemia–reperfusion injury in the bowel, and reduced lipid and the production of peroxynitrite during reperfusion. Treatment with the peroxynitrite decomposition catalyst also markedly reduced the intensity and degree of P-selectin and ICAM-1 staining in tissue sections from SAO-shocked rats and improved survival.

As mentioned above, peroxynitrite is highly toxic to various cell types. Peroxynitrite infusion causes a reduction in myocardial contractility in

isolated perfused hearts (Schulz et al., 1995) and induces an impairment of the endothelium-dependent relaxant ability (Villa et al., 1994). Our group has investigated the effects of FP15, a novel, potent, porphyrinic peroxynitrite decomposition catalyst (Szabó et al., 2002) in various animal models of disease. We have recently demonstrated the efficacy of FP15 in a large animal model of myocardial ischemia and reperfusion. Infarct size was significantly reduced (by $\approx 40\%$) in the FP15 treated group. FP15 provided a significant suppression of tyrosine nitration (a marker of peroxynitrite reactivity) in the ischemic myocardium, further confirming its mode of action (Bianchi et al., 2002).

Taken together, the above data demonstrate that peroxynitrite is an important contributor to various forms of reperfusion injury. It will be important to test the effect of potent peroxynitrite decomposition catalysts in other forms of reperfusion injury also (including stroke, renal ischemia–reperfusion, hemorrhagic shock—which is widely considered a form of whole body ischemia–reperfusion—as well as in a variety of other disease models of ischemia and reperfusion).

5. The pathophysiological relevance of peroxynitrite: inflammation

The earliest evidence demonstrating the protective effect of the peroxynitrite decomposition catalyst 5,10,15,20-tetrakis(2,4,6-trimethyl-3,5-disulfonatophenyl)porphyrinato iron (III), was shown in the carrageenan-induced paw edema model, a model of acute inflammation in which peroxynitrite may play a major role (Salvemini et al., 1996). When tested in this system, the compound caused a dose-dependent reduction in swelling and lactate dehydrogenase release as well as a detectable shift to nitrate formation in paw tissue (Salvemini et al., 1998). Subsequent studies demonstrated the protective effect of the same compound in a model of experimental autoimmune encephalomyelitis, an animal model of the human disease multiple sclerosis. Mice receiving the peroxynitrite decomposition catalyst displayed less severe clinical disease, and less

inflammation and demyelination than control mice (Cross et al., 2001). In our own studies, we have found that FP15 also exerts protective effects in a wide variety of inflammation models, including murine models of endotoxic shock, collagen-induced arthritis and various experimental models of colitis (Mabley et al., 2002).

Taken together, the above data demonstrate that peroxynitrite is an important contributor to various forms of inflammation. It will be important to test the effect of potent peroxynitrite decomposition catalysts in other forms of inflammation also (including arthritis, endotoxic or septic shock—which is widely considered a systemic inflammatory disease—as well as in a variety of other models of inflammation).

References

- Aykac-Toker, G., Bulgurcuoglu, S., Kocak-Toker, N., 2001. Effect of peroxynitrite on glutaredoxin. *Hum. Exp. Toxicol.* 20, 373–376.
- Bai, Y., Suzuki, A.K., Sagai, M., 2001. The cytotoxic effects of diesel exhaust particles on human pulmonary artery endothelial cells in vitro: role of active oxygen species. *Free Radic. Biol. Med.* 30, 555–562.
- Barlett, D., Church, D.F., Bounds, P.L., Koppenol, W.H., 1995. The kinetics of the oxidation of L-ascorbic acid by peroxynitrite. *Free Radic. Biol. Med.* 18, 85–90.
- Beckman, J.S., Beckman, T.W., Chen, J., Marshall, P.A., Freeman, B.A., 1990. Apparent hydroxyl radical production by peroxynitrite: implication for endothelial injury from nitric oxide and superoxide. *Proc. Natl. Acad. Sci. USA* 87, 1620–1624.
- Bianchi, C., Wakiyama, H., Faro, R., Khan, T., McCully, J., Levitsky, S., Szabó, C., Sellke, F., 2002. A novel peroxynitrite decomposition catalyst (FP-15) reduces heart infarct size in a model of acute ischemia–reperfusion. *Ann. Thorac. Surg.* 74, 1201–1207.
- Choe, N., Tanaka, S., Kagan, E., 1998. Asbestos fibers and interleukin-1 upregulate the formation of reactive nitrogen species in rat pleural mesothelial cells. *Am. J. Respir. Cell Mol. Biol.* 19, 226–236.
- Cross, A.H., San, M., Stern, M.K., Keeling, R.M., Salvemini, D., Misko, T.P., 2001. A catalyst of peroxynitrite decomposition inhibits murine experimental autoimmune encephalomyelitis. *J. Neuroimmunol.* 107, 21–28.
- Cuzzocrea, S., Zingarelli, B., O'Connor, M., Salzman, A.L., Szabó, C., 1998. Effect of L-buthionine-(S, R)-sulphoximine, an inhibitor of gamma-glutamylcysteine synthetase on peroxynitrite- and endotoxic shock-induced vascular failure. *Br. J. Pharmacol.* 123, 525–537.
- Cuzzocrea, S., Misko, T.P., Costantino, G., Mazzon, E., Micali, A., Caputi, A.P., Macarthur, H., Salvemini, D., 2000. Beneficial effects of peroxynitrite decomposition catalyst in a rat model of splanchnic artery occlusion and reperfusion. *FASEB J.* 14, 1061–1072.
- Douki, T., Cadet, J., 1996. Peroxynitrite mediated oxidation of purine bases of nucleosides and isolated DNA. *Free Radic. Res.* 24, 369–380.
- Eiserich, J.P., Hristova, M., Cross, C.E., Jones, A.D., Freeman, B.A., Halliwell, B., van der Vliet, A., 1998. Formation of nitric oxide-derived inflammatory oxidants by myeloperoxidase in neutrophils. *Nature* 391, 393–397.
- Goldstein, S., Czapski, G., 1995. The reaction of NO[•] with O₂^{•-} and HO₂[•]: a pulse radiolysis study. *Free Radic. Biol. Med.* 19, 505–510.
- Goldstein, S., Czapski, G., 2000. Reactivity of peroxynitrite versus simultaneous generation of (*NO and O(2)(*)(–) toward NADH. *Chem. Res. Toxicol.* 13, 736–741.
- Greenacre, S.A., Ischiropoulos, H., 2001. Tyrosine nitration: localization, quantification, consequences for protein function and signal transduction. *Free Radic. Res.* 34, 541–581.
- Groves, J.T., 1999. Peroxynitrite: reactive, invasive and enigmatic. *Curr. Opin. Chem. Biol.* 3, 226–235.
- Grune, T., Blasig, I.E., Sitte, N., Roloff, B., Haseloff, R., Davies, K.J., 1998. Peroxynitrite increases the degradation of aconitase and other cellular proteins by proteasome. *J. Biol. Chem.* 273, 10857–10862.
- Halfpenny, E., Robinson, P.L., 1992. Pernitrous acid: the reaction between hydrogen peroxide and nitrous acid, and the properties of an intermediate product. *J. Chem. Soc.* 1952, 928–938.
- Halliwell, B., 1997. What nitrates tyrosine? Is nitrotyrosine specific as a biomarker of peroxynitrite formation in vivo? *FEBS Lett.* 11, 157–160.
- Hausladen, A., Fridovich, I., 1994. Superoxide and peroxynitrite inactivate aconitases, but nitric oxide does not. *J. Biol. Chem.* 269, 29405–29408.
- Ischiropoulos, H., Zhu, L., Chen, J., Tsai, M., Martin, J.C., Smith, C.D., Beckman, J.S., 1992. Peroxynitrite-mediated tyrosine nitration catalyzed by superoxide dismutase. *Arch. Biochem. Biophys.* 298, 431–437.
- Jaeschke, H., Gores, G.J., Cederbaum, A.I., Hinson, J.A., Pessayre, D., Lemasters, J.J., 2002. Mechanisms of hepatotoxicity. *Toxicol. Sci.* 65, 166–176.
- Kerry, N., Rice-Evans, C., 1998. Peroxynitrite oxidises catechols to *o*-quinones. *FEBS Lett.* 437, 167–171.
- Klebl, B.M., Ayoub, A.T., Pette, D., 1998. Protein oxidation, tyrosine nitration, and inactivation of sarcoplasmic reticulum Ca²⁺-ATPase in low-frequency stimulated rabbit muscle. *FEBS Lett.* 422, 381–384.
- Konorev, E.A., Hogg, N., Kalyanaraman, B., 1998. Rapid and irreversible inhibition of creatine kinase by peroxynitrite. *FEBS Lett.* 427, 171–174.
- Lin, J.K., Chen, K.J., Liu, G.Y., Chu, Y.R., Lin-Shiau, S.Y., 2000. Nitration and hydroxylation of aromatic amino acid and guanine by the air pollutant peroxyacetyl nitrate. *Chem. Biol. Interact.* 127, 219–236.

- Liu, G.Y., Chen, K.J., Lin-Shiau, S.Y., Lin, J.K., 1999. Peroxyacetyl nitrate-induced apoptosis through generation of reactive oxygen species in HL-60 cells. *Mol. Carcinog.* 25, 196–206.
- Liu, Z., Martin, L.J., 2001. Motor neurons rapidly accumulate DNA single-strand breaks after in vitro exposure to nitric oxide and peroxynitrite and in vivo axotomy. *J. Comp. Neurol.* 432, 35–60.
- Mabley, J.G., Liaudet, L., Pacher, P., Southan, G.J., Salzman, A.L., Szabó, C., 2002. Beneficial effects of the peroxynitrite decomposition catalyst FP15 in murine models of arthritis and colitis. *Mol. Med.* 8, 581–590.
- MacMillan-Crow, L.A., Crow, J.P., Thompson, J.A., 1998. Peroxynitrite-mediated inactivation of manganese superoxide dismutase involves nitration and oxidation of critical tyrosine residues. *Biochemistry* 37, 1613–1622.
- Matata, B.M., Galinanes, M., 2001. Peroxynitrite is an essential component of cytokine production mechanism in human monocytes through modulation of NF- κ B DNA-binding activity. *J. Biol. Chem.* 277, 2330–2335.
- Mihm, M.J., Yu, F., Carnes, C.A., Reiser, P.J., McCarthy, P.M., Van Wagoner, D.R., Bauer, J.A., 2001. Impaired myofibrillar energetics and oxidative injury during human atrial fibrillation. *Circulation* 104, 174–180.
- Miles, A.M., Bohle, D.S., Glassbrenner, P.A., Hansert, B., Wink, D.A., Grisham, M.B., 1996. Modulation of superoxide-dependent oxidation and hydroxylation reactions by nitric oxide. *J. Biol. Chem.* 271, 40–47.
- Miller, R.T., 2002. Dinitrobenzene-mediated production of peroxynitrite by neuronal nitric oxide synthase. *Chem. Res. Toxicol.* 15, 927–934.
- Milstien, S., Katusic, Z., 1999. Oxidation of tetrahydrobiopterin by peroxynitrite: implications for vascular endothelial function. *Biochem. Biophys. Res. Commun.* 263, 681–684.
- Mohr, S., Stamler, J.S., Brune, B., 1994. Mechanism of covalent modification of glyceraldehyde-3-phosphate dehydrogenase at its active site thiol by nitric oxide, peroxynitrite and related nitrosating agents. *FEBS Lett.* 348, 223–227.
- Morio, L.A., Hooper, K.A., Brittingham, J., Li, T.H., Gordon, R.E., Turpin, B.J., Laskin, D.L., 2001. Tissue injury following inhalation of fine particulate matter and hydrogen peroxide is associated with altered production of inflammatory mediators and antioxidants by alveolar macrophages. *Toxicol. Appl. Pharmacol.* 177, 188–199.
- Muriel, P., Sandoval, G., 2000. Nitric oxide and peroxynitrite anion modulate liver plasma membrane fluidity and Na(+)/K(+)-ATPase activity. *Nitric Oxide* 4, 333–342.
- Okamoto, T., Akaike, T., Sawa, T., Miyamoto, Y., van der Vliet, A., Maeda, H., 2001. Activation of matrix metalloproteinases by peroxynitrite-induced protein S-glutathiolation via disulfide S-oxide formation. *J. Biol. Chem.* 276, 29596–29602.
- Padmaja, S., Squadrito, G.L., Lemercier, J.N., Cueto, R., Pryor, W.A., 1996. Rapid oxidation of DL-selenomethionine by peroxynitrite. *Free Radic. Biol. Med.* 21, 317–324.
- Park, S.H., Aust, A.E., 1998. Participation of iron and nitric oxide in the mutagenicity of asbestos in hgprt⁻, gpt⁺ Chinese hamster V79 cells. *Cancer Res.* 58, 1144–1148.
- Pryor, W., Squadrito, G., 1996. The chemistry of peroxynitrite: a product from the reaction of nitric oxide with superoxide. *Am. J. Physiol.* 268, L699–L722.
- Radi, R., Beckman, J.S., Bush, K.M., Freeman, B.A., 1991. Peroxynitrite-induced membrane lipid peroxidation: the cytotoxic potential of superoxide and nitric oxide. *Arch. Biochem. Biophys.* 288, 481–487.
- Ramezani, M.S., Padmaja, S., Koppenol, W.H., 1996. Nitration and hydroxylation of phenolic compounds by peroxynitrite. *Chem. Res. Toxicol.* 9, 232–237.
- Rojas-Walker, T., Tamir, S., Ji, H., Wishnok, J.S., Tannenbaum, S.R., 1995. Nitric oxide induces oxidative damage in addition to deamination in macrophage DNA. *Chem. Res. Toxicol.* 8, 473–477.
- Rubbo, H., Radi, R., Trujillo, M., Telleri, R., Kalyanaraman, B., Barnes, S., Kirk, M., Freeman, B.A., 1994. Nitric oxide regulation of superoxide and peroxynitrite-dependent lipid peroxidation. Formation of novel nitrogen-containing oxidized lipid derivatives. *J. Biol. Chem.* 269, 26066–26075.
- Salvemini, D., Wang, Z.-Q., Bourdon, D.M., Stern, M.K., Currie, M.G., Manning, P.T., 1996. Evidence of peroxynitrite involvement in the carrageenan-induced rat paw edema. *Eur. J. Pharmacol.* 303, 217–220.
- Salvemini, D., Wang, Z.-Q., Stern, M.K., Currie, M.G., Misko, T.P., 1998. Peroxynitrite decomposition catalysts: therapeutics for peroxynitrite-mediated pathology. *Proc. Natl. Acad. Sci. USA* 95, 2659–2663.
- Schulz, R., Dodge, K.L., Lopasschuk, G.D., Clanachan, A.S., 1995. Peroxynitrite depresses cardiac efficiency in the isolated working heart. *Circulation* 92, I-45.
- Szabó, C., 1996. The role of peroxynitrite in the pathophysiology of shock, inflammation and ischemia-reperfusion injury. *Shock* 6, 79–88.
- Szabó, C., Ohshima, H., 1997. DNA injury induced by peroxynitrite. *Nitric Oxide Biol. Chem.* 1, 323–385.
- Szabó, C., Zingarelli, B., O'Connor, M., Salzman, A.L., 1996. DNA strand breakage, activation of poly-ADP ribosyl synthetase, and cellular energy depletion are involved in the cytotoxicity in macrophages and smooth muscle cells exposed to peroxynitrite. *Proc. Natl. Acad. Sci. USA* 93, 1753–1758.
- Szabó, C., Cuzzocrea, S., Zingarelli, B., O'Connor, M., Salzman, A.L., 1997. Endothelial dysfunction in endotoxic shock: importance of the activation of poly (ADP ribose) synthetase (PARS) by peroxynitrite. *J. Clin. Invest.* 100, 723–735.
- Szabó, C., Mabley, J., Moeller, S.M., Shimanovich, R., Pacher, P., Virág, L., Soriano, F.G., VanDuzer, J.H., Williams, W., Salzman, A.L., Groves, J.T., 2002. Pathogenetic role of peroxynitrite in the development of diabetes and diabetic vascular complications: studies with FP15, a novel potent peroxynitrite decomposition catalyst. *Mol. Med.* 8, 571–580.

- Tanaka, S., Choe, N., Hemenway, D.R., Zhu, S., Matalon, S., Kagan, E., 1998. Asbestos inhalation induces reactive nitrogen species and nitrotyrosine formation in the lungs and pleura of the rat. *J. Clin. Invest.* 102, 445–454.
- Tuo, J., Wolff, S.P., Loft, S., Poulsen, H.E., 1998. Formation of nitrated and hydroxylated aromatic compounds from benzene and peroxynitrite, a possible mechanism of benzene genotoxicity. *Free Radic. Res.* 28, 369–375.
- Villa, L.M., Salas, E., Darley-Usmar, M., Radomski, M.W., Moncada, S., 1994. Peroxynitrite induces both vasodilatation and impaired vascular relaxation in the isolated perfused rat heart. *Proc. Natl. Acad. Sci. USA* 91, 12383–12387.
- Virág, L., Scott, G.S., Cuzzocrea, S., Marmer, D., Salzman, A.L., Szabó, C., 1998. Peroxynitrite-induced thymocyte apoptosis: the role of caspases and poly (ADP-ribose) synthetase (PARS) activation. *Immunology* 94, 345–355.
- Virág, L., Szabó, C., 2002. The therapeutic potential of PARP inhibition. *Pharmacol. Rev.* 54, 375–429.
- Yamakura, F., Taka, H., Fujimura, T., Murayama, K., 1998. Inactivation of human manganese-superoxide dismutase by peroxynitrite is caused by exclusive nitration of tyrosine 34 to 3-nitrotyrosine. *J. Biol. Chem.* 273, 14085–14089.
- Yamazaki, C., Hoshino, J., Sekiguchi, T., Hori, Y., Miyauchi, S., Mizuno, S., Horie, K., 1998. Production of superoxide and nitric oxide by alveolar macrophages in the bleomycin-induced interstitial pneumonia mice model. *Jpn J. Pharmacol.* 78, 69–73.
- Zhu, S., Manuel, M., Tanaka, S., Choe, N., Kagan, E., Matalon, S., 1998. Contribution of reactive oxygen and nitrogen species to particulate-induced lung injury. *Environ. Health Perspect.* 106 (Suppl. 5), 1157–1163.
- Zhuang, S., Simon, G., 2000. Peroxynitrite-induced apoptosis involves activation of multiple caspases in HL-60 cells. *Am. J. Physiol. Cell Physiol.* 279, C341–C351.
- Zingarelli, B., O'Connor, M., Wong, H., Salzman, A.L., Szabó, C., 1996. Peroxynitrite-mediated DNA strand breakage activates poly-ADP ribosyl synthetase and causes cellular energy depletion in macrophages stimulated with bacterial lipopolysaccharide. *J. Immunol.* 156, 350–358.
- Zingarelli, B., Salzman, A.L., Szabó, C., 1998. Genetic disruption of poly (ADP-ribose) synthetase inhibits the expression of *P*-selectin and intercellular adhesion molecule-1 in myocardial ischemia/reperfusion injury. *Circ. Res.* 83, 85–94.
- Zouki, C., Zhang, S.L., Chan, J.S., Filep, J.G., 2001. Peroxynitrite induces integrin-dependent adhesion of human neutrophils to endothelial cells via activation of the Raf-1/MEK/Erk pathway. *FASEB J.* 15, 25–27.