

## INTERACTIONS BETWEEN SUPEROXIDE AND NITRIC OXIDE: IMPLICATIONS IN DNA DAMAGE AND MUTAGENESIS

David Jourdeuil, David Kang and Matthew B. Grisham<sup>1</sup>

Department of Molecular and Cellular Physiology, Louisiana State University Medical Center, Shreveport, LA 71130, USA

### TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Nitric oxide and N-nitrosation reactions
4. Superoxide-nitric oxide interactions: oxidative reactions
5. Superoxide, Fenton chemistry and nitric oxide
6. Conclusion
7. Acknowledgments
8. References

### 1. ABSTRACT

Chronic inflammation is known to be associated with enhanced production of both nitric oxide (NO) and reactive oxygen species such as superoxide ( $O_2^-$ ) and hydrogen peroxide ( $H_2O_2$ ). Patients with long-standing ulcerative colitis are also known to be at increased risk of developing colorectal cancer. Although NO and reactive oxygen intermediates alone have been known to damage DNA and to promote a wide array of mutagenic reactions, there is increasing evidence to suggest that the interaction between  $O_2^-$  and NO may dictate the type of mutagenic reactions produced at sites where both these free radicals are produced. In the absence of  $O_2^-$ , NO will engage in nitrosative chemistry to yield stable N-nitrosamine derivatives of secondary amines and promote nitrosative deamination of DNA bases. As the flux of  $O_2^-$  is increased, nitrosation reactions are suppressed and oxidative chemistry is enhanced. Thus, depending upon the fluxes of each radical either nitrosation or oxidation chemistry may predominate. The fundamental understanding between  $O_2^-$  and NO may provide new insight in the mechanisms responsible for inflammation-induced mutagenesis.

### 2. INTRODUCTION

Active episodes of ulcerative colitis are characterized by infiltration of large numbers of phagocytic leukocytes into the mucosal interstitium. This enhanced inflammatory infiltrate is accompanied

by extensive injury to the mucosa. A growing body of clinical and experimental data suggests that severe long-standing inflammation of the colon is associated with an increase risk of colorectal cancer (1-3). In addition, investigators have shown that inflammation enhances the formation of colonic tumors in experimental animals given known carcinogens (4,5). Despite these studies, the mechanisms by which inflammation promotes tumor formation remain poorly understood. It has been suggested that certain leukocyte-derived products may act as endogenous carcinogens or tumor promoters *in vivo* (1). Recent studies have shown that neutrophils and macrophages are capable of generating the free radical nitric oxide (NO) via an L-arginine-dependent pathway (6-9). Nitric oxide is unstable in the presence of molecular oxygen and will rapidly and spontaneously decompose to yield a variety of nitrogen oxides that are known to be potent nitrosating agents that will N-nitrosate primary and secondary amines to yield carcinogenic nitrosamines (10). Secondary nitrosamines require metabolic activation to yield alkylating agents that have been shown to activate certain oncogenes via the covalent modification of certain DNA bases (11). Nitrosative deamination of primary aromatic amines has been another suggested pathway by which NO-derived N-nitrosating agents produce transition and transversion mutations (12). The objective of this review is to discuss the chemical interactions between superoxide ( $O_2^-$ ) and NO and to examine how these interactions may be involved in inflammation-induced DNA damage and mutagenesis.

### 3. NITRIC OXIDE AND N-NITROSATION REACTIONS

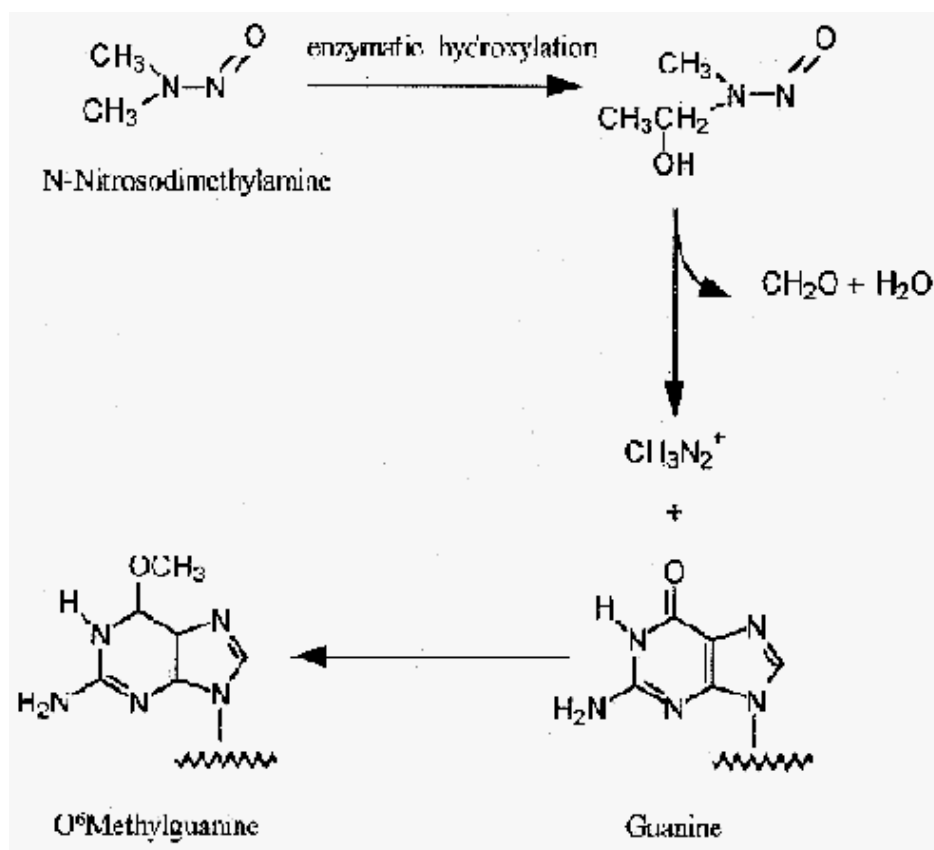
Exposure of critical genes to mutagenic conditions increases the probability of tumor development. Chronic inflammation is one such environment which promotes

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<sup>1</sup> To whom correspondence should be addressed, at:

Department of Molecular and Cellular Physiology LSU Medical Center 1501 Kings Highway Shreveport, LA 71130 USA Tel.: (318) 675-6021 Fax: (318) 675-4156 Email: mgrish@lsuic.edu

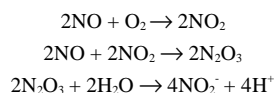


**Figure 1:** Nitrosamine-mediated alkylation of DNA bases. Secondary nitrosamines such as N-nitrosodimethylamine induce point mutations by alkylation of DNA bases such as guanine to form O<sup>6</sup>methylguanine residues.

malignant transformation. First, tissues neighboring inflammatory foci undergo increased cell division

Consequently, mutagenic effects associated with chronic inflammation can become multiplicative, as the chance of mis-repair and DNA exposure increase (13). Secondly, certain leukocyte-derived metabolites and inflammatory mediators cause genomic damage, thereby increasing the probability of nicks, deletions, and point mutations (14).

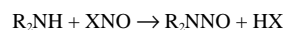
In situations of chronic inflammation, target cells may be exposed to large amounts of NO (as much as 10<sup>4</sup> molecules/cell/s) (15). Nitric oxide will autooxidize in the presence of molecular oxygen (O<sub>2</sub>) to yield a variety of nitrogen oxides:



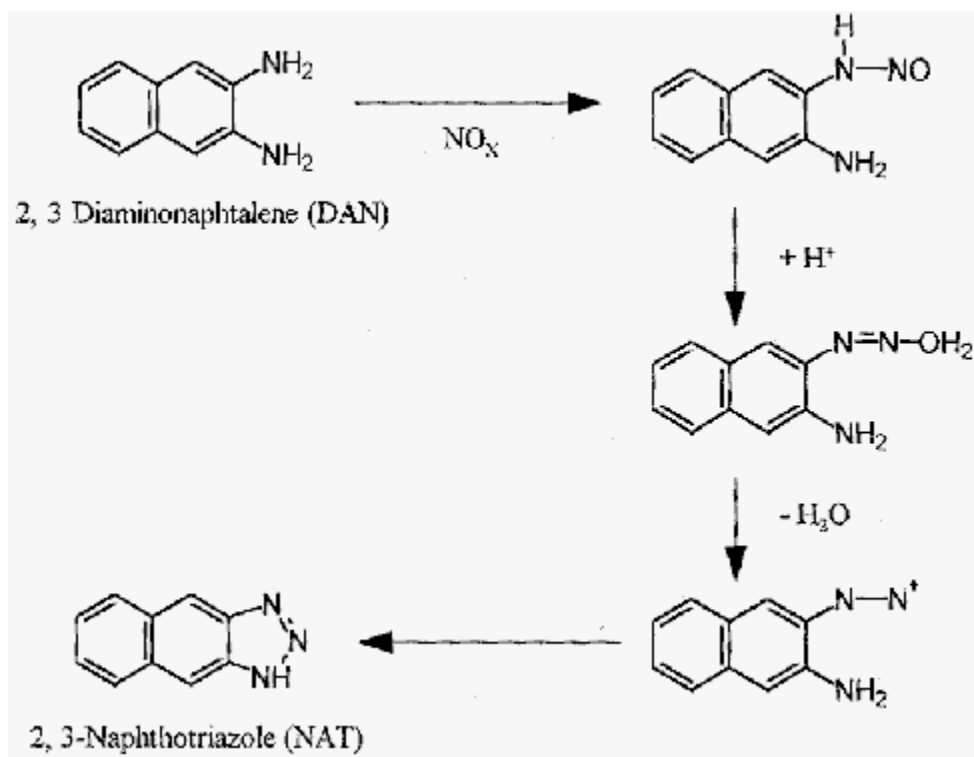
where NO<sub>2</sub>, N<sub>2</sub>O<sub>3</sub>, and NO<sub>2</sub><sup>-</sup> represent nitrogen dioxide, dinitrogen trioxide, and nitrite, respectively. Of these, NO<sub>2</sub> and N<sub>2</sub>O<sub>3</sub> have drawn particular interest due to their ability to N-nitrosate certain nucleophilic substrates such as primary and secondary amines (16).

Such nitrosating species have been shown to promote the nitrosative deamination of primary aromatic amines, including purines and pyrimidines, via the formation of nitrosamine and diazonium ion intermediates (17). Deamination of cytosine, methyl cytosine, adenine, or guanine results in the formation of uracil, thymine, hypoxanthine, and xanthine respectively. Base conversion of cytosine and methyl cytosine can lead ultimately to a base pair substitution mutation, while deamination of adenine and guanine results in transversion mutations. Moreover, the instability of hypoxanthine and xanthine in the DNA structure leads to rapid depurination and consequent single strand breaks. Even crosslinking with other nucleic acids or proteins have been suggested via reaction of a nucleophilic site on an adjacent macromolecule and the diazonium ion of the modified base (18).

Nitrosation of secondary aliphatic and aromatic amines can also produce potentially carcinogenic nitrosamines. Secondary nitrosamines are more stable than their primary amine counterparts:



## Nitric oxide and superoxide in DNA damage



**Figure 2:** N-nitrosation of 2,3-diaminonaphthalene (DAN) to yield 2,3-naphthotriazole (NAT) by an NO-derived N-nitrosating agent (NO<sub>x</sub>).

Such nitrosamines, like many chemical carcinogens, are thought to promote mutagenesis and carcinogenesis via their ability to alkylate specific sites in DNA. For example, these types of nitrosamines undergo enzymatic alpha-hydroxylation. The alpha-hydroxy nitrosamine decomposes to form the alkyl diazonium ion and free alkyl carbocation (Fig 1). The alkyl diazonium salt or carbocations then can react with nucleophilic sites in DNA.

To date, alkylation of DNA has been noted on the ring-nitrogen positions in the bases (adenine, guanine, cytosine, thymine), the oxygen atoms of hydroxyl or carbonyl groups (guanine, thymine, and cytosine) as well as on the phosphate groups (19).

Thus, the limitation of NO-mediated genomic damage, rests primarily on the localized diffusion of the small molecule, the degree of reactivity and nitrosation, and ultimately the cells' replicative and DNA repair machinery. However, even in the latter case, DNA repair proteins such as O6-methylguanine-DNA-methyltransferase and Fpg,

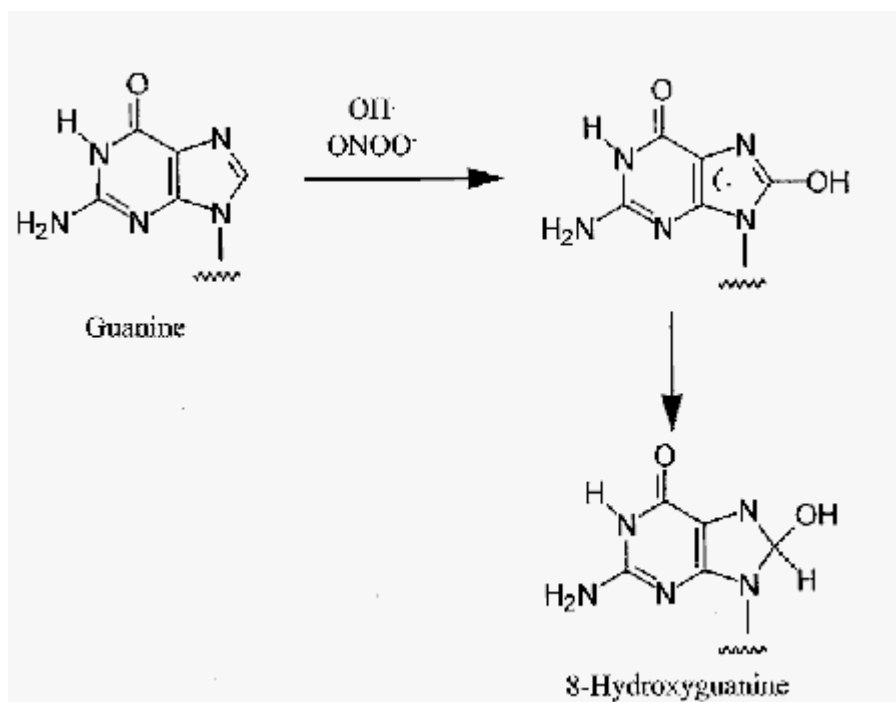
have been shown to be inhibited by NO-derived nitrosating agents such as N<sub>2</sub>O<sub>3</sub> *in vitro* and *in vivo* (20, 21).

Coincident with the sustained overproduction of NO, inflammatory foci are also sites of enhanced production of

reactive oxygen species, such as O<sub>2</sub><sup>-</sup> and H<sub>2</sub>O<sub>2</sub>. Because O<sub>2</sub><sup>-</sup> is known to rapidly react with NO, it was of interest to determine whether this reactive oxygen species may modulate NO-dependent N-nitrosation of primary aromatic amines. Consequently, we have used 2,3-diaminonaphthalene (DAN) as a model to study the effect of O<sub>2</sub><sup>-</sup> on the NO-dependent N-nitrosation of this primary aromatic amine. DAN is N-nitrosated by nitrosating agents derived from NO to yield its highly fluorescent triazole derivative 1-naphtho(2,3)triazole (NAT) (Fig 2).

We have demonstrated that the addition of a O<sub>2</sub><sup>-</sup> generator such as hypoxanthine/xanthine oxidase virtually eliminated the NO-dependent N-nitrosation of DAN. Inhibition was maximal when equimolar fluxes of NO and O<sub>2</sub><sup>-</sup> were produced (17). We also noted that this inhibition was reversed by the addition of superoxide dismutase, but not catalase suggesting that O<sub>2</sub><sup>-</sup> and not H<sub>2</sub>O<sub>2</sub> was responsible for the inhibition. We proposed that at equimolar fluxes, O<sub>2</sub><sup>-</sup> reacts rapidly with NO to generate products such as peroxynitrite (ONOO<sup>-</sup>) or derivatives thereof - which have only a limited ability to N-nitrosate amino compounds (16,17). Although we found that O<sub>2</sub><sup>-</sup> inhibits the potentially mutagenic N-nitrosation of primary and secondary amines, the formation of ONOO<sup>-</sup> could conceivably promote oxidative (and nitrate) modifications of DNA bases, switching NO-mediated DNA

## Nitric oxide and superoxide in DNA damage



**Figure 3:** Hydroxyl radical (OH) and peroxynitrite (ONOO)-mediated oxidation of guanine.

damage from a nitrosative to a more oxidative pattern of mutagenic reactions (22).

### 4. SUPEROXIDE-NITRIC OXIDE INTER-ACTIONS: OXIDATIVE REACTIONS

Nitric oxide reacts with  $O_2^-$  to yield  $ONOO^-$  with a second order rate constant of  $6.7 \times 10^9 \text{ M}^{-1}\cdot\text{s}^{-1}$  (23). Once formed,  $ONOO^-$  is protonated resulting in its rapid decomposition to nitrate ( $NO_3^-$ ) in the absence of any oxidizable substrate. Therefore,  $O_2^-$  will rapidly inhibit NO-mediated N-nitrosation reactions in favor of reactions catalyzed by  $ONOO^-$ .

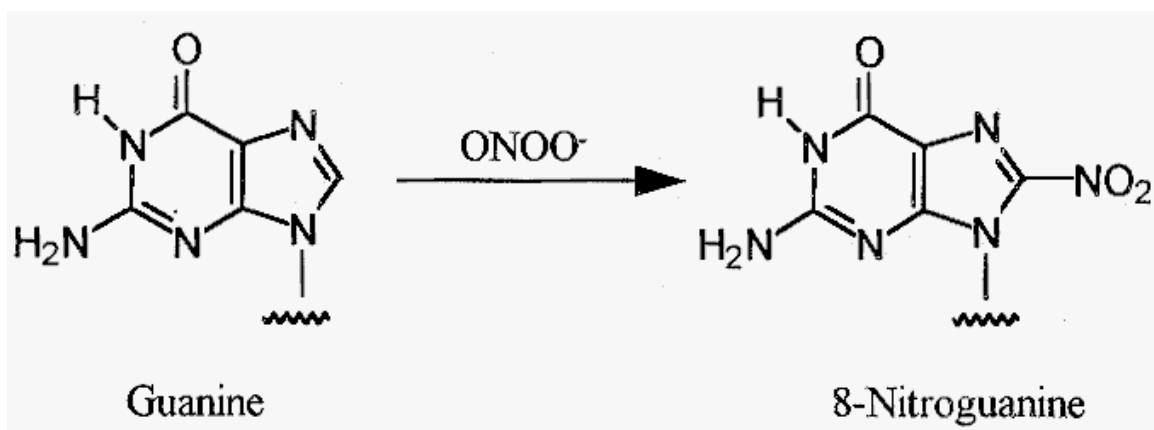
Peroxynitrite exists in equilibrium with its conjugate acid peroxynitrous acid ( $ONOOH$ , pKa 6.6):



Peroxynitrous acid is very unstable and at physiological pH decomposes to form derivatives with potent oxidizing properties. This may involve the homolysis of  $ONOOH$  to nitrogen dioxide radical ( $NO_2^\cdot$ ) and hydroxyl radical (OH) within a solvent cage, the two free radicals diffusing out of the solvent cage to mediate oxidation reactions (24). Hydroxyl radical is an extremely reactive species, interacting with virtually all biomolecules at diffusion limited rates ( $\sim 10^7 - 10^9 \text{ M}\cdot\text{sec}^{-1}$ ) (25). Nitrogen dioxide can initiate lipid peroxidation and N-nitrosate certain amines to yield nitrosamines (26). However, a second more probable

mechanism that may account for the oxidative properties of  $ONOO^-$  is suggested to involve the formation of an activated isomer of peroxynitrous acid,  $ONOOH^*$  which would possess  $NO_2^\cdot$  and OH-like properties (27). From the standpoint of biologically relevant reactions,  $ONOO^-$  is a potent oxidizing, hydroxylating, and nitrating agent. In regard to DNA modifications,  $ONOO^-$  has been found to oxidize and nitrate isolated DNA resulting in DNA strand breaks (28).

Little is known regarding the detailed reactions of  $ONOO^-$  with DNA. Because of the multiplicity of DNA modifications produced during oxidative reactions, it has been difficult to establish the specificity of mutations engendered by individual oxidants such as  $ONOO^-$ . Among oxidative-mediated DNA base modifications, a major product is 8-hydroxydeoxyguanosine (8-OHdG) (Fig 3) (29). The occurrence of this alteration has been associated with a number of conditions leading to increased oxidative stress including higher basal metabolic rate, gamma-irradiation, and hydrogen peroxide-mediated oxidative stress (30). Nitric oxide and iron have also been implicated in the formation of 8-OHdG in asbestos-treated human lung epithelial cells (31). Peroxynitrite also mediates the oxidation of deoxyguanosine (32). In all of those conditions, the formation of 8-OHdG might lead to mutations by inducing misreading of the base itself and of the adjacent bases (29) which may represent an important source of mutations (33). Peroxynitrite induces G:C to T:A mutations for the supF gene in *E. coli* and in human AD293 cells (34).



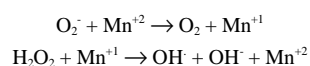
**Figure 4:** Peroxynitrite (ONOO<sup>-</sup>)-mediated nitration of guanine to form 8-nitroguanine.

In addition to oxidative reactions, recent data suggest that the interaction of ONOO<sup>-</sup> with DNA results in the nitration of guanine to form 8-nitroguanine (Fig 4) (35). This modification is potentially mutagenic, the epurination of 8-nitroguanine yielding apurinic sites with the resultant possibility of G:C to T:A transversions (36). However, whether ONOO<sup>-</sup> mediates such DNA damage in cells and tissues is yet to be determined. The identification of 8-nitroguanine as a marker of ONOO<sup>-</sup>-induced nitration may represent an important tool for the detection of such alteration *in vivo*.

It is important to note that the evaluation of ONOO<sup>-</sup>-mediated mutagenic properties has been assessed *in vitro* using bolus amounts of chemically generated oxidant. However, it is becoming increasingly evident that the formation of ONOO<sup>-</sup> at sites where both O<sub>2</sub><sup>-</sup> and NO are produced may depend upon the relative fluxes of NO and O<sub>2</sub><sup>-</sup>. Using the hypoxanthine/ xanthine oxidase system to generate both O<sub>2</sub><sup>-</sup> and H<sub>2</sub>O<sub>2</sub> and the spermine/NO adduct to generate various fluxes of NO, we found that the simultaneous production of equimolar fluxes of O<sub>2</sub><sup>-</sup> and NO dramatically increased the oxidation of the oxidant-sensitive probe dihydrorhodamine (DHR) (22). The oxidation of DHR was inhibited by superoxide dismutase but not catalase suggesting that O<sub>2</sub><sup>-</sup> and not NO interacted with NO to form ONOO<sup>-</sup> (25). In these same experiments, ONOO<sup>-</sup>-mediated oxidation of DHR was quenched by excess formation of either NO or O<sub>2</sub><sup>-</sup> suggesting that excess production of either radical may act as an endogenous modulator of ONOO<sup>-</sup> chemistry. Subsequent experiments demonstrated that NO (or O<sub>2</sub><sup>-</sup>) interacted with and decomposed ONOO<sup>-</sup>. Although this hypothesis suggests that NO and O<sub>2</sub><sup>-</sup> may modulate steady state concentrations of ONOO<sup>-</sup>, there has yet to be any direct evidence demonstrating such modulation of ONOO<sup>-</sup>-mediated oxidative and/or nitrating reactions under physiological conditions.

## 5. SUPEROXIDE, FENTON CHEMISTRY AND NITRIC OXIDE

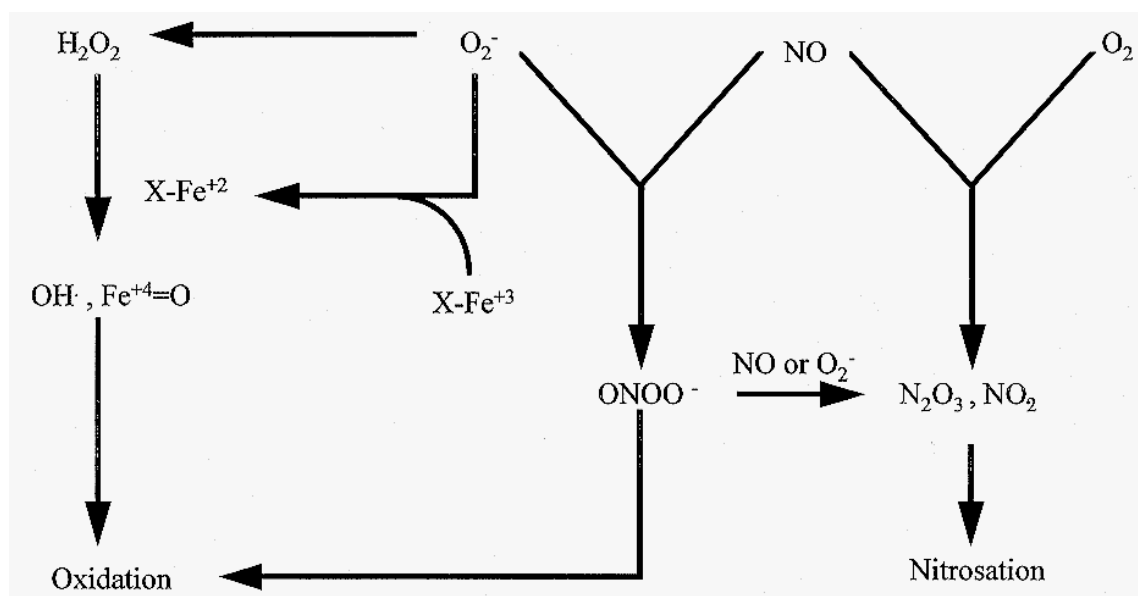
In general, O<sub>2</sub><sup>-</sup> per se is not thought to be highly toxic to cells and tissues since it is a better reducing agent than an oxidant (37). However, O<sub>2</sub><sup>-</sup> will rapidly dismutate to form H<sub>2</sub>O<sub>2</sub>. Although H<sub>2</sub>O<sub>2</sub> is an oxidizing agent, most of the H<sub>2</sub>O<sub>2</sub>-dependent oxidizing activity is mediated by secondary radicals such as OH<sup>•</sup> generated from metal (Mn)-catalyzed reactions. It is known that O<sub>2</sub><sup>-</sup> and H<sub>2</sub>O<sub>2</sub> interact with chelates of iron or copper to yield the potent oxidant OH<sup>•</sup> or OH-like species via the superoxide-driven Fenton reaction:



The interaction of free radicals derived from superoxide-driven Fenton reactions with DNA has received considerable interest (38). Recent reports have focused on the ability of copper to participate in mutagenic reactions *in vivo* via Fenton-catalyzed reactions. Copper is an important structural metal in chromatin (39) that in fact induces more DNA bases damage in the presence of H<sub>2</sub>O<sub>2</sub> than does iron (40).

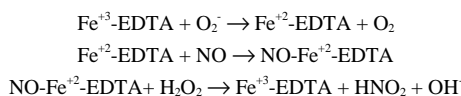
There is now evidence to suggest that NO may modulate Fenton-driven oxidative reactions. We have recently investigated the ability of different fluxes of nitric oxide to modulate iron complex (10) and hemoprotein-catalyzed oxidative reactions (41). We found that generation of O<sub>2</sub><sup>-</sup> and H<sub>2</sub>O<sub>2</sub> in the presence of 5 μM Fe<sup>+3</sup>-EDTA stimulated oxidation of DHR producing approximately 15 μM rhodamine (10). Catalase and superoxide dismutase were both effective at inhibiting this classic Fenton-driven reaction. Addition of NO increased DHR oxidation such that a 2:1 NO/ O<sub>2</sub><sup>-</sup> ratio increased DHR oxidation by 30%. As the ratio NO/ O<sub>2</sub><sup>-</sup> further increased to 4.5, DHR oxidation was reduced by 40%. Kanner *et al.* (42) proposed that NO may inhibit iron-mediated

## Nitric oxide and superoxide in DNA damage



**Figure 5:** Modulation of oxidative and nitrosative reactions by NO and  $O_2^-$ .

oxidative reactions by forming nitrosyl complexes with ferrous iron:



The efficiency of such interaction may explain the decrease in DHR oxidation obtained in the presence of high fluxes of NO. We have also assessed the ability of myoglobin to oxidize DHR in the presence or the absence of  $O_2^-$ ,  $H_2O_2$  and/or NO (33). In the presence of equimolar fluxes of  $H_2O_2$  and  $O_2^-$ , the addition of myoglobin dramatically enhanced DHR oxidation via the formation of ferryl ( $Fe^{4+}$ ) myoglobin. This oxidative reaction was as expected inhibited by catalase but not Superoxide dismutase. Addition of NO to this system further enhanced DHR oxidation which was inhibited by superoxide dismutase suggesting that  $O_2^-$  reacted with NO to form a potent oxidant such as peroxynitrite in addition to the  $H_2O_2$ -dependent formation of ferryl myoglobin. Further increases in NO flux dramatically inhibited DHR oxidation which was found to be due to the NO-mediated reduction of ferryl heme to the met form. Taken together, these data suggest that NO may enhance or inhibit iron complex and hemoprotein-catalyzed oxidative reactions depending upon the relative fluxes of  $O_2^-$ ,  $H_2O_2$  and NO. In accordance with our results, Pacelli *et al.* have shown that NO can inhibit DNA strand breaks induced by  $H_2O_2$  and certain transition metals (43).

## 6. CONCLUSION

It is well known that chronic intestinal inflammation is associated with an increased risk of

malignancy (1-3). This pathological condition might represent one prime example in which the chemistry we have described may play an important role. Indeed, the phagocytic leukocytes that accumulate within the chronically inflamed colon produce large amounts of  $O_2^-$  and NO which may mediate mutagenesis and possibly malignant transformation (1). Thus, the fundamental understanding of the interplay between  $O_2^-$  and NO may give us new insights into the chemical role that these two free radicals play during mutagenesis. In the absence of  $O_2^-$ , NO will N-nitrosate certain primary and secondary amines to yield potentially mutagenic nitrosamine intermediates (see Fig 5). In the presence of both NO and  $O_2^-$ , N-nitrosation chemistry may be suppressed but oxidation reactions may predominate.

## 7. ACKNOWLEDGMENTS

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## 8. REFERENCES

- Weitzman, S.A. & L.I. Gordon: Inflammation and cancer: role of phagocyte-generated oxidants in carcinogenesis. *Blood* 76, 655-663 (1990)
- Collins, R.H. Feldman, M. & J.S. Fordtran: Colon cancer, dysplasia and surveillance in patients with ulcerative colitis: a critical review. *N Engl J Med* 316, 1654-1658 (1987)
- Korelitz B.I. Carcinoma of the intestinal tract in Crohn's disease: results of a survey conducted by the National

## Nitric oxide and superoxide in DNA damage

- Foundation for Ileitis and Colitis. *Am J Gastroenterol* 78,44-46 (1983)
4. Pozharisski, K.M. The significance of non-specific injury for colon carcinogenesis in rats. *Cancer Res* 35, 3824 (1975)
  5. Chester, J.F. Gaissert, H.A. Ross, J.S. Malt, R.A. & S.A. Weitzman: Colonic cancer induced by 1,2-dimethylhydrazine: promotion by experimental colitis. *Br J Cancer* 59, 704-705 (1989)
  6. Moncada, S. Palmer, R.M.J. & E.A. Higgs: Nitric oxide: physiology, pathophysiology, and pharmacology. *J Pharmacol Exp Ther* 43, 109-142 (1991)
  7. McCall, T.B. Boughton-Smith, N.K. Palmer, R.M.J. Whittle, B.J.R. & S. Moncada: Synthesis of nitric oxide from L-arginine by neutrophils, release and interaction with superoxide anion. *Biochem J* 261, 293-296 (1989)
  8. Salvemini, D. De Nucci, G. Gryglewski, R.J. & J.R. Vane: Human neutrophils and mononuclear cells inhibit platelet aggregation by releasing a nitric oxide-like factor. *Proc Natl Acad Sci USA* 86,6328-6332 (1989)
  9. McCall, T.B. Feelisch M. Palmer, R.M.J. & A. Moncada: Identification of N-iminoethyl-L-ornithine as an irreversible inhibitor of nitric oxide synthase in phagocytic cells. *Br J Pharmacol* 102, 234-238 (1991)
  10. Marletta, M.A.: Mammalian synthesis of nitrite, nitrate, and N-nitrosating agents. *Chem Res Toxicol* 1, 249-257 (1988)
  11. Bartsh, H.E. Hietanen, E. & C. Malaveille: Carcinogenic nitrosamines: free radical aspects of their action. *Free Radical Biol Med* 7, 637-644 (1989)
  12. Wink, D.A. Kasprzak, K.S. Maragos, C.M. Elespuru, R.K. Misra, M. Dunams, T.M. Cebula, T.A. Koch, W.H. Andrews, A.W. Allen, J.S. and L. Keefer: DNA deamination ability and genotoxicity of nitric oxide and its progenitors. *Science* 254, 1001-1003 (1991)
  13. Cohen, S.M. Purilo, D.T. & L.B. Ellwein: Pivotal role of increased cell proliferation in human carcinogenesis. *Mod Pathol* 4, 371-382 (1991)
  14. Shacter, E. Beecham, E.J. Covey, J.M. Kohn, K.W. & M. Potter: Activated neutrophils induce prolonged DNA damage in neighboring cells. *Carcinogenesis* 9, 2297-2304 (1988)
  15. Tamir, S. Burney, S. & S.R. Tannenbaum: DNA damage by nitric oxide. *Chem Res Toxicol* 9, 821-827 (1996)
  16. AM Miles, Y Chen, MW Owens, & MB Grisham: Fluorometric determination of nitric oxide. In: *Methods: A Companion to Methods in Enzymology*. 7, 40-47. Eds: Everse J, Grisham MB, Academic Press, NY (1995)
  17. Miles, A.M. Gibson, M.F. Kirshna, M. Cook, J.C. Parcelli, R. Wink, D. & M.B. Grisham: Effects of superoxide on nitric oxide-dependent N-nitrosation reactions. *Free Rad Res* 23, 379-390 (1995)
  18. Tamir, S. Rojas-Walker, T. Wishnor, J.S. & S.R. Tannenbaum: DNA damage and genotoxicity by nitric oxide. *Methods Enzymol* 269, 230-242 (1996)
  19. In: Nitrosation. Eds: Williams DLH, Cambridge University Press, Cambridge (1988)
  20. Laval, F. & D.A. Wink: Inhibition by nitric oxide of the repair protein, O6-methylguanine-DNA-methyltransferase. *Carcinogenesis* 15, 443-447 (1995)
  21. Wink, D.A. & J. Laval: The Fpg protein, a DNA repair enzyme, is inhibited by the biomediator nitric oxide in vitro and in vivo. *Carcinogenesis* 15, 2125-2129 (1994)
  22. Miles, A.M. Bohle, D.S. Glassbrenner, P.A. Hansert, B. Wink, D.A. & M.B. Grisham: Modulation of superoxide-dependent oxidation and hydroxylation reactions by nitric oxide. *J Biol Chem* 271, 40-47 (1996)
  23. Huie, R.E. & S. Padmaja: The reaction of NO with superoxide. *Free Rad Res* 18, 195-199 (1993)
  24. Pryor, W.A. & G.L. Squadrito: The chemistry of peroxy-nitrite: a product from the reaction of nitric oxide with superoxide. *Am J Physiol* 268, L699-L722 (1995)
  25. Pryor, W.A.: Oxy-radicals and related species: their formation, lifetimes, and reactions. *Ann Rev Physiol* 48, 657-667 (1986)
  26. Pryor, W.A.: Mechanisms of nitrogen dioxide reactions: initiation of lipid peroxidation and the production of nitrous acid. *Science* 214, 435-437 (1981)
  27. Koppenol, W.H. Moreno, J.J. Pryor, W.A. Ischiropoulos, H. & J.S. Beckman. Peroxynitrite, a cloaked oxidant formed by nitric oxide and superoxide. *Chem Res Toxicol* 5, 834-842 (1992)
  28. Tamir, S. Burney, S. & S.R. Tannenbaum: DNA damage by nitric oxide. *Chem Res Toxicol* 9, 821-827 (1996)
  29. Halliwell, B. & O.I. Aruoma: DNA damage by oxygen-derived species. *FEBS Lett* 281, 9-19 (1991)
  30. Kasai, H. Crain, P.F. Kuchino, Y. Nishimura, S. Ootsuyama, A. & H. Tanooka: Formation of 8-hydroxyguanine

## Nitric oxide and superoxide in DNA damage

moiety in cellular DNA by agents producing oxygen radicals and evidence for its repair. *Carcinogenesis* 7,1849-1851 (1986)

31. Chao, C.C. Park, S.H. & A.E. Aust: Participation of nitric oxide and iron in the oxidation of DNA in asbestos-treated human lung epithelial cells. *Arch Biochem Biophys* 326, 152-157 (1996)

32. King, P.A. Jamison, E. Strahs, D. Anderson, V.E. & M. Brenowitz: 'Footprinting' proteins on DNA with peroxynitrous acid. *Nucleic Acid Res* 21, 2473 (1993)

33. Cheng, K.C. Cahill, D.S. Kasai, H. Nishimura, S. & L.A. Loeb: 8-hydroxyguanine, an abundant form of oxidative DNA damage, causes G→T and A→C substitutions. *J Biol Chem* 267, 166-172 (1992)

34. Juedes, M.J. & G.N. Wogan: Peroxynitrite-induced mutation spectra of pSP189 following replication in bacteria and human cells. *Mut Res* 349, 51-61 (1996)

35. Yermilov, V. Rubio, J. Becchi, J. Friesen, M.D., Pignatelli, B. & H. Ohshima: Formation of 8-nitroguanine by the reaction of guanine with peroxynitrite. *Carcinogenesis* 16, 2045-2050 (1995)

36. Yermilov, V. Rubio, J. & H. Ohshima: Formation of 8-nitroguanine in DNA treated with peroxynitrite *in vitro* and its rapid removal from DNA by depurination. *FEBS Lett* 376, 207-210 (1995)

37. Buettner, G.R.: The pecking order of free radicals and antioxidants: lipid peroxidation, alpha-tocopherol, and ascorbate. *Arch Biochem Biophys* 300, 535-543 (1993)

38. Imlay, J.A. & S. Linn: DNA damage and oxygen radical toxicity. *Science* 24:1302-1309 (1988)

39. Geierstanger, B.H. Kagawa, T.F. Chen, S. Quigley, G.J. & P.S. Ho: Base-specific binding of copper (II) to Z-DNA. *J Biol Chem* 266, 20185-20191 (1991)

40. Dizdaroglu, M. Rao, G. Halliwell, B. & E. Gajewski: Damage to the DNA bases in mammalian chromatin by hydrogen peroxide in the presence of ferric and cupric ions. *Arch Biochem Biophys* 285, 317-324 (1991)

41. Grisham, M.B. Mills, L. Miles, A.M. & D. Jourdain: Effect of nitric oxide on reactive oxygen metabolite-dependent hemoprotein-catalyzed oxidative reactions. *FASEB J* 11, A643 (1997)

42. Kanner, J. Harel, S. & R. Granit: Nitric oxide as an antioxidant. *Arch Biochem Biophys* 289, 130-136 (1991)

43. Pacelli, R. Krishna, M.C. Wink, D.A. & J.B. Mitchell: Nitric oxide protects DNA from hydrogen peroxide-induced double strand cleavage. *Proc Am Assoc Cancer Res* 35, 540 (1994)