

Received: 2002.01.14  
Accepted: 2002.01.23  
Published: 2002.02.22

**Authors' Contribution:**

- A** Study Design
- B** Data Collection
- C** Statistical Analysis
- D** Data Interpretation
- E** Manuscript Preparation
- F** Literature Search
- G** Funds Collection

## Tonal nitric oxide and health: anti-bacterial and -viral actions and implications for HIV

Danielle Benz <sup>■</sup>, Patrick Cadet <sup>■</sup>, Kirk Mantione <sup>■</sup>,  
Wei Zhu <sup>■</sup>, George B. Stefano <sup>■</sup>

Neuroscience Research Institute, State University of New York at Old Westbury,  
Old Westbury, New York 11568, USA

**Source of support:** In part by the following grants: NIDA 09010 and the Cell Dynamics Corporation.

RA

### Summary

Nitric oxide has been shown to have important physiological regulatory roles, i. e, vasodilation, neurotransmitter release, etc. Now, we review its role as an antibacterial and antiviral agent. Nitric oxide has also been identified as an important factor in the development of non-specific immunity. And accordingly, nitric oxide synthase (NOS), the catalytic enzyme producing nitric oxide, is a key element in the protective activities of nitric oxide. The expression of inducible (i) NOS is regulated by cytokines. iNOS-derived nitric oxide was found to contribute to both early and late phases of antibacterial activity. Enzymes, such as proteases (reverse transcriptases, and ribonucleotide reductase, etc.) containing cysteine residues, appear to be targets for nitric oxide nitrosylation, as well as viral-encoded transcription factors that are involved in viral replication. It would appear that this multifunctional signaling molecule is not only involved with signaling between cells, it also appears to maintain the immediate environment free of microbial agents.

**Key words:** nitric oxide • bacteria • virus • tonal nitric oxide • HIV • immunocytes • reverse transcriptases • ribonucleotide reductase

**Full-text PDF:** [http://www.MedSciMonit.com/pub/vol\\_8/no\\_1/2433.pdf](http://www.MedSciMonit.com/pub/vol_8/no_1/2433.pdf)

**File size:** 79 kB  
**Word count:** 1770  
**Tables:** 1  
**Figures:** –  
**References:** 46

**Author's address:** George B. Stefano Neuroscience Research Institute, State University of New York at Old Westbury, Old Westbury, New York 11568, USA; Phone 516-876-2732; Fax 516-876-2727; email gstefano@optonline.net

## NITRIC OXIDE AS AN ANTIBACTERIAL AGENT

The physiological roles of nitric oxide (NO) are numerous [1]. A great deal of recent research has centered on the examination of its antibacterial and antiviral activities. Investigations into the effects of exogenous NO on pulmonary infections have proved promising. In addition, this can also suggest a possible use of inhaled NO therapy in the future. Inhaled NO has produced a marked reduction in bacterial load of rats that had been infected with *Pseudomonas aeruginosa pneumonia*, as well as decreased pulmonary leukocyte infiltration in vivo [2] (Table 1). In vitro experimentation has also shown that exposure to exogenous NO results in selective bactericidal actions. Extracellular *Mycobacterium tuberculosis* were killed, in both a dose- and time-dependent manner, by low (< 100 ppm) concentrations of NO for short periods of time (24 h or less) [3], while *Staphylococcus aureus* and group B *Streptococcus* (GBS) were significantly affected by greater doses (120 ppm) as compared to controls [4]. In cystic fibrosis patients, *Burkholderia* (formerly *Pseudomonas*) cepacia is an important pulmonary pathogen that survives in the lung. Research has shown that NO and H<sub>2</sub>O<sub>2</sub> together were a potent bactericidal combination against *B. cepacia*, decreasing the bacterial count by >1000-fold over 75 minutes [5]. Smith, Green et al suggest that the lack of expression of inducible nitric oxide synthase (iNOS) in the airway epithelial cells of CF patients, and, therefore, the lack of NO in these same patients could contribute to the survival of this pathogen [5].

NO donors have also been shown to have inhibitory effects on selective bacterial strains. NO derived from S-nitroso-acetyl-penicillamine (SNAP) had inhibitory properties against bacterial fish pathogens *Aeromonas salmonicida*, *Renibacterium salmonarum* and *Yersinia ruckeri*, while peroxyxynitrite had no effect [6] (Table 1).

Antiviral effects of NO were demonstrated by a NO donor, glycerin trinitrate (GTN), against viral haemorrhagic septicemia virus (VSHV), a fish rhabdovirus. VSHV replication was significantly inhibited [7]. Another compound, (Z)-1-[N-(2-aminoethyl)-N-(2-ammonioethyl)amino]diazene-1-ium-1, 2-diolate (DETA-NO), which releases NO at a predictable rate, was demonstrated to inhibit representative strains of *Candida albicans*, *C. krusei*, *C. parapsilosis*, *C. tropicalis*, *C. glabrata*, and *C. dubliniensis* (*C. parapsilosis* and *C. krusei*, being most sensitive), while DETA-NO combined with azoles (ketoconazole, fluconazol, and miconazole) proved to be synergistic [8]. Furthermore, examinations have shown that acidified nitrite, which generates NO under various conditions, has also proved to be a successful antimicrobial agent. The effects of nitrite under acidic conditions were demonstrated against cariogenic bacteria (*Streptococcus mutans*, *Lactobacillus casei*, and *Actinomyces naeslundii*) [9], periodontal bacteria (*Fusobacterium nucleatum*, *Eikenella corrodens* and *Porphyromonas gingivalis*) [10], dermatophyte fungi, *Candida* and bacterial skin pathogens (*Trichophyton mentagrophytes*, *T. rubrum*, *Candida albicans*, *Staphylococcus aureus* and *Propionibacterium acnes*) [11], and the viral molluscum contagiosum [12].

**Table 1.** Bacteria and viruses affected by nitric oxide.

Bacteria	Viruses	Other microorganisms
<i>Pseudomonas aeruginosa</i>	Hepatitis B virus (HBV)	<i>Candida albicans</i>
Salmonella	Herpes simplex virus (HSV)	
<i>Klebsiella pneumoniae</i>	Coxsackievirus	
<i>Escherichia coli</i>	lymphocytic choriomeningitis virus (LCMV)	
<i>Streptococcus mutans</i>	Marek's disease virus (MDV)	
<i>Staphylococcus aureus</i>	Murine cytomegalovirus	
group B <i>Streptococcus</i>	Rhabdovirus	
<i>Lactobacillus casei</i>	Viral haemorrhagic septicemia virus	
<i>Mycobacterium tuberculosis</i>	bovine herpesvirus 1 (BHV-1)	

Interestingly, *Scutellaria baicalensis* (SB), a member of the mint family, and a plant used in traditional Chinese herbal medicine, is purported to have antibacterial and antiviral properties. Researchers examined the effects of SB on the production of NO and have found that the marked increase in NO production that was observed in rIFN-gamma-primed mouse peritoneal cells was the result of SB-induced TNF-alpha secretion [13]. Another plant extract, *Phyllanthus tenellus*, is also traditionally used for the treatment of viral, bacterial and parasitic infections; and, it was shown that, in vitro, a concentration of 100 microg/ml fresh extract stimulated a significant NO production (P< or =0. 05) in all assays and in 10 and 50 mg/kg fresh extract which was injected twice intraperitoneally primed macrophages in vivo [14]. Actually, much research has centered on the interactions of NO and the levels of various cytokines involved in immune responses [15].

## NITRIC OXIDE AS AN ANTIVIRAL AGENT

NO, from the NO donor 3-(2-hydroxy-2-nitroso-1-propyl-hydrazino)-1-propanamine (NONOate), was shown to inhibit granulocyte macrophage colony-stimulating factor (GM-CSF), which was induced by exposure of human bronchial epithelial cells to human rhinovirus (HRV), a common cause of exacerbation in asthma. Hence, demonstrating an important anti-inflammatory role of NO in this viral infection [16] [17]. HRV infections in vitro, and in vivo, were also shown to induce increased epithelial NOS2 expression, which would indicate that increased production of NO plays an important part in host defense [18]. Hepatitis B virus (HBV) replication was inhibited by IFN-gamma in HBV transgenic mice during infection with *Schistosoma mansoni* [19] through what appears to be a NO mediated manner. Another experiment showed the same effects against HBV: without the *Schistosoma mansoni* infection, as well as a NO-mediated inhibition against lymphocytic choriomeningitis virus (LCMV). This was demonstrated by an increased replication of LCMV in the livers of iNOS deficient mice as compared to controls [20]. NO, derived from NOS2, was also found to modulate the

cytokine profile within mice infected with *Mycobacterium avium*, and to regulate the number, size and cellular composition of *M. avium*-induced granulomas independently of antibacterial effects [21].

NO has also been identified as an important factor in the development of non-specific immunity [22], and accordingly, NOS, as the catalytic enzyme producing NO, is a key element in the protective activities of NO. The expression of iNOS is regulated by cytokines. iNOS-derived NO was found to contribute to both early and late phases of antibacterial activity against *Salmonella*, with peroxyxynitrite (ONOO<sup>-</sup>), in addition to reactive oxygen species, involved in the early oxidative bacterial killing, followed by a sustained period of nitrosative chemistry limiting bacterial growth [22]. In that same experiment, IFN-gamma was found to enhance antibacterial activity by increasing NO production [23]. With regard to constitutive NO, it has been suggested that low levels negatively regulate the expression of IFN alpha and beta in mouse peritoneal macrophages, and that NO could act as a homeostatic agent for these pathways [24].

Japanese encephalitis virus infection was shown to increase NOS/iNOS activity in the brains of treated mice, and pre-treatment with a NOS inhibitor, L-NMMA, decreased the survival rates of the infected mice, furthering the evidence of the protective actions of NOS [25]. When infected with murine cytomegalovirus, the peritoneal macrophages of NOS2 deficient mice showed a lower antiviral activity, while non deficient mice who were treated with a selective NOS2 inhibitor showed even greater decrease in antiviral activity, both resulting in higher mortality and greater MCMV replication [26].

Regarding NO and viruses in general, mice deficient in iNOS were susceptible to herpes simplex virus-1 infection and they also exhibited a delayed clearance of the virus from dorsal root ganglia [27]. Furthermore, macrophage NO is implicated in resistance to a number of viruses, including ectromelia virus and vaccinia virus, see [28]. Gamma interferon-induced production of NO also inhibited growth of murine hepatitis virus type 3 in a murine macrophage cell line (RAW 264. 7) an action also found with the NO donor SNAP [28]. NO has also been implicated in an anti-hepatitis C infection [29]. In support of these observations the antiretroviral agent (R)-9-(2-phosphonomethoxypropyl)adenine stimulates cytokine and NO production [30]. In HIV-1-associated dementia iNOS levels were elevated and coincided with increased expression of the HIV-1 coat protein gp41 that has been shown to induce iNOS in primary cultures of mixed rat neuronal and glial cells [31]. Bukrinsky and colleagues [32] found NO expressed in HIV-1-infected monocyte cultures. In another report, Hermann and colleagues (1997) [33] found that infection of human monocyte-derived macrophages with HIV-1 did not seem to induce detectable NO release or iNOS mRNA accumulation.

This conflict may be better understood within the framework of experimental design. For example, superoxide (O<sub>2</sub><sup>-</sup>) and NO metabolites may also cause harm to the host besides exerting their antimicrobial and antiviral actions. Low levels of these agents and their metabolites can also facilitate viral replication because of their mitogenic effects on cells (see [34]). Additionally, most viruses stimulate their host cells since they grow better in proliferating cells (see [34,35]). Indeed, we have demonstrated that gp120 may exert such a stimulatory influence in diverse cells, suggesting that its immune and vascular cell activation is intentional and comprises an important step in the infection process [36–38]. Furthermore, universal mechanisms in this regard probably are not found and variation does exist. Additionally, these studies did not measure or take cNOS stimulated NO into consideration. However, there is general agreement on the point that NO may exert antiviral actions on particular viruses.

It was also shown that myocarditis in mice infected with Coxsackie group B virus (CVB) lead to an increased expression of iNOS mRNA in inflammatory cells, as compared to controls, suggesting that high NO production is part of the host immune defense as an antiviral agent [39]. Bovine peripheral blood mononuclear cells (PBMCs) and monocytes were demonstrated to produce iNOS-derived NO in response to stimulation by bovine herpesvirus 1 (BHV-1), LPS and concanavalin A (Con A), and the NO was found to exhibit antiviral activity toward the BHV-1 [40]. As can be seen, the up-regulation of NO production in response to viral and bacterial infections has been documented numerous times. Another example can be seen in an experiment in which Japanese encephalitis virus (JEV) and JEV-induced macrophage derived neutrophil chemotactic factor (MDF) both induced increased NO production in splenic macrophages of mice and the MDF stimulated macrophages inhibited virus replication with high levels of NO production [41]. In addition to its antibacterial and antiviral properties, NO has also been shown to produce gene activation. In *Drosophila melanogaster*, NO introduced into the hemocoel caused activation of the gene encoding the antimicrobial peptide Diptericin [42]. This type of reaction has also been documented in the activation of neutral endopeptidase [43].

#### POSSIBLE MECHANISM AND IMPLICATIONS

The question still remains as to how this NO „killing“ mechanism is working. Since viral life cycles are dependent upon proteases that cleave polypeptides into smaller individual units, it has been suggested that NO-mediated S-nitrosylation of viral and host macromolecules may be a possible answer to this question [44]. They have identified enzymes (such as proteases, reverse transcriptases, and ribonucleotide reductase) containing cysteine residues as targets for NO nitrosylation, as well as host and viral-encoded transcription factors that are involved in viral replication [44]. Another group documented NO inactivating the Coxsackie virus protease 3C by S-nitrosylating the cysteine residue in the active site of the protease thereby inhibiting its activity and

interrupting the viral life cycle [45]. This effect has even been documented on human immunodeficiency virus type 1 (HIV-1) protease (HIV-PR), which has two cysteine residues located near the surface of the protease [46]. The researchers found that treatment of HIV-PR with different NO congeners resulted in loss of its proteolytic activity [46]. Their findings that sodium nitroprusside inhibited HIV-PR up to 70% and SNAP completely inhibited the protease within 5 min of treatment could possibly lead to new role for NO in mediating resistance to HIV-1 infection [46].

## REFERENCES:

1. Urazaev AK, Zefirov AL: The physiological role of nitric oxide. *Uspekhi Fiziologicheskikh Nauk*, 1999; 30: 54-72
2. Webert KE, Vanderzwan J, Duggan M et al: Effects of inhaled nitric oxide in a rat model of *Pseudomonas aeruginosa* pneumonia. *Critical Care Medicine*, 2000; 28: 2397-405
3. Long R, Light B, Talbot JA: Mycobacteriocidal action of exogenous nitric oxide. *Antimicrobial Agents & Chemotherapy*, 1999; 43: 403-5
4. Hoehn T, Huebner J, Paboura E et al: Effect of therapeutic concentrations of nitric oxide on bacterial growth in vitro. *Critical Care Medicine*, 1998; 26: 1857-62
5. Smith AW, Green J, Eden CE, Watson ML: Nitric oxide-induced potentiation of the killing of *Burkholderia cepacia* by reactive oxygen species: implications for cystic fibrosis. *Journal of Medical Microbiology*, 1999; 48: 419-23
6. Campos-Perez JJ, Ellis AE, Secombes CJ: Toxicity of nitric oxide and peroxy-nitrite to bacterial pathogens of fish. *Diseases of Aquatic Organisms*, 2000; 43: 109-15
7. Tafalla C, Figueras A, Novoa B: Role of nitric oxide on the replication of viral haemorrhagic septicemia virus (VHSV), a fish rhabdovirus. *Veterinary Immunology & Immunopathology*, 1999; 72: 249-56
8. McElhaney-Feser GE, Rauli RE, Cihlar RL: Synergy of nitric oxide and azoles against *Candida* species in vitro. *Antimicrobial Agents & Chemotherapy*, 1998; 42: 2342-6
9. Silva Mendez LS, Allaker RP, Hardie JM, Benjamin N: Antimicrobial effect of acidified nitrite on cariogenic bacteria. *Oral Microbiology & Immunology*, 1999; 14: 391-2
10. Allaker RP, Silva Mendez LS, Hardie JM, Benjamin N: Antimicrobial effect of acidified nitrite on periodontal bacteria. *Oral Microbiology & Immunology*, 2001; 16: 253-6
11. Weller R, Price RJ, Ormerod AD et al: Antimicrobial effect of acidified nitrite on dermatophyte fungi, *Candida* and bacterial skin pathogens. *Journal of Applied Microbiology*, 2001; 90: 648-52
12. Ormerod AD, White MI, Shah SA, Benjamin N: Molluscum contagiosum effectively treated with a topical acidified nitrite, nitric oxide liberating cream. *British Journal of Dermatology*, 1999; 141: 1051-3
13. Kim HM, Moon EJ, Li E et al: The nitric oxide-producing activities of *Scutellaria baicalensis*. *Toxicology*, 1999; 135: 109-15
14. Ignacio SR, Ferreira JL, Almeida MB, Kubelka CF: Nitric oxide production by murine peritoneal macrophages in vitro and in vivo treated with *Phyllanthus tenellus* extracts. *Journal of Ethnopharmacology*, 2001; 74: 181-7
15. Bogdan C, Rollinghoff M, Diefenbach A: Reactive oxygen and reactive nitrogen intermediates in innate and specific immunity. *Current Opinion in Immunology*, 2000; 12: 64-76
16. Sanders SP, Kim J, Connolly KR et al: Nitric oxide inhibits rhinovirus-induced granulocyte macrophage colony-stimulating factor production in bronchial epithelial cells. *American Journal of Respiratory Cell & Molecular Biology*, 2001; 24: 317-25
17. Sanders SP: Asthma, viruses, and nitric oxide. *Proceedings of the Society for Experimental Biology & Medicine*, 1999; 220: 123-32
18. Sanders SP, Siekierski ES, Richards SM et al: Rhinovirus infection induces expression of type 2 nitric oxide synthase in human respiratory epithelial cells in vitro and in vivo. *Journal of Allergy & Clinical Immunology*, 2001; 107: 235-43
19. McClary H, Koch R, Chisari FV, Guidotti LG: Inhibition of hepatitis B virus replication during schistosoma mansoni infection in transgenic mice. *Journal of Experimental Medicine*, 2000; 192: 289-94
20. Guidotti LG, McClary H, Loudis JM, Chisari FV: Nitric oxide inhibits hepatitis B virus replication in the livers of transgenic mice. *Journal of Experimental Medicine*, 2000; 191: 1247-52
21. Ehlers S, Kutsch S, Benini J et al: NOS2-derived nitric oxide regulates the size, quantity and quality of granuloma formation in *Mycobacterium avium*-infected mice without affecting bacterial loads. *Immunology*, 1999; 98: 313-23
22. Bondarenko VM, Vinogradov NA, Maleev VV: The antimicrobial activity of nitric oxide and its role in the infectious process. *Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii*, 1999; 61-7
23. Vazquez-Torres A, Jones-Carson J, Mastroeni P et al: Antimicrobial actions of the NADPH phagocyte oxidase and inducible nitric oxide synthase in experimental salmonellosis. I. Effects on microbial killing by activated peritoneal macrophages in vitro. *Journal of Experimental Medicine*, 2000; 192: 227-36
24. Guillemard E, Varano B, Belardelli F et al: Inhibitory activity of constitutive nitric oxide on the expression of alpha/beta interferon genes in murine peritoneal macrophages. *Journal of Virology*, 1999; 73: 7328-33
25. Saxena SK, Mathur A, Srivastava RC: Induction of nitric oxide synthase during Japanese encephalitis virus infection: evidence of protective role. *Archives of Biochemistry & Biophysics*, 2001; 391: 1-7
26. Noda S, Tanaka K, Sawamura S et al: Role of nitric oxide synthase type 2 in acute infection with murine cytomegalovirus. *Journal of Immunology*, 2001; 166: 3533-41
27. MacLean A, Wei XQ, Huang FP et al: Mice lacking inducible nitric oxide synthase are more susceptible to herpes simplex virus infection despite enhanced Th1 cell responses. *J Gen Virol*, 1998; 79: 825-30
28. Pope M, Marsden PA, Cole E et al: Resistance to murine hepatitis virus strain 3 is dependent on production of nitric oxide. *J Virol*, 1998; 72: 7084-90
29. Sharara AI, Perkins DJ, Misukonis MA et al: Interferon (IFN) -alpha activation of human blood mononuclear cells in vitro and in vivo for nitric oxide synthase (NOS) type 2 mRNA and protein expression: possible relationship of induced NOS2 to the anti-hepatitis C effects of IFN-alpha in vivo. *J Exp Med*, 1997; 186: 1495-502
30. Zidek Z, Holy A, Frankova D: Antiretroviral agent (R)-9-(2-phosphonomethoxypropyl) adenine stimulates cytokine and nitric oxide production. *Eur J Pharmacol*, 1997; 331: 245-52
31. Adamson DC, Wildemann B, Sasaki M et al: Immunologic NO synthase: elevation in severe AIDS dementia and the induction by HIV-1 gp41. *Science*, 1996; 274: 1917-21
32. Bukrinsky M, Schmidtnerova H, Zybarch G et al: A critical role of nitric oxide in human immunodeficiency virus type 1-induced hyperresponsiveness of cultured monocytes. *Mol Med*, 1996; 2: 460-8
33. Hermann E, Idziorek T, Kusnierz JP et al: Role of nitric oxide in the regulation of lymphocyte apoptosis and HIV-1 replication. *Int J Immunopharmacol*, 1997; 19: 387-97
34. Peterhans E: Reactive oxygen species and nitric oxide in viral diseases. *Biol Trace Element Res*, 1997; 56: 107-16
35. Torre D, Ferrario G: Immunological aspects of nitric oxide in HIV-1 infection. *Med Hypotheses*, 1996; 47: 405-7
36. Stefano GB, Smith EM, Cadet P, Hughes TK: HIV GP120 alteration of DAMA and IL-1a induced chemotactic responses in human and invertebrate immunocytes. *J Neuroimmunol*, 1993; 43: 177-84
37. Stefano GB, Salzet M, Bilfinger TV: Long-term exposure of human blood vessels to HIV gp120, morphine and anandamide increases endothelial adhesion of monocytes: Uncoupling of Nitric Oxide. *J Cardiovasc Pharmacol*, 1998; 31: 862-8
38. Stefano GB, Salzet M, Rialas C et al: Macrophage behavior associated with acute and chronic exposure to HIV GP120, morphine and anandamide: endothelial implications. *Int J Cardiol*, 1998; 64: S3-S13
39. Robinson NM, Zhang HY, Bevan AL et al: Induction of myocardial nitric oxide synthase by Coxsackie B3 virus in mice. *European Journal of Clinical Investigation*, 1999; 29: 700-7
40. Gupta PK, Saini M, Gupta LK, Garg SK: Induction of nitric oxide in bovine peripheral blood mononuclear cells by bovine herpesvirus 1. *Acta Virologica*, 1999; 43: 321-4

41. Saxena SK, Singh A, Mathur A: Antiviral effect of nitric oxide during Japanese encephalitis virus infection. *International Journal of Experimental Pathology*, 2000; 81: 165-72
42. Nappi AJ, Vass E, Frey F, Carton Y: Nitric oxide involvement in *Drosophila* immunity. *Nitric Oxide*, 2000; 4: 423-30
43. Stefano GB, Salzet M: Invertebrate opioid precursors: evolutionary conservation and the significance of enzymatic processing. *Int Rev Cytol*, 1999; 187: 261-86
44. Colasanti M, Persichini T, Venturini G, Ascenzi P: S-nitrosylation of viral proteins: molecular bases for antiviral effect of nitric oxide. *Jubmb Life*, 1999; 48: 25-31
45. Saura M, Zaragoza C, McMillan A et al: An antiviral mechanism of nitric oxide: inhibition of a viral protease. *Immunity*, 1999; 10: 21-8
46. Basu A, Sehajpal PK, Ogiste JS, Lander HM: Targeting cysteine residues of human immunodeficiency virus type 1 protease by reactive free radical species. *Antioxidants & Redox Signaling*, 1999; 1: 105-12