

## METHOD OF INHIBITING NITRIC OXIDE FORMATION

### CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of application Ser. No. 08/328,925, filed on Oct. 25, 1994 now abandoned, which is a continuation-in-part of application Ser. No. 08/110,915, filed Aug. 24, 1993, now U.S. Pat. No. 5,358,969 which is in turn, a continuation-in-part of application Ser. No. 07/843,387, filed Feb. 28, 1992 now U.S. Pat. No. 5,246,971 and application Ser. No. 07/906,632, filed Jun. 30, 1992, now U.S. Pat. No. 5,246,970 each of which are a continuation-in-part of application Ser. No. 07/807,912, filed Dec. 16, 1991 now abandoned.

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### BACKGROUND OF THE INVENTION

This invention relates to a method of-inhibiting nitric oxide formation in warm blooded mammals and, more particularly, to the administration of methyl-, dimethyl-, or amino-substituted guanidines as inhibitors of nitric oxide production in a host afflicted with acute or chronic inflammatory disease.

Nitric oxide synthase catalyzes the mixed functional oxidation of t-arginine to t-citrulline and nitric oxide (NO. 5 '1) [Stuehr et al., Proc. Natl. Acad. Sci. U.S.A. 88, 7773 (1991)]. NO appears to function as either a signaling or an effector molecule depending on the isoform of the enzyme. The constitutive isoform of nitric oxide synthase produces small amounts of NO. which activate guanylate cyclase resulting in the formation of cGMP which mediates endothelium-dependent relaxation [Moncada et al., Pharmacol. Reviews 43, 109 (1991)] and neural transmission [Garthwaite, Trends Neurol. Sci. 14, 60 (1991)]. NO. is produced in much larger amounts by the cytokine and endotoxin inducible isoform of nitric oxide synthase, and in macrophages functions as an effector molecule which appears to mediate the cytotoxic actions of macrophages on target cells [Hibbs et al., Nitric Oxide from L-Arginine: A Bioregulatory System, S. Moncada and E. Higgs, Eds. Elsevier, N.Y., pp. 189-223 (1990)]. Since NO. is a potent vasodilator and increases blood flow, and since vasoactive agents (such as histamine and bradykinin), which stimulate NO. production increase both blood flow and vascular permeability, NO. may be a candidate for mediating increases in blood flow and vascular permeability induced by diabetes and elevated glucose [Pugliese et al., Diabetes/Metabolism Reviews 7, 35 (1991)].

Recently, Interleukin-1 (IL-1) has been shown to induce the expression of the cytokine inducible isoform of nitric oxide synthase in pancreatic islets. The production of NO. has been proposed to be the effector molecule which mediates IL-1's inhibitory effects on islet function [Southern et al., FEBS. Lett. 276, 42 (1990) and Corbett et al., Biochemical J. 287,229 (1992)]. Generation of an IL-induced EPR detectable iron-nitrosyl complex, which is prevented by N<sup>G</sup> monomethyl-t-arginine (NNMA), has been used to confirm the formation of nitric oxide by islets [Corbett et al., J. Biol. Chem. 266, pp. 21351-21354 (1991)]. Also, the protein synthesis inhibitor, cycloheximide has been shown to block IL-1-induced nitrite formation, cGMP accumulation, and EPR detectable iron-nitrosyl complex formation by islets, thus establishing that IL-1 induces the cytokine inducible

isoform of nitric oxide synthase in pancreatic islets [Corbett et al., Biochemical J. 287, 229 (1992)].

The pathogenesis of diabetic complications has been linked to imbalances in sorbitol, myo-inositol, and 1,2-diacyl-sn-glycerol metabolism, and to non-enzymatic glycation of cellular and extracellular constituents [Pugliese et al., Diabetes/Metabolism 35 Reviews 7, 37 (1991)]. The glycation link is supported by evidence that aminoguanidine, a nucleophilic hydrazine compound, interferes with the formation of these glycation products and also attenuates the development of several diabetes-induced vascular [Pugliese et al., Diabetes/Metabolism Reviews 7, 35 (1991); Williamson et al., Diabetes & Metab. 16, 3369 (1990); Soulis-Liparota et al., Diabetes 40, 1328 (1991)], neural [Kihara et al., Proc. Natl. Acad. Sci. U.S.A. 88, 6107 (1991)] and collagen changes [Brownlee et al., New Encl. J. Med. 318, 1315 (1988) and Brownlee et al., Science 232, 1629 (1986)]. Bucala et al., J. Clin. Invest. 87, 432 (1991) recently, reported that quenching of NO. in vitro by glycated albumin is attenuated by aminoguanidine (present during exposure of albumin to glycation agents) and suggested that glycation products may impair endothelium-dependent relaxation by attenuating NO. activity.

### BRIEF DESCRIPTION OF THE INVENTION

In accordance with the present invention a novel method of inhibiting nitric oxide formation in warm blooded mammals afflicted with acute or chronic inflammatory diseases is provided. The method comprises administering to warm blooded mammals afflicted with acute or chronic inflammatory diseases a small but nitric oxide inhibitory effective amount of methyl-, dimethyl-, or amino-substituted guanidines. These inhibitory compounds are also chemically named as aminoguanidine, N, N'-diaminoguanidine, methylguanidine and 1, 1-dimethylguanidine.

It will be understood that pharmaceutically acceptable salts of these compounds, e.g., the HCl, HCO<sub>3</sub> and sulfate salts, can also be administered to the host in accordance with the method of the invention. Inflammation can be conveniently divided into acute and chronic conditions. Acute inflammation is generally of relatively short duration and lasts for about a few minutes to about one to two days. Its main characteristics are increased blood flow, exudation of fluid and plasma proteins (edema) and emigration of leukocytes, predominantly neutrophils. Chronic inflammation is of longer duration and is associated histologically with the presence of lymphocytes and macrophages and with proliferation of blood vessels and connective tissue. Inflammation is manifested by heat, redness, swelling, pain and loss of function. See, e.g., Cotran, Kumar and Robbins, Robbins Pathologic Basis of Disease, 4th ed., W. B. Saunders Company, pp.40-41(1989); Chandrasoma and Taylor, Concise Pathology, First Edition, pp. 35-44, Appleton & Lange (1991).

The causes of inflammation are numerous and include such factors as microbial infections (e.g., bacterial and fungal infections), physical agents such as burns, radiation and trauma, chemical agents such as toxins and caustic substances, necrotic tissue and various types of immunologic reactions.

The present invention is directed to the prevention/ treatment of a broad spectrum of diseases which may be linked to the production of nitric oxide by leukocytes (neutrophils and macrophages) and other cells of nonhemopoietic origin as distinguished from diseases mediated by immunologic reactions as claimed in copending application