

HEMOPROTEINS FOR INHIBITION OF NITRIC OXIDE-MEDIATED HYPOTENSION AND SEPTIC SHOCK

This application is a continuation-in-part of U.S. Ser. No. 07/838,603, filed Feb. 19, 1992, now U.S. Pat. No. 5,296,466, which is incorporated by reference herein.

FIELD OF THE INVENTION

The invention is directed to a method for the prophylaxis or treatment of an animal for systemic hypotension induced by a biological response modifier. Examples of such biological response modifiers include but are not limited to a cytokine and an endotoxin. The invention is also directed to a method for the treatment of septic shock.

BACKGROUND OF THE INVENTION

Endothelium-Derived Relaxing Factor

Endothelial cells have been shown to produce a potent vasodilator known as Endothelium-Derived Relaxing Factor (EDRF). Many naturally occurring substances which act as physiological vasodilators mediate all or part of their action by stimulating the release of EDRF. Examples of such substances include acetylcholine, histamine, bradykinin, leukotrienes, ADP, and ATP. Recent studies have identified EDRF as nitric oxide, a short lived, unstable compound (Ignarro et al., 1987, *Proc. Natl. Acad. Sci. U.S.A.* 84:9265-9269 and Palmer et al., 1987, *Nature* 327:524-526).

L-Arginine is the metabolic precursor of EDRF (Schmidt et al., 1988, *Eur. J. Pharmacol.* 154:213-216). N^G-methyl-L-arginine is a competitive inhibitor of the biosynthetic pathway of EDRF (Palmer et al., 1988, *Nature* 333:664-666). Administration of N^G-methyl-L-arginine to guinea pigs and rabbits has been shown to increase blood pressure (Aisaka et al., 1989, *Biochem. Biophys. Res. Commun.* 160:881-886). Nitric oxide (NO) appears to be synthesized from L-arginine by the enzyme, NO synthase; the coproduct is L-citrulline (Moncada et al., 1991, *J. Cardiovascular Pharmacol.* 17 (Suppl. 3):S1-S9). NO is an endogenous stimulator for soluble guanylate cyclase.

Nitric oxide has been found to be produced by macrophages, endothelial cells, neutrophils, Kupffer cells and hepatocytes, murine fibroblasts stimulated with cytokines, and EMT-6 cells, a spontaneous murine mammary adenocarcinoma cell line when treated with cytokine (reviewed in Moncada et al., 1991, *Pharmacol. Reviews* 43:109-142). Specifically, macrophage cells become activated by 12-36 hour treatments with gamma-interferon, bacterial endotoxin and various cytokines (reviewed in Collier and Vallance, 1989, *Trends in Pharmacol. Sci.* 10:427-431).

Endothelial cells in the presence of gamma-interferon, have been found to secrete large quantities of arginine-derived nitrogen oxides after activation by tumor necrosis factor (TNF) or endotoxin (Kilbourn and Belloni, 1990, *J. Natl. Cancer Inst.* 82:772-776). TNF causes marked hypotension in mammals (Tracey et al., 1986, *Gynecol. Obstet.* 164:415-422; Old, 1985, *Science* 230:630-632). Additionally, TNF is thought to mediate the vascular collapse resulting from bacterial endotoxin (Beutler et al., 1985, *Science* 229:869-871). It has recently been shown that arginine derivatives inhibit systemic hypotension associated with nitric oxide production, specifically treatment with TNF, gamma interferon, interleukin-2, and bacterial endotoxin (Kilbourn et al., U.S. Pat. No. 5,028,627, issued Jul. 2,

1991; PCT Application no. WO 91/04023, published Apr. 4, 1991; and Kilbourn et al., 1990, *Proc. Natl. Acad. Sci. U.S.A.* 87:3629-3632). Nitric oxide overproduction is also thought to be involved in numerous other pathogenic or potentially pathogenic syndromes. For example, some of these syndromes are thought to be associated with malaria, senescence and diabetes. The procedures of the present invention may also be used to prevent, inhibit and/or alleviate such NO-related syndromes.

Interaction of Hemoglobin with Endothelium-Derived Relaxation Factor

Nitric oxide reacts with hemoglobin to form nitrosylhemoglobin (Kosaka et al., 1989, *Free Radical Biology* 7:653-658). Nitrosylhemoglobin reacts with oxygen to yield nitrate and methemoglobin, which is rapidly reduced by methemoglobin reductase. At least part of the nitric oxide is oxidized by oxygen to NO₂, which is in turn converted to nitrite and nitrate.

The formation of nitrosylhemoglobin has been used to quantify nitric oxide present in mice given bacterial endotoxin, specifically by using electron paramagnetic resonance (EPR) spectroscopy to detect NO liganded to hemoglobin (Wang et al., 1991, *Life Sciences* 49:55-60). Although bacterial endotoxin induces septic shock, hypotension was not observed in the Wang et al. study. Those skilled in the art recognize that such EPR studies may be used to quantitate the binding of NO to other hemoproteins as well.

Hemoglobin has also been found to inhibit nonvascular relaxant responses to EDRF (Buga et al., 1989, *Eur. J. Pharmacol.* 161:61-72). Furthermore, hemoglobin at 1 μM reduced and at 10 μM abolished the endothelium-dependent relaxation induced by acetylcholine or by A23187 in rabbit aortic rings (Martin et al., 1985, *J. Pharmacol. Exp. Ther.* 232:708-716). It was hypothesized by Martin et al. that the hemoglobin inhibits endothelium-dependent induced relaxation by binding nitric oxide.

Septic Shock

Septic shock is characterized by inadequate tissue perfusion and is usually caused by gram-negative enteric bacilli such as *Escherichia coli*, *Klebsiella*, *Enterobacter*, *Pseudomonas*, *Serratia*, and *Bacteroides* (see Harrison's INTERNAL MEDICINE, 10th Ed., vol. 1, Petersdorf et al., eds., McGraw Hill, N.Y. 1983). Gram-negative bacilli possess endotoxin, also known as lipopolysaccharide (LPS), which is a cell-wall component that can activate leukocytes in minute amounts in the blood.

Septic shock is characterized by chills, fever, nausea, vomiting, diarrhea, and prostration. The subsequent development of septic shock is characterized by tachycardia, tachypnea, hypotension, peripheral cyanosis, mental obtundation, and oliguria. As shock progresses, oliguria persists, and heart failure, respiratory insufficiency, and coma supervene. Death usually results from pulmonary edema, generalized anoxemia secondary to respiratory insufficiency, cardiac arrhythmia, disseminated intravascular coagulation with bleeding, cerebral anoxia, or a combination of the above.

Most of the damage caused by septic shock is thought to be caused by endotoxin. It has also been hypothesized that nitric oxide plays a major role in effecting hypotension in those exposed to endotoxin (Kilbourn et al. *Biochem. and Biophys. Res. Comm.* 1990, vol. 172:1132-1138). Studies have shown that the hypotension and loss of vascular responsiveness resulting from endotoxin administration is reversed by the administration of analogues of L-arginine which inhibit nitric oxide production (Parratt and Stoclet,