

**METHOD OF USING ARGININE DERIVATIVES
TO INHIBIT SYSTEMIC HYPOTENSION
ASSOCIATED WITH NITRIC OXIDE
PRODUCTION OR ENDOTHELIAL DERIVED
RELAXING FACTOR**

Certain research relating to the development of this invention was supported by the United States Public Health Service grants which may give the United States government certain rights in the present invention.

This application relates to a patent application filed on the same date (Sept. 13, 1989) entitled "Isolating Aminoarginine and Use to Block Nitric Oxide Formation in Body" by Owen W. Griffith, having an inventor and assignee in common and incorporated by reference herein.

BACKGROUND OF THE INVENTION

The present invention relates to the prophylaxis and alleviation of hypotension induced by nitrogen oxide production.

In 1980, Furchgott and Zawadzki (Nature 288: 373-376) demonstrated that endothelial cells, which line blood vessels, can be stimulated to release a substance which relaxes vascular smooth muscle i.e., causes vasodilatation. Since the chemical nature of this substance was completely unknown, it was simply named endothelium-derived relaxing factor (DRF). It is now widely accepted that many naturally-occurring substances which act as physiological vasodilators mediate all or part of their action by stimulating release of EDRF; these substances include, acetylcholine, histamine, bradykinin, leukotrienes, ADP, ATF, substance P, serotonin, thrombin and others. Although the extremely short lifetime of EDRF (several seconds) hampered efforts to chemically identify this molecule, in 1987 several laboratories suggested that EDRF may be nitric oxide (NO), which spontaneously decomposes to nitrate and nitrite. A fundamental problem in accepting this NO hypothesis was that mammalian systems were not known to contain an enzymatic pathway which could synthesize NO, additionally, a likely precursor for NO biosynthesis was unknown. After observing that the arginine analog L-N^G-methylarginine (L-NMA) could inhibit vascular EDRF/NO synthesis induced by acetylcholine and histamine, and that EDRF/NO synthesis could be restored by adding excess L-arginine, certain of the present inventors proposed that arginine is the physiological precursor of EDRF/NO biosynthesis (Sakuma et al., PNAS 85: 8664-8667, 1988). Additional evidence supporting this proposal was reported almost simultaneously. Certain of the present inventors later demonstrated that inhibition of EDRF/NO synthesis in the anesthetized guinea pig raises blood pressure, suggesting that EDRF/NO is an important physiological regulator of blood pressure (Aisaka et al., BBRC 160: 881-886, 1989).

Other laboratories had demonstrated that macrophage cells become "activated" by 12-36 hour treatment with gamma-interferon, bacterial endotoxin and various cytokines. This "activation" is associated with initiation of tumor cell killing and generation of nitrite and nitrate from L-arginine. We observed that activated macrophage actually make NO from L-arginine (just like endothelial cells) and that this NO subsequently reacts with oxygen to form more oxidized nitrogen metabolites which appear to be physiologically inert

(Stuehr et al., J. Exp. Med. 169: 1011-1020, 1989). The enzyme responsible for NO synthesis (nitric oxide synthetase) has been partially characterized by some of the present inventors (Stuehr et al. BBRC161: 420-426, 1989) and acts to oxidize the terminal amino group of arginine, resulting in production of NO and citrulline. It is now believed that macrophage-derived NO is an important tumoricidal and bactericidal agent. Since bacterial endotoxin, gamma-interferon and other cytokines can trigger NO generation by macrophage cells it appeared that: 1) endothelial cell NO generation may be stimulated by similar stimuli and 2) septic shock (i.e., systemic vasodilatation induced by bacterial endotoxin) may result from massive activation of NO biosynthesis. Speculation that the latter hypothesis was correct was fueled by a prior report that urinary nitrate levels are grossly elevated by treatment of rats with bacterial endotoxin (Wagner et al., PNAS 80: 4518-4521, 1983).

Cytokines are well known to cause morphological and functional alterations in endothelial cells described as "endothelial cell activation". Distinct immunemediators such as tumor necrosis factor (TNF), interleukin-1 (IL-1), and gamma-interferon (IFN) appear to induce different but partially overlapping patterns of endothelial cell activation including increased procoagulant activity (Bevilaqua, 1986), PGI₂ production (Rossi, 1985 Science 229,174), HLA antigen expression (Pober 1987) and lymphocyte adhesion molecules (Harlan 1985; Cavender 1987). Although these cytokines are reported to cause hypotension, vascular hemorrhage, and ischemia, the underlying mechanisms of altered vasoactivity is unclear (Goldblum et al. 1989; Tracey et al. Science 234:470, 1986). A potential mediator of altered vasoactivity is endothelial-derived relaxing factor (EDRF).

In both clinical and animal (Dvorak, 1959) studies on the effects of biological response modifiers a major dose limiting toxicity has been hypotension and vascular leakage.

SUMMARY OF THE INVENTION

The present invention involves a method for prophylaxis or treatment of an animal for systemic hypotension induced by a biological response modifier. Said method involves administering, preferably intravascularly, a therapeutically effective amount of an inhibitor of nitrogen oxide formation from arginine. Although preferable administration is intravascular, it is contemplated that other parenteral administration routes such as peritoneal, intramuscular or subdermal injection, for example, may prove useful. Enteral or topical administration may also prove beneficial for certain clinical conditions.

In one embodiment the inhibitor is N^G-substituted arginine or an N^G,N^G-disubstituted arginine which is administered to an animal possibly developing or having such induced systemic hypotension. The arginine antagonists of the present invention are preferably of the L configuration and include any pharmaceutically acceptable addition salts as commensurate with planned treatments.

Biological response modifiers which may induce such hypotension include gamma-interferon, tumor necrosis factor, interleukin-1 or interleukin-2. A particular use of the method of the present invention is for prophylaxis or treatment of systemic hypotension in a patient induced by chemotherapeutic treatment with at least one of tumor necrosis factor and interleukin-2. In this aspect the method involves intravascularly administering to