

**PREVENTING CONVERSION OF
CITRULLINE TO ARGININOSUCCINATE TO
LIMIT PATHOLOGICAL NITRIC OXIDE
OVERPRODUCTION**

This is a divisional of application Ser. No. 08/354,585 filed on Dec. 12, 1994 now U.S. Pat. No. 5,545,625.

This invention was made at least in part with Government support under National Institutes of Health Grant HL46403 and under National Institutes of Health Grant DK37116.

TECHNICAL FIELD

This invention is directed to a novel approach for inhibiting biological nitric oxide production.

BACKGROUND OF THE INVENTION

For several decades nitroglycerin has been administered to humans as a vasodilating agent in the treatment of cardiovascular disease. It has been shown that nitroglycerin so administered is converted in the body to nitric oxide which is the pharmacologically active metabolite. Recently, nitric oxide has been shown to be formed enzymatically as a normal metabolite from arginine in vascular endothelium to provide an important component of endothelium-derived relaxing factors (EDRFs) which are currently being intensively studied as participating in regulation of blood flow and vascular resistance. Macrophages have also been shown to produce nitric oxide in the body as a component of their cell killing and/or cytostatic function.

More recently it has been established that the enzyme forming nitric oxide from arginine, i.e., nitric oxide synthase, occurs in at least two distinct types, namely the constitutive forms and an inducible form. Constitutive forms are present in normal endothelial cells, neurons and some other tissues. Formation of nitric oxide by a constitutive form in endothelial cells is thought to play a role in normal blood pressure regulation. The inducible form of nitric oxide synthase has been found to be present in activated macrophages and is induced in endothelial cells and vascular smooth muscle cells, for example, by various cytokines and/or microbial products. It is thought that in sepsis or cytokine-induced shock, overproduction of nitric oxide by the inducible form of nitric oxide synthase plays an important role in the observed life-threatening hypotension. Furthermore, it is thought that overproduction of nitric oxide by the inducible form of nitric oxide synthase is a basis for insensitivity to clinically used pressor agents such as α_1 -adrenergic agonists in the treatment of septic or cytokine-induced shock patients. Moreover, it is thought that overproduction of nitric oxide by inducible form of nitric oxide synthase is involved in inflammation incident to an immune response.

Overproduction of nitric oxide is due either to overstimulation of a constitutive nitric oxide synthase (cNOS) or to overexpression of inducible nitric oxide synthase (iNOS). In either case, overproduction of nitric oxide is also dependent on adequate availability of arginine, the substrate of nitric oxide synthase. Arginine supply is maintained in three ways: (i) protein degradation, (ii) uptake of arginine from plasma, (iii) conversion of citrulline to arginine by pathways involving conversion of citrulline and aspartate to argininosuccinate (ASA) by argininosuccinate synthetase and conversion of ASA to arginine and fumarate by argininosuccinate lyase. In the case of iNOS, enzyme activity and overproduction of nitric oxide is also dependent on availability of required cofactors including tetrahydrobiopterin.

To date, pathological overproduction of nitric oxide has been controlled by administration of nitric oxide synthase inhibiting arginine antagonists, plasma arginine depleting enzymes and tetrahydrobiopterin induction or utilization blocking agents.

SUMMARY OF THE INVENTION

It has been discovered herein that administration of argininosuccinate synthetase activity reducing agents is useful to prevent or treat sepsis or cytokine-induced systemic hypotension, is useful in the treatment of sepsis or cytokine-induced systemic hypotension to restore vascular sensitivity to the effects of α_1 -adrenergic agonists, is useful to suppress an immune response, e.g., in treating inflammation, and is useful to prevent or treat a subject for a stroke.

One embodiment herein is directed to a method of prophylaxis or treatment of a subject for systemic hypotension caused by pathological overproduction of nitric oxide from arginine in vascular cells in said subject induced by therapy with a cytokine or by exposure to a bacterial endotoxin, said method comprising administering to a subject expected to develop or having such systemic hypotension a therapeutically effective amount of an argininosuccinate synthetase activity reducing agent. The agent can be either an argininosuccinate synthetase induction blocking agent or an argininosuccinate synthetase inhibitor. A therapeutically effective amount of an argininosuccinate synthetase activity reducing agent in this method is one that causes reduction in induced nitric oxide production to the extent of causing an increase in blood pressure.

Another embodiment herein is directed to a method for treatment of a subject for systemic hypotension caused by pathological overproduction of nitric oxide from arginine in vascular cells in said subject induced by therapy with a cytokine or by exposure to a bacterial endotoxin, said method comprising administering to a subject having such systemic hypotension a therapeutically effective amount of an α_1 -adrenergic agonist, and an amount of argininosuccinate synthetase activity reducing agent to restore vascular contractile sensitivity to the effects of said α_1 -adrenergic agonist. The argininosuccinate synthetase activity reducing agent can be either an argininosuccinate synthetase induction blocking agent or an argininosuccinate synthetase inhibitor. A therapeutically effective amount of α_1 -adrenergic agonist is one that causes increase in blood pressure.

Still another embodiment herein is directed to a method for treating a subject for systemic hypotension caused by pathological overproduction of nitric oxide from arginine in vascular cells in said subject induced by therapy with a cytokine or by exposure to a bacterial endotoxin, said method comprising administering to said subject a therapeutically effective amount of an arginine antagonist of nitric oxide synthesis by nitric oxide synthase and a therapeutically effective amount of an argininosuccinate synthetase activity reducing agent selected from the group consisting of argininosuccinate synthetase induction blocking agents and argininosuccinate synthetase inhibitors which do not block the activity of inducible nitric oxide synthase (by inhibiting inducible nitric oxide synthase by binding to it or its heme cofactor). The therapeutically effective amount of arginine antagonist of nitric oxide synthesis by inducible nitric oxide synthase is an induced nitric oxide production limiting amount, i.e., a blood pressure raising amount. The therapeutically effective amount of the argininosuccinate synthetase activity reducing agent in this method is that