

METHOD OF INHIBITING NITRIC OXIDE SYNTHASE

CROSS-REFERENCE TO RELATED APPLICATIONS

Not applicable

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

Not applicable

REFERENCE TO A MICROFICHE APPENDIX

Not applicable

BACKGROUND OF THE INVENTION

1. Field of Invention

This invention relates to novel inhibitors of nitric oxide synthase (NOS) that act at the level of enzymatic activity inhibition. In particular, this invention relates to the treatment of acute and chronic inflammatory conditions by the administration of NOS inhibitors.

2. Background

Nitric oxide (NO) is a gas radical produced by the enzyme nitric oxide synthase (NOS). There are three NOS enzymes, or isoforms, in mammals. A low level of NO is continuously produced by isoforms I and III, the constitutive or cNOS that are activated by calcium and the calcium-binding protein calmodulin. The NO produced is functional in vascular and nervous systems, and is important in the regulation of blood pressure and in neurotransmission. A high level of NO is produced by isoform II, the inducible or iNOS that is regulated by gene expression. The high level of NO is functional in the immune system as a means of host defense.

In the immune system, NO produced by one particular type of leukocytes, namely the macrophages, contributes to leukocyte killing of bacteria, fungi and tumor cells. Although meant to be protective, excess NO and NO metabolites, or reactive nitrogen intermediates, may also contribute to the destructive aspects of an immune response, particularly in chronic inflammation, by non-specific destruction of cellular metabolic machinery within a circumscribed area of NO release. Such non-specific destruction, if excessive, can lead to any one of a number of inflammatory diseases or syndromes, including autoimmune diseases, such as rheumatoid arthritis.

Interfering with the production of NO provides a means of modulating inflammatory reactions and of inhibiting destructive sequelae of a chronic inflammatory immune response. However, given that NO is highly reactive by nature, inhibitors which inhibit the NO radical directly would not be expected to be as effective as an inhibitor which blocks the synthesis of the NO radical.

A variety of inhibitors of the nitric oxide synthases has been reported, as seen from the following examples. U.S. Pat. No. 5,585,402 to Moncada and Palmer (1996) discloses N-monomethyl-L-arginine. U.S. Pat. No. 5,674,907 to Southan, Salzman and Szabo (1997) discloses mercapto derivatives as NOS inhibitors. U.S. Pat. No. 5,449,688 to Wahl, Allen and McCartney-Francis (1995) discloses a method of treating chronic inflammatory diseases using compounds including nitric oxide synthase inhibitors. However, these compounds are mostly generated by chemical synthesis, and may have additional adverse effects besides inhibiting the NOS enzymes. Hence, inhibitors from

natural sources are being sought after. It is anticipated that additional compounds may prove to have fewer side effects and greater selectivity in inhibiting the inducible nitric oxide synthase enzyme.

5 Epigallocatechin-3-gallate (EGCG) is the major polyphenol from green tea. Since tea is one of the most widely consumed beverages, second only to water, tea-derived EGCG may be much safer. Several beneficial effects of EGCG are known, as seen from the following examples. 10 U.S. Pat. Nos. 5,318,986 and 5,670,154 both granted to Hara and Honda (1994, 1997) disclose that tea polyphenols including EGCG inhibit the enzyme activity of alpha amylase and tyrosinase. U.S. Pat. No. 5,605,929 to Liao and Liang (1997) discloses catechins including EGCG inhibit 15 the enzyme activity of 5 alpha reductase. U.S. Pat. No. 5,391,568 to Chung (1995) discloses that EGCG inhibits lung cancer in a mammal. However, the effect of EGCG on the level of enzyme activity of nitric oxide synthase is unknown.

20 It is the object of the present invention to provide a method for the treatment of chronic and acute inflammatory conditions. More specifically, it is an object of the present invention to provide a method for the treatment of conditions wherein an agent that inhibits nitric oxide synthase is administered. 25

These and other objects and advantages of the present invention, as well as additional inventive features, will be apparent from the description of the invention provided herein. 30

BRIEF SUMMARY OF THE INVENTION

This invention is directed to a pharmacologically acceptable composition for inhibiting nitric oxide synthase (NOS) in a mammal. The composition includes a catechin derivative and a pharmaceutically acceptable carrier, with the active agent present in the composition in an effective amount to inhibit NOS in the mammal. 35

The present invention provides a method for treating a mammal, preferably a human, having an inflammatory condition, especially chronic, wherein an effective amount of an agent, which is capable of decreasing the amount of nitric oxide present, is administered. Preferably, the agent is an inhibitor of NOS. More preferably, the NOS inhibitor is a catechin derivative, such as the green tea polyphenol epigallocatechin-3-gallate (EGCG). 40

The invention may be more specifically regarded as inhibiting NOS at the level of gene expression and enzyme activity. Catechin derivatives, such as EGCG from green tea, competitively inhibit binding of the substrate arginine and the cofactor tetrahydrobiopterin to the NOS enzyme; and they also decrease the messenger RNA level of NOS. The invention for the composition and the method includes the catechin EGCG, both in natural and synthetic forms, and its structural derivatives generated by chemical and molecular biological processes such as via combinatorial library. 45 50 55

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a graph of the effect of epigallocatechin gallate (EGCG) on the relative level of inducible nitric oxide synthase (iNOS) gene expression in murine peritoneal cells.

FIG. 2 is a graph of the effect of EGCG on the enzyme activity of iNOS in cell lysate and in a partially purified fraction.

FIG. 3 is a graph on the kinetic analysis of the action of EGCG on iNOS activity with respect to arginine (substrate) and tetrahydrobiopterin (co-factor). 60 65