

**METHODS OF TREATING
OSTEOARTHRITIS WITH INDUCIBLE
NITRIC OXIDE SYNTHASE INHIBITORS**

**CROSS-REFERENCE TO RELATED
APPLICATION**

This application claims priority from U.S. Provisional Application Ser. No. 60/099,412, filed Sep. 8, 1998.

FIELD OF INVENTION

This invention is generally related to nitric oxide synthase inhibitors and more specifically related to treating patients having osteoarthritis with nitric oxide synthase inhibitors.

BACKGROUND OF THE INVENTION

Nitric oxide (NO) is an inorganic reactive gas molecule, important in many physiological and pathological processes where it is synthesized by cells mediating vital biological functions.

Nitric oxide serves as a neurotransmitter in the brain, produced in small amounts on an intermittent basis in response to various endogenous molecular signals. Endothelial cells lining the blood vessels also produce nitric oxide in small amounts, relaxing smooth muscle and regulating blood pressure. Indeed, the production of nitric oxide has a significant effect on the function of circulating blood cells such as platelets and neutrophils as well as on smooth muscle including blood vessels and other organs. Nitric oxide is also synthesized in the immune systems. Endotoxin and cytokines induce the production of large amounts of nitric oxide in response to infectious and inflammatory stimuli, contributing to both host defense processes such as killing of bacteria and viruses as well as pathology associated with acute and chronic inflammation in a wide variety of diseases.

Nitric oxide is formed from L-arginine oxydation by at least three different isoforms of nitric oxide synthases (NOS) in mammalian cells divided into two distinct classes, constitutive and inducible. The three NOS isoforms have been identified as:

- (i) Endothelial Nitric Oxide Synthase (eNOS); (Type III NOS), a constitutive, Ca^{++} /calmodulin dependent enzyme, located in the endothelium releasing nitric oxide in response to receptor or physical stimulation;
- (ii) Neuronal Nitric Oxide Synthase (nNOS); (Type I NOS), a constitutive, Ca^{++} /calmodulin dependent enzyme, located in the brain releasing nitric oxide in response to receptor or physical stimulation; and
- (iii) Inducible Nitric Oxide Synthase (iNOS); (Type II NOS), a Ca^{++} independent enzyme which is induced after activation of vascular smooth muscle, macrophages, endothelial cells, and a number of other cells by endotoxin and cytokines. Once expressed, this inducible NOS synthesizes large amounts of nitric oxide (NO) for long periods.

Nitric oxide generated by the constitutive enzymes acts as a transduction mechanism underlying several physiological responses. For example, eNOS is critical for production of nitric oxide, originally identified as endothelium derived relaxation factor (ERDF). The nitric oxide generated by eNOS regulates blood pressure in animals, blood flow in man, and prevents leucocyte adhesion.

On the other hand, the nitric oxide produced in large amounts by the inducible enzyme in is a cytotoxic effector molecule. As disclosed in U.S. Pat. No. 5,629,322, incorporated herein by reference, iNOS has been cloned from human liver and identified in more than a dozen other cells and tissues including smooth muscle cells, the kidney, and numerous epithelial cells in a variety of tissues including the lung and colon. This enzyme is induced upon exposure to lipopolysaccharide (LPS) and cytokines such as gamma interferon (IFN- γ), interleukin-1 β (IL-1 β), and tumor necrosis factor (TNF). Once induced, nitric oxide production by iNOS continues over a prolonged period of time, and the activity of iNOS is relatively independent of intracellular Calcium concentrations.

iNOS is implicated in conditions leading to cytokine-induced hypotension including septic shock, hemodialysis and IL-2 therapy in cancer patients. The excess production of nitric oxide generated by the inducible form of nitric oxide synthase also appears to contribute to cytokine-mediated inflammation, cytotoxicity and tissue damage. accordingly, certain conditions have been identified where inhibiting nitric oxide production is advantageous. These conditions include arthritis, inflammatory bowel disease, cardiovascular ischemia, diabetes, congestive heart failure, myocarditis, atherosclerosis, migraine, reflux esophagitis, diarrhea, irritable bowel syndrome, cystic fibrosis, emphysema, asthma, bronchiectasis, hyperalgesia (allodynia), cerebral ischemia (both focal ischemia, thrombotic stroke and global ischemia (secondary to cardiac arrest), multiple sclerosis and other central nervous system disorders, for example Parkinson's disease and Alzheimer's disease, and other disorders mediated by NO including opiate tolerance in patients needing protracted opiate analgesics, and benzodiazepine tolerance in patients taking benzodiazepines, and other addictive behavior, for example, nicotine and eating disorders.

Further conditions in which there is an advantage in inhibiting NO production from L-arginine include systemic hypotension associated with septic and/or toxic shock induced by a wide variety of agents; therapy with cytokines such as TNF, IL-1 and IL-2; as an adjuvant to short term immunosuppression in transplant therapy; and as a chemopreventative.

Although the potential uses for NOS inhibitors has been implicated in numerous diseases, the efficacy and outcome of using a NOS inhibitor to prevent, treat and cure many diseases has never been identified. For example, U.S. Pat. No. 5,629,322 at Col. 15 beginning line 60 lists an enormity of disease types where the NOS inhibitors may be used to treat a disease. However, the disease types are named as a result of speculation, without examples or analysis. Examples of other compounds which inhibit the production of nitric oxide can be found in U.S. Pat. Nos. 5,684,008 and WO 93/13055, each incorporated by reference as if written herein.

The effect and efficacy of NOS inhibitors and specifically selective iNOS inhibitors in vivo on disease progression for the many diseases has not been addressed. Therefore, the outcome and consequence of the use of the inhibitors on disease progression in vivo in many cases remains unknown. Although some information has been generated in vivo in