

## METHOD OF TREATING CHRONIC INFLAMMATORY DISEASES

### TECHNICAL FIELD OF THE INVENTION

The present invention relates to a method of treating acute and chronic inflammatory conditions, including autoimmune diseases. In particular, the present invention relates to a method of reducing the amount of nitric oxide present at a site of inflammation by administering a nitric oxide synthase inhibitor, a nitric oxide scavenger, or an inhibitor of tetrahydrobiopterin synthesis.

### BACKGROUND OF THE INVENTION

Inflammatory and immune reactions depend upon the recruitment and migration of circulating leukocytes to sites of injury or antigen exposure. Accumulation and activation of leukocytes result in the generation of numerous cytokines, growth factors, enzymes, and mediators, which participate in the further recruitment and activation of leukocytes, thereby augmenting and perpetuating the defense of the injured or antigen-exposed mammal.

When a particular type of leukocyte, namely the macrophage, is activated by bacteria, bacterial products, T lymphocyte-derived cytokines, and antigens, it responds by converting arginine into nitric oxide (NO). NO is just one of a number of highly toxic free-radicals, which include oxygen ( $O_2^-$ ), peroxide ( $H_2O_2$ ), and hydroxyl radicals (OH $\cdot$ ). When released from macrophages as part of the host defense mechanism, NO contributes to leukocyte killing of bacteria, fungi and tumor cells. The extracellular release of NO from other cells and tissues, such as endothelium, may cause vasodilation and tissue damage.

NO is produced by the action of a NO synthase. Some cells, such as macrophages, express an inducible NO synthase, which produces large quantities of NO upon stimulation. In contrast, cells, such as neurons and endothelial cells, possess a constitutive form of this enzyme. In other words, the NO synthase possessed by these cells produces NO continuously at a constant level.

Although meant to be protective, reactive nitrogen intermediates can actually suppress host defenses during some infections, such as listeriosis and brucellosis, and thus play a pathogenic role in some infectious diseases. The NO pathway also may contribute to the destructive aspects of an immune response, particularly in chronic inflammation, by the nonspecific destruction of cellular metabolic machinery within a circumscribed area of NO release. Such nonspecific 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 the 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.

It is an object of the present invention to provide a method for the treatment of chronic and acute inflammatory conditions, including autoimmune diseases. More specifically, it is an object of the present invention to provide a method for the treatment of such condi-

tions wherein an agent that decreases the amount of nitric oxide present is administered. 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.

### SUMMARY OF THE INVENTION

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 nitric oxide synthase (NOS), a nitric oxide scavenger, or an inhibitor of tetrahydrobiopterin synthesis. More preferably, the NOS inhibitor is a L-arginine analog, such as N<sup>G</sup>-monomethyl-L-arginine, N-nitro-L-arginine methyl ester, N<sup>G</sup>-nitro-L-arginine, N-iminoethyl-L-ornithine, N<sup>G</sup>-amino-L-arginine, L-canavanine, citrulline, canaline, homocitrulline, or aminoguanidine, or a cytokine, such as MDF, TGF- $\beta$ 1, TGF- $\beta$ 2, TGF- $\beta$ 3, IL-4, or IL-10. Even more preferably, the NOS inhibitor is N-iminoethyl-L-ornithine, citrulline, canaline, homocitrulline, aminoguanidine, MDF, or IL-10. The NO scavenger is preferably hemoglobin or ferrous diethyldithiocarbamate (DETC). The inhibitor of tetrahydrobiopterin synthesis is preferably 2,4-diamino-6-hydroxy-pyrimidine.

The inflammatory condition to be treated may be any one of a number of inflammatory conditions, such as rheumatoid arthritis, osteoarthritis, systemic lupus erythematosus, psoriasis, discoid lupus, collagen vascular disease, diabetes mellitus, myositis, polyarteritis, scleroderma, sarcoidosis, granulomatous lesions such as hepatic granulomas, inflammatory bowel disease, thyroiditis, multiple sclerosis, graft versus host disease, organ transplant rejection, sepsis, acute respiratory distress syndrome, cirrhosis, periodontitis, gingivitis, AIDS dementia, primary biliary cirrhosis, granulomatous hepatitis, Wegener's granulomatosis, chronic granulomatous disease, allergic granulomatosis, granulomatous arteritis-Folymyalgia rheumatica, and inflammation of the central nervous system.

### BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a graph of articular index versus days after injection with either SCW alone (SCW) or SCW in combination with NMMA (SCW+NMMA).

FIG. 2 is a bar graph of nitrite concentration ( $\mu$ M), citrulline (% of total cpm), and urea (% of total cpm), versus synovial tissue 3 days and 17 days post-injection and a control sample.

FIG. 3 is a graph of articular index versus days after injection with SCW. The SCW line follows the articular index over 22 days after injection with SCW, whereas the SCW+NMMA line follows the articular index over 12 days after injection with SCW, at which point the animals were injected with NMMA, and then over the 10 days following injection with NMMA.

FIG. 4 is a bar graph of articular index versus treatment with SCW alone (SCW), SCW and aminoguanidine (SCW+AG), and SCW and hemoglobin (SCW+Hb).

FIG. 5 is a bar graph of nitrite concentration ( $\mu$ M) versus untreated (PBL, control) and SCW-treated (PBL, SCW) peripheral blood leukocytes and untreated