

## METHODS FOR INHIBITING INFLAMMATORY ISCHEMIC, THROMBOTIC AND CHOLESTEROLEMIC DISEASE RESPONSE WITH METHIONINE COMPOUNDS

This application is a continuation-in-part of Application Ser. No. 07/179,226, filed on Apr. 8, 1988 now abandoned.

### TECHNICAL FIELD

This invention concerns novel methods employing antioxidant and antiinflammatory dietary or therapeutic compositions containing the amino acid methionine (also known as "Met"), and/or one or more related compounds including certain metabolic precursor compounds for inhibiting inflammatory ischemic, thrombotic and cholesterolemic disease response in man and animals.

### BACKGROUND OF THE INVENTION

A variety of efforts have been made over many years to elucidate the mechanisms and origins of inflammation and the various forms of disease it may cause. Limited success has been achieved in alleviating the symptoms of diseases having inflammatory components. Oxidative stress has been implicated in many of these diseases, and antioxidant therapy has been recommended as one method to alleviate the damage it causes (Cross, et. al., *Annals of Internal Medicine*, 107:526-45, 1987).

Recently, several investigators have focused on the role of sulfhydryl compounds in the mechanism of treatment of some forms of arthritis. Cuperus, (*Arthritis and Rheumatism* 28: 1228-33 1985) showed that d-penicillamine, tiopronin, aurothiomalate and aurothioglucose were scavengers of the products of activated granulocytes, and Bailey and Sheffner (*Biochemical Pharmacology* 16: 1175-82, 1967) showed that acetylcysteine and acetylpenicillamine reduced experimental dermal inflammation but that methionine did not. Methionine is known to be oxidized to its sulfoxide by granulocytes but not by hydrogen peroxide at physiological concentrations. Persons deficient in the enzyme myeloperoxidase do not make hypochlorous acid in lymphocytes and appear not to suffer unusually from infections. By contrast, persons with deficient production of hydrogen peroxide are adversely affected. (Stendahl, et al., *J. Clin. Invest.*, 73:366-73, 1984).

Cuperus, *supra*, describes a feature of inflamed synovial fluid, such as that occurring in arthritis patients, as the accumulation of polymorphonuclear (PMN) leukocytes. One function of the leukocytes is the destruction of invading elements such as microorganisms. For this destruction, the leukocyte releases hydrogen peroxide and enzymes, e.g., myeloperoxidase, into the extracellular fluid. In the presence of hydrogen peroxide and chloride ion, myeloperoxidase catalyzes the formation of reactive hypochlorous acid (HOCl) which can oxidize tissue components and plasma protease inhibitors. Oxidation and subsequent inactivation of these protease inhibitors in vivo may lead to unrestrained proteolysis, resulting in severe tissue damage. (Weissmann, et.al., *Jour. Investigative Dermatology*, 71:95-9, 1978).

Several investigators have noted that patients with severe rheumatoid arthritis have lower levels of serum SH groups (Hall, *Journal of Rheumatology* 9:593-6, 1982). Ambanelli (*Scand. Jour. Rheumatology*

11:203-7, 1982) showed that serum SH groups went up in patients that responded to tiopronin therapy. The mechanism of serum SH groups in relation to the severity of arthritis has not been established. The correlation could be explained by the failure of particular individuals to counteract the production of oxidizing substances by immunocytes.

McKenna, (*British J. Rheumatology* 25:132, 1986), saw benefit for only 2 of 15 patients given cysteine methyl ester for rheumatoid arthritis, a direct sulfhydryl agent.

Delrieu, et al., (*Revue du Rhumatisme*, 55:995-7, Dec., 1988) found no statistical difference between treatment and controls in a 24 patient study of rheumatoid arthritis using 5 and 10 grams of l-methionine per day for 4 and 2 months, respectively. Clinical tolerance was good, but gastrointestinal distress was encountered by a majority of the patients.

Gualano (*Pharmacology Research Comm.* 15:683-96, 1983) showed antiinflammatory activity of S-adenosyl methionine but attributed its effects to mechanisms of aspirin-like drugs. Davis, (*Jour. Am. Pod. Assoc.* 68:24-30, 1978) studied the effects of certain amino acids on inflammation measured as edema and found that methionine was not effective in reducing edema while cystine was effective. Marcolongo (*Current Therapeutic Research* 37:82-94, 1985) showed beneficial effects of S-adenosyl methionine slightly better than ibuprofen in the treatment of hip and knee osteoarthritis. Stramentinoli (*Am. Jour. Medicine*, 83 Suppl 5A:35-42, 1987) shows that S-adenosyl methionine will inhibit the swelling in carrageenin-induced rat paw edema, while l-methionine in equimolar doses is completely ineffective.

Other studies involving the oral administration of S-adenosyl methionine have shown that treatment does not increase the blood levels of methionine (Baldesarini, et. al., *Arch. Gen. Psychiatry*, 36:303-7, 1979). In animal studies blood level increases of methionine are reflected by parallel increases in brain levels of methionine, but a 10 fold increase in brain methionine produces only a 50% increase in brain S-adenosyl methionine (Rubin, et al., *J. Neurochemistry* 23: 227-231, 1974).

In a study of the immunosuppressive activity of D-amino acids, Inoue, et al. showed that there was no immunosuppressive effect for d-methionine in their mouse assay at a dose of 10 mg per kg body weight. (*Japanese J. Experimental Medicine*, 51:363-6, 1981).

Regarding acute inflammation, the complement system of the human body (see Spector, W. G., *Intro. to General Pathology*, p. 58-75, Churchill Livingstone, New York, 1980) is part of a cascade of enzyme reactions that are responsive to external injury in which complement is activated and generates peptides known as C3a and C5a which are response-inducing or chemotactic for white cells.

The S-methyl derivative of methionine, S-methyl methionine, also known as vitamin U has been shown to have benefit as an anti-ulcer compound and to have benefit for allergies. The same benefit is shown for carboxyl esters and N-acyl derivatives (Kowa, DT 2821-704). However, in this teaching no distinction is made for the d- and l-isomers of S-methyl methionine or its derivatives and no claim is made that these compounds act through anti-inflammatory mechanisms.