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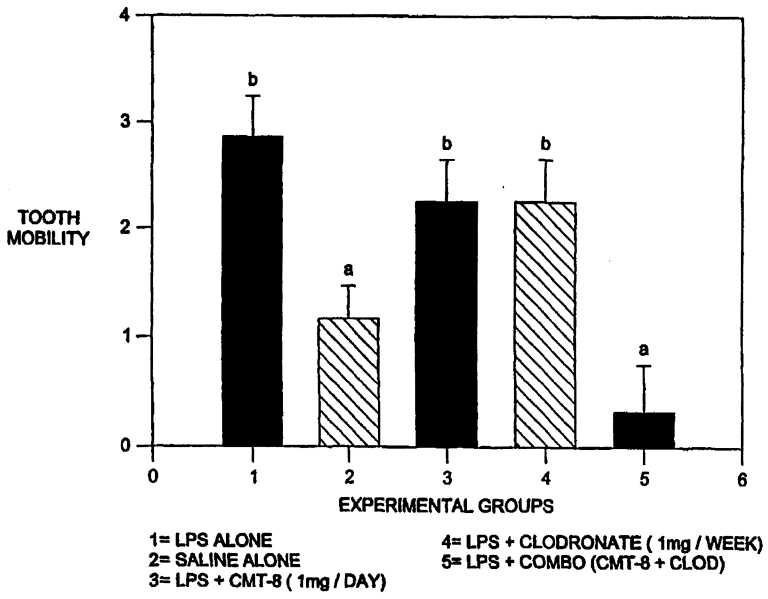

(57) Abstract

Tissue–destructive conditions related to excess proteinase activity in a biological system are treated or prevented by administering to the system a composition which combines a tetracycline and a bisphosphonate in synergistic proteinase inhibiting amounts. The effectiveness of such compositions can be demonstrated in standard tests, for example by measuring tooth mobility.

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COMBINATION OF BISPHOSPHONATE AND TETRACYCLINE

This invention was made with government support under R37DE03987 awarded by the National Institute of Dental Research (NIH). The government has certain rights in the invention.

The invention relates to a combination of tetracyclines and bisphosphonates which act synergistically to inhibit, reduce, down-regulate and/or prevent degradation of connective tissue, basement membrane as well as other factors in subjects susceptible to this type of tissue degradation.

BACKGROUND OF THE INVENTION

Proteolytic activity is responsible for damage to connective tissues and basement membranes as a complication of the inflammatory and/or immune response and other disease processes, such as cancer cell invasion and metastasis. The inflammatory response contributes, for example, to the pathological changes in a number of acute and chronic processes involving diverse organs and tissues such as the lungs, bone, heart, joints, skin and periodontium, etc.

The proteinases involved in these responses or disease processes include matrix metalloproteinase (MMP's), MMP-like proteinases and related proteinases, serine proteinases and other proteinases. The MMP's are zinc and calcium-dependent for hydrolytic cleavage of substrate proteins and are secreted or released by a variety of host cells (e.g., polymorphonuclear neutrophils (PMN's), macrophages, bone cells, epithelium and fibroblasts). Certain other genetically distinct MMP's called membrane-type MMP's (MT-MMP's) are cell membrane-bound; others are secreted into the extracellular matrix (ECM). With serine proteinases, the amino acid serine acts as a nucleophile for hydrolytic cleavage of substrate protein. Serine proteinases are released, e.g., by triggered leukocytes, more specifically by the azurophilic granules of PMN's, and other cells including malignant tumor cells.

Several studies have shown that the expression and activities of MMPs are pathologically elevated over the body's endogenous anti-proteinase shield in a variety of diseases such as cancer metastasis, rheumatoid arthritis, multiple sclerosis, periodontitis, osteoporosis, osteosarcoma, osteomyelitis, bronchiectasis, chronic pulmonary obstructive disease, skin and eye diseases. Proteolytic enzymes, especially MMPs, are believed to contribute to the tissue destruction damage associated with these diseases.

Some metalloproteinases (MMP's) and their association with diseases are discussed by M.E. Ryan, et al., *Curr. Op. Rheum.*, 1996, 8:238-247. More than twenty MMP's have been identified and the number is growing. These include Interstitial Collagenases MMP-1 (fibroblast-type), MMP-8 (polymorphonuclear leukocyte - PMNL- type or collagenase-2), MMP-13 (collagenase-3); Gelatinases MMP-2 (72-kD gelatinase A) and MMP-9 (92-kD gelatinase B); Stromelysins MMP-3 (stromelysin - 1), MMP-10 (stromelysin -2), and MMP-7 (matrilysin or putative metalloproteinase (PUMP) -1); Membrane Type (MT-MMP's), MMP-14 (MT_1-MMP), MMP-15 (MT_2-MMP), MMP-16 (MT_3-MMP); others are, for example, MMP-11 (stromelysin -3), MMP-12 (macrophage metalloelastase) and MMP-20. Enamelysin (MMP-20) is described by Llano et al., *Biochem.* 1997, 36:15101-15108, and can also be expressed by human cancer cells such as squamous carcinoma cells of the human tongue indicating its potential contribution to cancer progression and invasion (Salo et al., *J. Dent. Res.* 1998, 77:829, Abstr. No. 1978). Related proteinases include TACE's and ADAM's fertilin or meltrin (metalloproteinase/disintegrin).

MMP's, MMP-like and related proteinases such as TACE's, ADAM's, etc., are involved in processing and modification of molecular phenomena such as tissue remodeling (Birkedal-Hansen, *Current Opin. Cell Biol.* 1995, 7:728-735; JF Woessner, Jr., *FASEB J.* 1991, 5:2145-2154), cytokine actions (S. Chandler et al., *J. Neuroimmunol.* 1997, 72:155-161), cell-cell fusion (RH van Huijsduijen, *Gene* 1998, 206:273-282; Huovila et al., *Curr. Opin. Cell Biol.* 1996, 8:692-699; Yagami-Hiromasa et al., *Nature* 1995, 377:662-656), angiogenesis, growth factor actions,

integrin and other adhesion factors and their receptor processings. See also, A.C. Perry et al., *Biochem. Biophys Acta* 1994, 1207:134-137. The ADAM enzymes are membrane proteins with A Disintegrin and Metalloproteinase Domain (Wolfsberg et al., *Dev. Biol.* 1995, 169:378-383). TACE is tumor necrosis factor converting enzyme.

MMP-like proteinases and related proteinases are metalloproteinases distinct from classic MMP's and can be involved in cellular processing of pro-TNF alpha (Tumor Necrosis Factor), cellular shedding of cytokine receptors, adhesion molecules, etc. as described by S. Chandler et al., *J. Neuroimmunol.* 1997, 72:155-161. MMP's and MMP-like and related enzymes, e.g., ADAM's, TACE's, etc., also mediate the release of TNF alpha (Watanabe et al., *Eur. J. Biochem.* 1998, 253: 576-582) and are involved in membrane-bound processing of TNF alpha by monocytes induced by bacterial-virulence factors. This event is mediated by membrane-bound metalloproteinases. Shapira et al., *J. Period. Res.* 1997, 32:183-185.

There is extensive evidence for the association between proteinases and a large number of disease processes. Microbial proteinases can act in concert with host proteinases in the promotion of tissue destruction as seen in periodontium (Sorsa et al., *Infect. Immun.* 1992, 60: 4491-4495). Recent studies indicate that a serine protease, i.e., elastase, may play a role in connective tissue breakdown and tissue invasion in the Dunning rat model of cancer invasion and metastases (prostate cancer) (Lowe and Isaacs, *Cancer Res.* 1984, 44:744-52). Also involved in initiating the proteinase cascade that mediates tumor invasion and metastasis are trypsin and chymotrypsin-like activity (Sorsa et al., *J. Biol. Chem.* 1997, 272:21067-21074). Serine proteinase is expressed in human cancers such as ovarian carcinoma and cholangiosarcoma (Sorsa et al., *J. Biol. Chem.* 1997, 272:21067-21074).

The role of MMP's has been well-established in a great many disease states, e.g., tumor invasion and metastasis (Stetler-Stevenson et al., *Annu. Rev. Cell Biol.* 1993, 9:541-73; Tryggvason et al., *Biochim. Biophys. Acta* 1987, 907:191-217) and

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