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INHIBITION OF MATRIX METALLOPROTEINASES

THERAPEUTIC APPLICATIONS

Edited by Robert A. Greenwald, Stanley Zucker, and Lorne M. Golub

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The cover of the paper-bound edition of this volume shows the catalytic domain of a crystal structure of truncated MMP-3 bound to an MMP inhibitor. The inhibitor shown is PGE-116611, the chemical name of which is: (2R)-isobutyl-(3S)-[N-hydroxycarboxamido]-6-hydroxyhexanoic acid amide of (1N)-2-[methoxyethyl]-caprolactam-(3S)-amine. The cover illustration was generously provided by Drs. Biswanath De and Glen Mieling of Procter and Gamble Pharmaceuticals.

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Tetracycline Derivative CMT-3 Inhibits Cytokine Production, Degranulation, and Proliferation in Cultured Mouse and Human Mast Cells

KARI K. EKLUND[a,c] AND TIMO SORSA[b]

[a]*Institute of Biomedicine, Department of Medical Chemistry and Department of Internal Medicine, Helsinki University Hospital, Helsinki, Finland*

[b]*Department of Dentistry, Helsinki University, Helsinki, Finland*

INTRODUCTION

Activated mast cells (MC) produce a wide variety of inflammatory mediators such as histamine, eicosanoids, proteases, and several cytokines. Recent findings emphasize the pathogenetic role of MC not only in allergic diseases, but also in diseases associated with chronic inflammation, such as connective tissue diseases.[1]

Tetracyclines are commonly used antibiotics that have therapeutic properties other than those related to their antimicrobial activity. They have been shown to be potent inhibitors of collagenases and to have beneficial antiinflammatory effects in rheumatoid arthritis.[2,3] Chemically modified tetracyclines (CMT) are tetracycline derivatives that do not have the antimicrobial activity but have retained their other properties. We decided to study the effect of three CMTs (CMT-1, CMT-3, CMT-5) on key functions of mast cells to find out whether CMTs could also have antiallergic properties.

MATERIALS AND METHODS

Cell Culture

Mouse bone marrow–derived mast cells (mBMMC) were generated by culturing bone marrow cells of BALB/c mice (animal facilities of Helsinki University) for 2–5 weeks in enriched medium supplemented with 50% WEHI-3 cell (line TIB-68; ATCC, Rockville, MD) conditioned medium as a source of IL-3. Human mast cell line (HMC)-1 cells[4] were cultured in Iscove's medium supplemented with 10% FCS.

Activation of MC

mBMMC were activated with calcium ionofore A23187 (5 mM, Sigma), and supernatants and cells were analyzed for β-hexosaminidase activity. HMC-1 cells were

[c]Address for correspondence: Kari Eklund, Institute of Biomedicine, Department of Medical Chemistry, University of Helsinki, Siltavuorenpenger 10, 00170 Helsinki, Finland.

activated with a combination of phorbol 12-myristate 13-acetate (PMA) 50 ng/ml (Sigma) and calcium ionofore A23187 0.5 μM, and cytokines were analyzed 24 hr after activation of the cells using a commercial ELISA method (R&D Systems, London, England). The viability of the cells was assessed by counting the trypan blue excluding cells 24 hr after activation. Viability was not affected by the CMTs in the concentrations used.

RESULTS AND DISCUSSION

A clear dose-dependent inhibition of TNF-α and to a lesser degree of IL-8 production was observed in HMC-1 cells by CMT-3 but not by CMT-1 or CMT-5 (FIG. 1). In the presence of 25 μM CMT-3 75% and 40% inhibition of TNF-α and IL-8 production, respectively, was observed. The effect of CMT-3 on mBMMC degranulation, as revealed by the granule-associated β-hexosaminidase release, is shown in FIGURE 2. A clear inhibition of calcium ionofore–induced degranulation was observed in the presence of 25 μM and 50 μM CMT-3. No clear effect on degranulation could be observed by the CMT-1 or CMT-5 (data not shown).

The reason for the inhibition of degranulation and cytokine production by CMT-3 is not clear at present. Both serine and metalloproteinases have been implicated in the degranulation of mast cells. Tetracyclines are known also to have metal chelating properties, which could perhaps account for the inhibitory effect of CMT-3. To con-

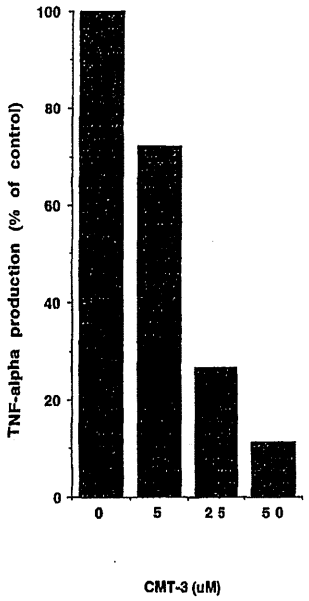

FIGURE 1. The effect of CMT-3 on TNF-α production in HMC-1 cells. Cells (1×10^6/ml) were activated with PMA (50ng/ml) and calcium ionofore A12387 (0.5 μM). CMT-3 was added 1 hr before the activators. Similar results were obtained in a replicate experiment.

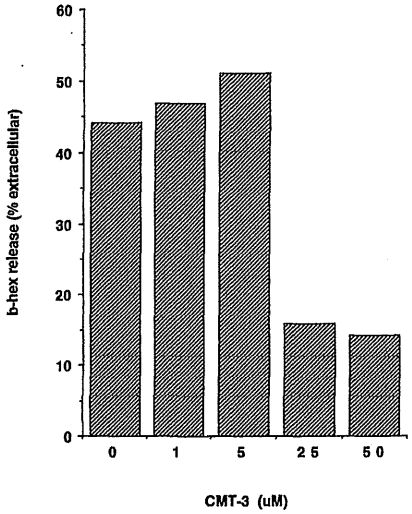

FIGURE 2. Degranulation of mouse bone marrow–derived mast cells (1×10^6/ml) in the presence of CMT-3. Degranulation was induced with calcium ionofore (A12387, 5 μM), and β-hexosaminidase activity of the cells and supernatants was analyzed 20 min later. Similar results were obtained in a replicate experiment.

clude, CMT-3 inhibits very efficiently several key functions of MC and could therefore have potential use in the treatment of mast cell–related diseases such as various allergic diseases.

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