Suppression of fibroblast activity by an inhibitor of dipeptidyl peptidase IV: A possible therapeutic strategy for rheumatoid arthritis

Sirs,

Dipeptidyl peptidase IV (DP IV) is an ectoenzyme that selectively cleaves X-Pro dipeptides from polypeptides or proteins and expressed on a variety of cell types. In the immune system, CD26, a co-stimulatory molecule highly expressed on activated T cells, possesses DP IV activity in the extracellular domain. Extensive studies have documented the involvement of CD 26 DP IV in immune responses (1, 2),although the natural substrates for this enzyme remain to be determined. In our previous study, administration of a specific inhibitor of DP IV, (2S,2S',2S")-2-[2'-[2"-amino-3"-(-indol-3"'-yl)-1"-oxopropyl]-1',2',3',4'-tetrahydro-6',8'-dihydroxy-7'-methoxyisoquinol-3-yl-carbonylamino]-4-hydroxymethyl-5-hydroxypentanoic acid (TMC-2), ameliorated adjuvant arthritis in rats without noticeable adverse effects (3). Because TMC-2 suppressed the synthesis of IL-2 and antigen-stimulated proliferation of T cells, one of its anti-arthritic mechanisms can be ascribed to suppression of T cell functions (4). It has recently been revealed, however, that fibroblast-like synovial cells of patients with rheumatoid arthritis (RA-FLS) also express DP IV abundantly (5, 6), and this prompted us to test the effect of TMC-2 on fibroblast activity.

After 48 h starvation in serum-free medium, TMC-2 was added to murine fibroblast-like cell line BALB/3T3 cultures, and 30 min later the cells were stimulated with 50-100 ng/ml platelet derived growth factor (PDGF, PeproTech, London, UK). TMC-2 at 100-300 µM suppressed proliferation of the cells without significant cytotoxicity. To study the biochemical mechanisms leading to this effect, the cells were lysed 15 min after PDGF stimulation, and the PDGF receptor was analyzed by western blotting using antibodies to PDGF receptor β and tyrosin-phosphorylated PDGF receptor β (Santa Cruz Biotechnology, Santa Cruz, CA, USA). MAP kinases were also analyzed using antibodies to phosphorylated c-Jun N-terminal kinase (JNK) and extracellular signal-regulated kinase (Erk) (Cell Signaling, Beverly, MA, USA). It was found that TMC-2 suppressed PDGF-induced tyrosine phosphorylation of the PDGF receptor (Fig.1 a), resulting in suppression of phosphorylation of downstream MAP kinases JNK and Erk (Fig. 1b).

It has been suggested that PDGF is involved in the pathogenesis of RA in several ways; for example, it stimulates proliferation of RA-FLS, secretion of cathepsin B and formation of fibrillar fibronectin matrix by RA-FLS (7-9). Further study is required to reveal the nature of the DP IV molecule on fibroblasts, whether it is identical to CD26 DP IV on T cells, and the linkage mechanism between the DP IV activity and PDGF receptor phosphorylation. The present study suggests the intriguing possibility that application of DP IV inhibitors may be useful to control hyperactivity of RA-FLS.

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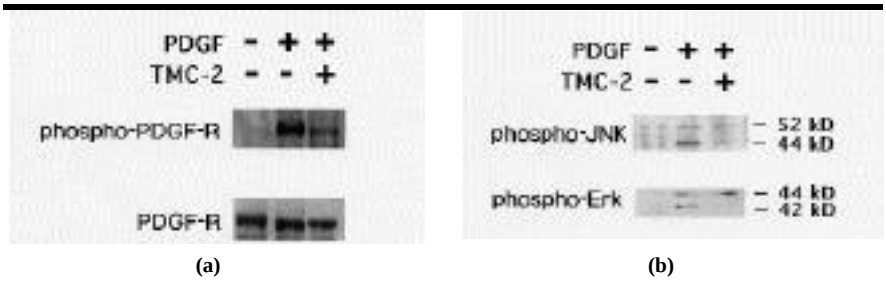

(a) **(b)**

Fig. 1. (**a**) Suppression of tyrosine phosphorylation of the PDGF receptor by TMC-2. Lysate of BALB/3T3 fibroblast-like cells harvested 15 min after stimulation with 100 ng/ml PDGF, in the presence or absence of TMC-2 (300 µM), was analyzed by western blotting using antibodies to PDGF receptor β and tyrosin-phosphorylated PDGF receptor β.
(**b**) Suppression of PDGF induced phosphorylation of MAP kinases by TMC-2. The same cell lysate prepared as above was analyzed using antibodies to phospho-JNK (Thr 183/Tyr 185) and phospho-Erk (Thr 202/Tyr 204).

Temporal arteritis masquerading as chronic myelomonocytic leukemia

Sirs,

Temporal or giant cell arteritis (GCA) is a chronic inflammatory disease of large- and medium-sized blood vessels occurring primarily in the elderly (1,2). We describe a patient presenting with constitutional symptoms and persistent peripheral blood monocytosis due to GCA.

An 86-year-old man was referred due to worsening pain and "stiffness" of the shoulder and pelvic girdles, frontal headaches, a low-grade fever, and a 5 kg-weight-loss over the previous 4 months. Three months before a "high monocyte blood count" was incidentally discovered, while a complete blood count was normal ten months earlier. Bone marrow examination showed changes of "myelodysplasia" and the patient was periodically followed with a presumptive diagnosis of chronic myelomonocytic leukemia (CMML).