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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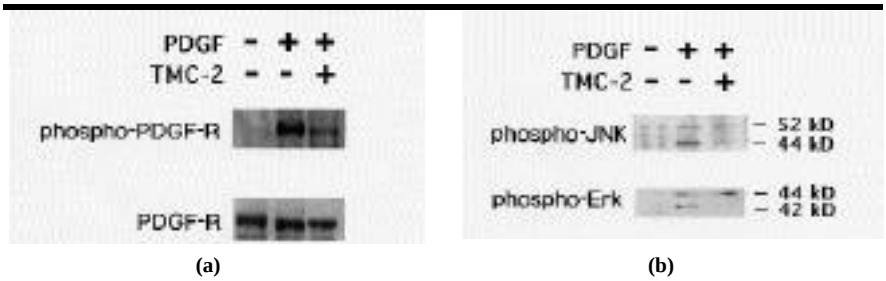

(a) **(b)**

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).

Physical examination revealed arthritis of the left wrist and right knee. There was scalp tenderness, and the temporal arteries were palpable. Current medications included quinapril and hydrochlorothiazide. Complete blood count showed: hematocrit 0.29 (MCV 84 fL, MCHC 330 g/L), white blood cells 8.7×10^9 /L and absolute monocytosis (monocytes 1.530×10^9/L) and platelet count 268×10^9/L. Erythrocyte sedimentation rate (ESR) was 101 mm/h and C-reactive protein (CRP) 52 mg/L. A thorough laboratory and imaging evaluation was within normal limits.

Biopsy and histopathological examination of the right temporal artery showed mixed-cell inflammatory infiltrate with disruption of the internal elastic lamina. The patient was treated with oral prednisone 60 mg daily and symptoms improved within the first week. A laboratory evaluation a month later showed: hematocrit 0.42, normal leukocyte and monocyte counts (9.9×10^9 /L, and 0.38×10^9 /L, respectively) and ESR 36 mm/h.

Although the presenting symptoms could raise the suspicion of GCA, the hematological findings of this patient were misleading. Besides a high ESR, one would expect anemia plus/minus thrombocytosis with normal white cell counts (1-3). A monocytosis is characteristic of the CMML, while immature myeloid cells usually occur in the less well-differentiated subgroups of myelodysplastic syndromes (4, 5). However, cytogenetic studies were not performed in this patient.

The pathogenesis of GCA remains obscure (2, 6). It has been proposed that an antigen-mediated T-cell response of the vessel wall promotes the differentiation of macrophages and the formation of giant cells. The overactivity of these cells could be harmful to the vessel wall (7). The findings of the temporal artery biopsies are either focal and segmental granulomatous inflammation with giant cell infiltration between the intima-media space, or mixed cell infiltration, composed mainly by lymphomononuclear cells, without giant cells (2). These findings show that an exaggerated inflammatory response takes place focally in the affected vessels. In this patient, blood monocytosis could be a manifestation of the inflammatory reaction. This effect might be attributed to a pro-inflammatory response and/or to an exaggerated reaction in response to circulating cytokines. In this context, monocyte chemoattractant protein-1 (MCP-1) has been found to be increased in mononuclear cells of temporal artery biopsies as well as in the plasma of patients with GCA (8). Moreover, GCA has been reported to occur concomitantly with various myelodysplastic syndromes (4,5), which is in concert with the "dysplastic changes" in the bone marrow biopsy examination of this patient and the early-suggested diagnosis of CMML. It seems that the systemic inflammatory response in this patient was manifested by arteriall wall infiltration in association with reactive changes in the bone marrow. Hence, corticosteroid treatment resulted in a dramatic resolution not only of symptoms, but also of peripheral blood abnormalities.

Conclusively, GCA may be associated with excessive peripheral blood monocyte counts. Clinical suspicion should be verified by temporal artery biopsy since prompt initiation of corticosteroid treatment is mandatory.

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