FR167653, a potent p38 MAPK inhibitor, suppresses the onset of collagen induced arthritis in rats

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Introduction  Rheumatoid arthritis (RA) is characterized as chronic and progressive inflammatory processes of affected joints with systemic immunological abnormalities leading to synovial hyperplasia. Cytokines which are produced from the inflamed rheumatoid synovial cells such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8) play crucial roles in the rheumatoid pathophysiology. FR167653 was first identified as an inhibitor of TNF-α and IL-1β production in lipopolysaccharide (LPS)-stimulated human monocytes and phytomelagginin M-stimulated human lymphocytes. Further investigation revealed that FR167653 inhibits the activation of p38 mitogen activated protein kinase (p38 MAPK) by suppressing the phosphorylation of p38 MAPK. It is known that p38 MAPK plays an important role in IL-6 and IL-8 production induced by TNF-α and IL-1β. In this study we investigated the effect of FR167653 on collagen-induced arthritis (CIA), a widely used experimental model of polyarthritis that has many histopathological features in common with RA.

Materials and Methods  CIA was induced using the modified method described by Trentham et al. Briefly, 6-week-old female Lewis rats were immunized intradermally with 0.5 mg of bovine type II collagen dissolved in 0.5 ml of 0.1M acetic acid at 4°C and emulsified in 0.5 ml of Freund's complete adjuvant. The rats received an intradermal booster injection of half the volume of the first immunization. Eight CIA rats were injected FR167653 subcutaneously, at a dose of 32 mg/kg in sterilized water every day from day 7 (FR167653 rats). Six CIA rats were not treated with anything (CIA rats). Four rats without CIA induction were used as normal control (normal rats).

Results  The extent of swelling in the hind paws and body weight were measured every 7 days. All CIA rats were day 21 and peripheral blood samples were collected for cytokine concentration assay. The hind paws of all rats were imaged on high-speed radiographic film. The following radiograph criteria were assessed: 0 = no bone damage, 1 = tissue swelling and edema, 2 = joint erosion, and 3 = bone erosion and osteophyte formation. The ankle joints were fixed in 4% paraformaldehyde and prepared for staining with hematoxylin and eosin. Histologically, the extent of arthritis in the ankle joints was assessed: 0 = normal synovium, 1 = synovial membrane hyperplasia and cell infiltrates, 2 = pannus and cartilage erosion, 3 = major erosion of cartilage and subchondral bone, and 4 = loss of joint integrity and ankylosis. T-cell population analysis was performed using bone marrow cells prepared by flushing bone marrow cavity of the femur removed from normal, FR167653, and CIA rats at day 21. Anti-CD4 FITC and anti-CD8a PE antibody (BD Pharmingen, San Diego, CA) were used for staining. Bone marrow cells from normal rats were used in in vitro osteoclast formation assay induced by M-CSF and sRANKL or TNF-α to investigate the effect of FR167653 on osteoclast differentiation. In addition, calcified matrix resorption activity of the osteoclast-like cells was tested using BD BioCoat Osteologic calcium hydroxyapatite coated slides (BD Biosciences, Bedford, MA). The unpaired t test, Mann-Whitney’s U test and ANOVA with Fisher’s LSD post-hoc test were used for the statistical analyses. The significant level was set at p<0.05.

Discussion  Collagen-induced arthritis is an established animal model for the human autoimmune disease, rheumatoid arthritis. The joint pathology associated with CIA is similar to the one observed in patients with RA. In this study, we demonstrated that FR167653 inhibited the onset of CIA in rats, with a significant reduction in inflammatory changes and in radiographic and histologic degree of joint injury. In this systemic arthritis model, the proinflammatory cytokines TNF-α and IL-1β have been shown to propagate the extension of local and systemic inflammatory processes. Our data showed that FR167653 effectively reduced the increased plasma levels of these two cytokines in CIA rats. These findings suggest that FR167653 inhibits the polyarticular inflammation process by inhibiting TNF-α and IL-1β production. It is reported that CD8+ T cells could play an important role in initiating CIA, but the role of CD8+ T cells in CIA is not fully understood. In this study the significant increase of CD4+CD8α- T cells were found in CIA rats compared with those of FR167653 and normal rats although the percentage of CD4+CD8α- and CD4+CD8α+ T cells showed no difference among the groups. These findings suggest that CD4+CD8α- T cells play an important role in initiating CIA and that the decrease of CD4+CD8α- T cells in local bone marrow may have contributed to the inhibition of arthritis by FR167653. Meanwhile, FR167653 inhibited the osteoclast differentiation and maturation in vitro induced by both sRANKL and TNF-α. Taken together, our findings suggest that FR167653 inhibits the joint destruction not only by suppressing inflammation in joints but also by inhibiting osteoclastic bone resorption directly. In conclusion, FR167653 inhibited the onset of collagen induced arthritis in rats, suggesting that p38 MAPK is a potential therapeutic target for RA.