Attenuation of pain and inflammation in adjuvant-induced arthritis by selective inhibition of proteasome

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Introduction: Rheumatoid arthritis (RA) is an inflammatory disease leads to various degree of destruction of bone and cartilage. Pain in rheumatoid arthritis is generally perceived to arise from inflammation. NF-kB is a key transcription factor required for the expression of proinflammatory mediators. Ubiquitin proteasome system is involved in the activation of NF-kB. Inhibitors of proteasome suppress the activation of NF-kB. Accumulating evidence suggests that the peripheral nervous system is involved in pro-inflammatory processes in addition to pain. The aim of the present study is to characterize the NF-kB in bone and joint tissue and to study the effect of proteasome inhibitor MG132 on inflammatory pain.

Materials and Methods: The study included 36 female Lewis rats divided into three groups: normal, arthritic rat treated with DMSO (dimethyl sulfoxide) and arthritic rats treated with MG 132 dissolved in DMSO. Arthritis was induced by injecting heat killed Mycobacterium butyricum. Rats were treated with MG 132 (1mg/kg/day), two weeks by subcutaneous daily injection. Approval was obtained by Animal Ethical Committee, Karolinska institute.

Pain Mechanical Test (hind paw withdraw latency-HPL) was applied to two arthritic groups and HPL thresholds were measured as described previously by Randall and Selitto. To assess the effect of treatment on degree of bone and joint changes in the arthritic rats, imaging was done with a dental X-ray machine.

Immunohistochemistry: Total of 18 Rats were perfused with phosphate buffered saline and with a fixative buffer. Bilateral ankle joints were dissected and demineralised. Immunostaining was performed according to the biotin-avidin system. The sections were incubated with normal goat serum before incubation overnight with antibody against SP.

Electrophoretic mobility shift assay (EMSA): Ankle joints from 18 rats were collected and immediately frozen on dry ice before homogenized and dissolved in buffer C supplemented with protease inhibitors. Binding reactions was performed with 32P-labelled oligonucleotide representing NF-kB binding sites of the HIV enhancer. The reaction mixtures were separated on native gel.

Results: Significant reduction in pain in the arthritic joints was observed in rats treated with MG 132 compared to the untreated group (Fig.1)

Similarly, radiographic analysis demonstrated a robust reductions in the soft tissue swelling and the destruction of the ankle joint (Fig. 2C) and obvious reduction in the expression of SP in the joints of rats treated with MG 132 compared to arthritic rats (Fig. 2F).

EMSA results showed a significant reduction in transcription factor NF-kB activity in ankle joints of arthritic rats after treatment. (Fig 3)

Discussion: We found that MG132 can reduce the pain behaviour and the destruction of bone and joint tissue in the adjuvant arthritis rat model. The data strongly suggests that the UPS plays critical role in the regulation of inflammatory pain, expression of sensory neuropeptides and bone turnover. The effects of Mg132 on inflammatory pain may be mediated through several cellular mechanisms; direct suppression of RANK and RANKL and the activation of osteoclasts, by altered synthesis or release of pro-inflammatory cytokines and nerve growth factors and indirectly stimulating the nerve degeneration. MG 132 might regulate the synthesis of neuronal mediators such as substance P which has been suggested to have a critical role in bone resorption and in nociception. These results will help developing a novel strategy for treatment of joint inflammation and pain.

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Fig. 1 Measurement of HPL to mechanical stimuli in adjuvant arthritic rats after treatment at week 1 and 2. Data is presented as the means ±SD, * = p<0.05

Fig. 2 Radiographic images (A-C) and IHC of neuromediator SP (D-E) in ankle joints of normal (A,D), arthritic rats (B, E) and arthritic rats treated with MG132 (C, F).

Fig. 3 The activity of NF-kB by EMSA (A) and the quantitative analysis (B) of NF-kB in normal, arthritic rats (DMSO) and arthritic rats (DMSO + MG132). * = p<0.05.