FURIN REDUCES CARTILAGE DEGRADATION IN A MURIN MODEL OF JOINT INSTABILITY

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Aim of the study: Cartilage degradation in OA is mainly due to a reduced matrix protein secretion and an increase of their degradation by matrix metalloprotease (MMP). Indeed during OA an enhanced expression and activation of MMPs is observed. The major MMPs involve in matrix protein degradation are ADAMTS-4, MMP-3 or MMP-13. However, inhibition of expression or activation of MMPs might reduce matrix degradation during OA. Furin, a proprotein convertase, is responsible for the conversion of various Pro-MMPs in active MMPs. Furin activates directly ADAMTS-4 and MMP-3, while promote indirectly the maturation of MMP-13 through other MMPs. Therefore, we hypothesised that Furin may have an impact in matrix homeostasis in joint instability. Moreover, we investigated the in vitro effect of Furin in MMP expression in murin chondrocytes.

Methods: DMM (destabilisation medial meniscus) was induced surgically by partial meniscectomy in 10-week-old C57Bl6 male mice. The day after surgery, the mice received twice weekly intraperitoneal injections of Furin (1U/mouse), or z1-PDX (the proprotein convertase inhibitor, 0.35 µg/g mice), or vehicle (control group) and sacrificed after 6 weeks. Joint sections were used to assess cartilage damage (OARSI score) and the number of ADAMTS-4 and MMP-13(+) cells as well as Furin expressing cells. We further assessed the signalling pathway induced by Furin in vitro. Murine primary chondrocytes (pre-treated with 10ng/ml IL-1) were cultured with Furin (10U/mL) or z1-PDX (8µM) for 48h and then analyzed the expression of MMP-13 and the phosphorylation of Smad2/3 in cell lysates by Western blot.

Results: We first observed a constitutive expression of Furin in cartilage of control mice while absent in DMM mice. Furin reduces OA score (5.64±1.54) but unchanged with z1-PDX (15.16±2.82) compared to vehicle (13.92±1.95, p<0.02). The percentage of ADAMTS-4(+) cells was unaffected by Furin or z1-PDX. However, the percentage of MMP-13(+) cells was significantly reduced with Furin (-76%, p<0.03) and unaffected by z1-PDX. In vitro, Western blot analysis revealed the presence of the active form of MMP-13 only in IL-1-activated chondrocytes compared to control suggesting a complete maturation of MMP-13. In contrast, Furin-treated cells showed a decrease in MMP-13 expression and maturation whereas z1-PDX had no effect on activated chondrocytes. Moreover, Furin enhanced the phosphorylation of Smad2/3 suggesting the activation of TGFβ pathway.

Conclusion: In mice, Furin reduced cartilage degradation induced by DMM as well as MMP-13 expression. Moreover, Furin decreased in the vitro maturation of MMP-13 which might be mediated by another signalling pathway.

EFFICACY OF THE SELECTIVE INDUCIBLE NITRIC OXIDE SYNTHASE INHIBITOR SD-6010 IN NONCLINICAL INFLAMMATORY, NEUROPATHIC, AND OSTEOARTHRITIS PAIN

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Purpose: To evaluate efficacy of SD-6010, a selective inducible nitric oxide synthase (iNOS) inhibitor, in four pain models.

Methods: Efficacy of oral SD-6010 was evaluated in acute inflammatory hyperalgesia (carrageenan-induced thermal hyperalgesia [CITH]); peripheral neuropathic pain (spinal nerve ligation [SNL] and chronic constriction injury [CCI]); and osteoarthritis (OA) pain (medial meniscal tear [MMT]) in male Sprague-Dawley rats. In the CITH study, efficacy of oral SD-6010 (50 mg/kg or 150 mg/kg), the selective iNOS inhibitor N-iminoethyl-L-lysine (L-NIL; 30 mg/kg; positive control), or vehicle (negative control; n = 6 per treatment) administered 4h after carrageenan injection was determined by measuring paw withdrawal response to a thermal stimulus at 1, 2, or 3 h thereafter. In studies that utilized the other models, tactile hypersensitivity of the hindpaws was assessed by measuring paw withdrawal threshold in response to probing with a series of calibrated von Frey filaments applied perpendicularly to the plantar aspect of the affected paw following oral administration of SD-6010 (SNL: 3, 10, 30, or 100 mg/kg, n=6 per treatment; CCI: 3, 10, or 30 mg/kg, n=6 per treatment; MMT: 15, 50, or 150 mg/kg, n=8 per treatment), gabapentin (SNL: 3, 10, 30 or 100 mg/kg; CCI: 30 mg/kg), or vehicle (SNL only). Efficacy of SD-6010 in relieving OA-like pain in the MMT model was evaluated as the weight bearing differential.

Results: A single dose of SD-6010 reduced acute inflammatory pain (thermal hyperalgesia) in a dose-dependent manner (Figure 1). In both models of neuropathic pain, SD-6010 produced dose-and time-dependent inhibition of pain. In the SNL model, doses of SD-6010 from 10 mg/kg to 100 mg/kg provided maximal efficacy at 6 hours after administration, comparable to gabapentin 30 mg/kg (Figure 2). Peak efficacy with both SD-6010 and gabapentin occurred between 4 and 6 hours after administration. In the CCI model, efficacy of 30 mg/kg of SD-6010 was comparable to 30 mg/kg gabapentin (Figure 3). Maximal efficacy with SD-6010 treatment occurred later than with gabapentin, but SD-6010 duration of efficacy was considerably longer in CCI. In the MMT model of OA pain, SD-6010 also reduced tactile hypersensitivity at doses of 50 mg/kg and 150 mg/kg and weight bearing at all doses (Figure 4).

Conclusions: The selective iNOS inhibitor SD-6010 reduces pain in rodent models of inflammatory, neuropathic, and osteoarthritis pain.