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INCREASED EXPRESSION OF ARTHRITIC MARKER GENES IN THE CARTILAGE OF SIRT1 NULL MICE
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Purpose: Osteoarthritis (OA), the most frequent age related disease present in the west, is a multi-factorial imbalance between cartilage anabolism and catabolism. To understand the mechanisms underlying OA, we focused on the protein deacetylase SirT1, a factor known to prolong organism lifespan. SirT1 has been previously shown to regulate apoptosis and cartilage-specific gene expression in human chondrocytes. Recent data also indicates that SirT1 is a potent inhibitor of the matrix metalloproteinases (MMPs). MMPs are the most well known of arthritis marker genes that play a central role in cartilage degeneration. In order to evaluate the role of SirT1 in cartilage homeostasis, we assessed MMP and ADAMTS expression in the cartilage of SirT1 null mice.

Method: We used SirT1 Wild-type (WT) and SirT1 Null mice in the analysis. The cartilage from hind paws in 1 to 3 week old mice was harvested for both immunohistochemistry and subculture of chondrocytes.

Results: Articular cartilage tissue sections from SirT1 Null mice exhibited low levels of type 2 collagen, aggrecan and glycosaminoglycans (GAG) compared to SirT1 WT mice at 1 week or 3 weeks. Protein levels of aggrecan and type 2 collagen were also decreased in the chondrocytes derived from SirT1 Null mice. In contrast, protein levels of MMP-3, MMP-8, MMP-9 and MMP-13 were elevated in the SirT1 Null mice compared to WT. Finally, DBC1 (deleted in breast carcinoma 1), a known Sirt1 associated anti-aging/anti-inflammatory protein, was found to be elevated in the SirT1 Null mice compared to WT.

Conclusion: The data from this animal model indicate that SirT1 is the SirT1 Null mice compared to WT. Finally, DBC1 (deleted in breast carcinoma 1), a known Sirt1 associated anti-arthritic gene is consistent with its general function as an anti-inflammatory protein.

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PHARMACOLOGY OF THE STR/OR T MOUSE AS A MODEL FOR OSTEOARTHRITIS: RESPONSIVENESS TO EP4 ANTAGONISTS AND AGGREGANASE INHIBITORS
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Purpose: The ST/or mouse can develop spontaneous osteoarthritis (OA) of the knee, and are believed to be a relevant model for human knee OA. We have recently shown that treatment with either glucosamine sulfate or an MMP and aggrecanase inhibitor reduced the severity of the OA histological score as well as histomorphometric parameters in this strain. To further validate this model for drug discovery purposes, we investigated its responsiveness to further interventions within strategies currently pursued for OA treatment. We investigated in two separate studies the effects of an EP4 specific agonist, or of a specific aggrecanase inhibitor, respectively, in ST/or mice.

Methods: As a specific EP4 receptor antagonist we used CJ-042,794 (Murase et al., 2008). Wyeth’s compound #800 described in patent WO 2007/008994 was used as an aggrecanase1/2 specific inhibitor (ASI). Six male Wistar rats were trained for one week to run in a rodent treadmill machine as follows: day 1, 10 minutes at 0.60 km/hr; day 2 15 minutes at 0.72 km/hr; day 3 20 minutes at 0.90 km/hr; day 4 30 minutes at 1.08 km/hr and day 5 35 minutes at 1.20 km/hr. The following 5 weeks, the rats were forced to run for five days a week, the first 10 minutes at 0.72 km/hr; day 3 20 minutes at 0.90 km/hr; day 4 30 minutes at 1.08 km/hr and day 5 35 minutes at 1.20 km/hr. The following 5 weeks, the rats were forced to run for five days a week, the first 10 minutes at 0.72 km/hr in order to warm-up and the following 50 minutes at 1.20 km/hr. All knee joints of all animals were scanned with CECT at the start of the experiment (t = 0) and directly after running at t = 1, 3, 6 weeks.

Results: After three (p=0.02) and six weeks (p=0.01) of running significant higher attenuation values were detected, indicating GAG loss from the total volume of condylar cartilage was significant reduced, indicating OA progression (p=0.02) (Figure 1). Patellar cartilage attenuation was not often inflammation and pannus. No improvement whatsoever was associated with treatment with the EP4 antagonist CJ-042,794 at either dose, at least in histopathological parameters, despite the animals were exposed to circulating levels well above its IC50 [approx. 10 nM] on EP4 receptors (e.g., 3 h after administration plasma concentration was 15 μg/ml [35 μM]).

Conversely, we observed a significant albeit slight decrease of OARSI score and total score following treatment with the AS1 at the higher dose (200 mg/kg). Pharmacokinetic analyses for AS1 (Cmax=102 μg/ml [214 μM], T1/2 =2h, AUC 0-24h=198 μg.h/ml), confirmed that these animals were exposed to circulating levels above the aggrecanase inhibiting concentrations [92 nM and 186 nM vs. aggrecanase 1 and 2, respectively].

Conclusions: The present studies confirm the ST/or mouse as a model of choice for evaluating OA disease modifying drugs. Moreover, they further establish aggrecanase inhibition as a disease-modifying approach, while suggest that EP4 antagonism is mainly an OA pain-controlling strategy.