Reduce Joint Contracture in Rabbit Model of Arthrofibrosis

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Introduction: Joint contracture due to trauma, surgery, arthritis and inflammatory conditions is functionally debilitating. Rehabilitation and surgery is may help improve range of motion, but residual or recurrent contractures are common. Pharmacological intervention for joint contracture is currently available. The purpose of this study was to show that the intra-articular delivery of an FDA-approved PPARgamma agonist via a hydrogel scaffold effectively reduces joint fibrosis in both in vitro and in vivo models.

Methods: Part A: In vitro cell testing. Human fibroblasts were exposed to selected concentrations of Rosiglitazone dissolved in DMSO (10, 20, 50 μM) (n=4). One hour prior to drug exposure, fibroblasts were stimulated with TGF-β to promote differentiation into myofibroblasts and activate ECM synthesis. After 24 hours mRNA was extracted, and Col-1A and SMA were measured using real-time PCR. XTT assays were performed to assess for apoptosis and cell toxicity.

Part B: Elution. Oligo(poly(ethylene glycol)-fumarate (OPF) hydrogels (n=5) were loaded with Rosiglitazone. Drug elution properties were characterized by soaking the scaffolds in 1 mL of minimum essential media (MEM) with 10% fetal bovine serum. Each scaffold was transferred into a new Eppendorf tube with fresh media over a span of 14 days. The media was utilized to measure the drug concentration via HPLC methods.

Part C: In vivo testing. 24 skeletally mature New Zealand White (NZW) female rabbits were used for the study utilizing a previously published rabbit model of joint contracture. All rabbits had their right knees operated on to create 3-mm defects in the non-cartilaginous portions of the femoral condyles, hyperextend the joint to disrupt the posterior capsule, and immobilize the joint in maximum flexion with a Kirschner-wire for 8 weeks. During the same procedure, single OPF scaffolds were implanted into the right knee. 12 rabbits received the OPF scaffold loaded with Rosiglitazone (1.67 mg in 10 μL DMSO) while the other twelve rabbits served as a control group by implantation of the OPF scaffold without the drug. After K-wire removal, rabbits were allowed free activity in a cage for an additional 16 weeks before being sacrificed. After sacrifice, the rabbit limbs were tested using a custom-made and validated joint measuring device. A two sample t-test was used for statistical analysis with P-values of 0.05 or less being significant.

Results: In vitro: Rosiglitazone suppressed Col-1A and SMA expressions in a dose dependent manner. DMSO (control group) did not suppress TGF-β activation. XTT assay showed no toxicity up to 50 μM. Drug elution analysis revealed sustained delivery of Rosiglitazone at high doses (~50μM) during a 14-day period.

In vivo: After 8 weeks of re-mobilization (week 16), the Rosiglitazone group showed a statistically significant decreased flexion contracture angle compared to the control group (89.4 degrees vs. 78.3 degrees, p<0.03). At the time of sacrifice (week 24) the Rosiglitazone group continued to exhibit less joint contracture (119.0 degrees versus 99.5 degrees, p=0.014). No macroscopic joint damage was observed in either group.

Discussion: Human fibroblasts exposed to Rosiglitazone in culture express less Col-1A and SMA in a dose-dependent manner without noticeable cell toxicity even at 50 μM doses. OPF scaffolds loaded with Rosiglitazone elute a high dose (~50µM) of Rosiglitazone in vitro up to at least 14 days. Rosiglitazone-loaded OPF scaffolds implanted intra-articularly decrease flexion contracture in a rabbit knee model of arthrofibrosis without inducing inflammatory or arthritic changes.

Significance: In-vivo and in-vitro results may be used for clinical trials in human beings in the future.

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Source
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