tant player in chondrocyte growth and differentiation. Regulation by methylation which is the most common DNA modification remains unknown for arthrotic cartilage.
The aim of this study was to evaluate the influence of demethylation on the gene expression of MMP - 13, BMP-2, Fli-1, Aggrecane, BMP-4 and collagen X.
Methods: We collected eight probes of human cartilage before undergoing total knee joint replacement under sterile conditions from osteoarthritis patients (5 female, 3 male). The average patient's age was 70 years (min 54, max 83). After digestion, the cells were spread out and half of them were treated with 10 uM of demethylation agent 5-AZA-deoxy-cytidine over a period of six days. After harvesting the cells, RNA was extracted using the Trizol method and cDNA was transcribed. Gene expression for MMP-13, BMP-2, BMP-4, Aggrecane, Fli-1 and collagen X was performed with the Taqman Realtime PCR Assay.
Results: Interestingly there was a remakable increase (4-fold) of the gene expression of MMP-13 and a 1, 5-fold increase of BMP-2 gene expression after treatment with 5-Aza-deoxy-cytidine in the human arthrotic cartilage cell cultures compared to the untreated controls. There was no obvious change in gene expression for BMP-4, collagen X, Fli-1 and Aggrecane.
Conclusions: Further investigations are needed to show if methylation plays a major role in regulating this pro-arthrotic enzyme. If so this may lead us to new therapeutic aspects of Osteoarthritis in the future.

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THE EFFECT OF DISTILLED METHYLSULFONYLMETHANE (MSM) ON HUMAN CHONDROCYTES IN VITRO

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Purpose: Osteoarthritis (OA) is a joint disease characterized by a degenerative change of articular cartilage and underlying subchondral bone and often accompanied by inflammation. MSM, a dietary supplement composed of about 34% sulfur and self-affirmed as GRAS (generally recognized as safe) in the U.S., is most utilized for treating osteoarthritis. A recent study by Kim et. al., OA & Cart, 2006, showed clinical effectiveness of MSM supplementation at 3 gm BID x 12 weeks compared to placebo. The objective of our study was to examine the effect of MSM at varying concentrations on cultured human healthy and osteoarthritic chondrocytes in vitro with a focus on catabolic and anabolic markers.
Methods: Human cartilage tissues were obtained from 22 knees, 72 hrs postmortem from donors with different grades of OA. We used the Outerbridge classification (Grades I - IV), Grade I for intact surface; Grade II for minimal fibrillation; Grade III for overt fibrillation; and Grade IV for erosion of the articular cartilage surface. Following gross assessment of the donor knees, the following knees were studied for Grade I (n=6) (aged 23-38); Grade II (n=9) (aged 50-77); for Grade III (n=5) (aged 32-70); and Grade IV (n=2) (aged 70-93). Cartilage tissues were harvested from femoral condyles and tibial plateaus, the matrix was dissolved with collagenase; the chondrocytes were then cultured for 2 weeks in culture media without MSM. After reaching confluence, the chondrocytes were harvested and 2 x 10^5 cells in 10 ml culture medium with varying concentrations of MSM (0, 1, 3, 6, 12, and 60 μg/ml) were cultured in 100 mm (in-diameter) plates at 37°C in 5% CO_2 for 3 days. The concentrations were estimated to correspond to human, oral dosing at between 0 and 30 grams of MSM per day. mRNA expression of various markers by RT-PCR including: TNF-alpha, IL-1, MMP-1, MMP-3, and MMP-13 was also determined for each OA grade and each concentration of MSM or control. Anabolic pathways examined included proteoglycan synthesis (by a pulse chase analysis of ^{35}SO4 incorporation) and chondrocyte mRNA expressions of Type-II collagen and aggrecan. A one-way ANOVA was performed to establish the level of statistical significance.
Results: In Grade II OA chondrocytes treated with MSM at the concentration of 12 μg/ml, there was a strong trend for MSM to reduce the mRNA expression of inflammatory markers: TNF-alpha (-33%, p=0.08) and IL-1 (-29%, p=0.08) when compared to lower concentrations of MSM and control. These results did not apply for OA chondrocytes of Grade III or IV. MSM did not show an increase in proteoglycan synthesis in cultured chondrocytes or an increase of cartilage matrix production in normal and osteoarthritic chondrocytes at the mRNA level.
Conclusions: MSM might have an ability to protect articular cartilage in early OA by reducing expression of inflammatory cytokines, i.e. TNF-alpha & IL-1. The effective concentration of 12 μg/ml MSM correlates with the dosage used in a recent clinical trial. MSM did not elicit an anabolic response in this study.

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DESPITE QUICK CLEARANCE OF BLOOD AFTER A HAEMARTHROS, ADVERSE EFFECTS ON CARTILAGE AND SYNOVIUM ARE INITIATED, COMPROMISING THE JOINT

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Purpose: Joint bleeds occur as a result of joint trauma or major joint surgery. These joint bleeds, or haemarthroses, have a direct devastating effect on the matrix turnover of articular cartilage, which is due to apoptosis of the chondrocytes, as we have previously shown. Also the synovium is affected by the joint bleeds. The synovium clears the blood from the joint, which leads to haemosiderin deposition and successive synovial inflammatory activities. This adds to the cartilage damage via the production of pro-inflammatory - and tissue destructive mediators.
Recently we showed that relatively low concentrations of blood present for a short period of time already lead to long-lasting damage of human articular cartilage when determined *in vitro*. The present study was undertaken to determine whether *in vivo* these negative effects occur as rapid as determined *in vitro*.
Methods: Autologous blood was injected into the knee joint of Beagle dogs, either 48 hours, 24 hours or 15 minutes before termination. The amount of red and white blood cells present in the joint cavity at the time of termination were determined. The proteoglycan synthesis rate and release of the cartilage were determined. Furthermore the cartilage destructive properties of the synovial tissue were tested by measuring the proteoglycan synthesis rate, -release and -content of healthy cartilage after exposure to culture supernatants of synovial tissue from the blood-exposed joints. All results were compared to the values of the uninjected joint.
Results: Fifteen minutes after the injection of autologous blood, the red blood cell count was $67*10^{12}$/L, comparable to the amount present in whole blood, and gradually decreased (50% at 24 hours) to $6{,}7*10^{12}$/L within 48 hours ($<$10%). The amount of white blood cells increased in the first 24 hours, and was still increased after 48 hours, although less than after 24 hours.
The proteoglycan synthesis rate and -release were adversely affected already within 24 hours (-15% and +23% respectively), and these effects were more severe 48 hours post-injection (-31% and +32% resp.). Synovial tissue culture supernatants