networks. Data was further analyzed by Ingenuity Pathways Analysis to construct molecular functional networks and signaling pathways to dissect pathways regulated by exercise. One-way ANOVA and a post hoc Tukey test were used for statistical analysis.

**Results.** Treadmill walking immediately following initiation of MIA (1 day post-MIA induction) demonstrated a significant prevention of MIA progression. However, the efficacy of this intervention was significantly reduced when implemented on knees showing close to Grade I or greater cartilage damage. On the contrary, TW accelerated damage in the knees with close to Grade II cartilage pathologies. Transcriptome-wide gene expression analysis revealed that exercise intervention started 1-day post-MIA inception significantly suppressed signaling networks that inhibit matrix synthesis. In parallel, TW upregulated gene networks associated with matrix synthesis. However, TW intervention following Grade I damage was less effective in preventing cartilage damage and regulating matrix synthesis. Interestingly, Asporin and networks associated with TGF-β expression and signaling were the major gene products that were regulated by TW to control matrix synthesis (Aggrecan, Collagen type II, Matrilin, FRZB, Col 9A2 and Col9A3) and prevent cartilage damage as evident by microscopic grading of cartilage.

**Conclusions.** The findings demonstrate that Asporin might be one of the critical genes controlled by TW, that in turn controls expression of TGF-β family of molecules, essential for cartilage matrix synthesis. The findings underscore the importance of physiotherapies by showing suppression of Asporin, an OA susceptibility gene by TW. Nevertheless, the extent of cartilage damage at the initiation of physiotherapy is an important determinant for the effectiveness of TW.

241

**ESTIMATION OF CHONDROITIN SULFATE AS A BIOMARKER IN ARTICULAR CARTILAGE FOR TREATMENT OF OSTEOARTHRITIS THROUGH ORAL DIACEREIN LOADED LIPID NANOPARTICLES IN RAT MODEL**


**Purpose:** Osteoarthritis (OA) is a disease which affects joints causing changes to joint structures specifically the cartilage. Current treatment recommendations for OA are intended to provide patient with symptomatic relief. Diacerein is novel chondroprotective /connective tissue structure modifying agent intended for the treatment of OA. Diacerein hydrolyses into its active metabolite rhein. It selectively inhibits the synthesis of interleukin-1 (IL-1) and down regulates the production of nitrous oxide (NO), which are the agents responsible for the cartilage degeneration. Nanotechnology is continuously providing new strategies to expand the opportunity for drug delivery. Inside it, solid lipid nanoparticles (SLNs) enhances lymphatic transport of drugs, reduces hepatic first pass metabolism and improves bioavailability. Chondroitin sulfate is a kind of glycosaminoglycan found in the extracellular matrix of the cartilage. Softening and erosion of cartilage the characteristic early lesions of OA occur in sites of decreased chondroitin sulfate concentration. Considering all these attributes, Diacerein loaded SLNs were prepared and thereafter it was indirectly targeted to joint cartilage using chondroitin sulfate as carrier. On these accounts, in the present study chondroitin sulfate was estimated in OA experimental rat model after oral administration of two different formulation of diacerein and compared with control.

**Methods:** Adult wistar rats (200±20g) were divided in three different groups (n=8) for the study. Prior approval from institutional Animal Ethics committee of Banaras Hindu University, Varanasi, India was taken. The three groups were treated orally with 0.2% sodium CMC suspension of pure drug (group 1), prepared SLNs without & with chondroitin sulfate (group 2 & 3) respectively. OA was induced chemically in the right knee of animals using 2mg/joint intra-articular injection of sodium iodoacetate before treatment. Treatment was started after 21 days of intra-articular injection. Left knee of all animals were used as control. After four weeks of treatment all animals were sacrificed and articular cartilage was obtained from knee at autopsy using a scalpel blade to cut as deeply as possible without removing underlying bone. Samples were removed from the posterior surface of the patella or from the condyles and patellar groove of the femur. Ethanol fractions were prepared from cartilage samples digestion in phosphate buffer pH 7 containing trypsin for 16 to 20 hrs at 37°C and then homogenized for 1-2 minute. Insoluble debris was removed by brief centrifugation. Supernatant was brought to 40%, 50% & 80% ethanol respectively by addition of ethanol and aqueous 20% potassium acetate solution. At each step supernatant was centrifuged. All precipitates were pooled, dissolved in water and were used for determination of chondroitin sulfate. Before treatment 6 animals (2 animals / group) were considered for arthritic control. Chondroitin sulfate was indirectly estimated by decreased in absorbance of methylene blue on complexation with chondroitin sulfate using spectrophotometry.

**Results:** Chondroitin sulfate concentration in articular cartilage of Normal control, arthritic control, group1, group 2 and group 3 were found to be 12.43±4.3, 5.53±2.6, 6.51±2.5, 7.5±2.2 and 8.87±2.2 µg/mg wet weight of cartilage respectively. All the values were average of six.

**Conclusions:** It was revealed from the study that chondroprotective activity of diacerein can be enhanced by administration of drug in the form of SLN and it can be further improved by delivering it with chondroitin sulfate as carrier.

242

**CARTILAGE REPAIR**

**PREVENTION AND REPAIR OF CARTILAGE DEGENERATION BY A NOVEL SMALL THIENONIAZOLE-DERIVATIVE COMPOUND**

F. Yang 1, Y. Hosaka 1, A. Fukushima 1, T. Saito 1, H. Hojo 1, S. Ohba 1, H. Kawaguchi 1, U.-I. Chung 1,2. 1 Sch. of Med., Univ. of Tokyo, Tokyo, Japan; 2 Dept. of Engineering, Univ. of Tokyo, Tokyo, Japan

**Purpose:** Osteoarthritis (OA) is a disease which affects joints causing changes to joint structures specifically the cartilage. Current treatment recommendations for OA are intended to provide patient with symptomatic relief. Diacerein is novel chondroprotective /connective tissue structure modifying agent intended for the treatment of OA. Diacerein hydrolyses into its active metabolite rhein. It selectively inhibits the synthesis of interleukin-1 (IL-1) and down regulates the production of nitrous oxide (NO), which are the agents responsible for the cartilage degeneration. Nanotechnology is continuously providing new strategies to expand the opportunity for drug delivery. Inside it, solid lipid nanoparticles (SLNs) enhances lymphatic transport of drugs, reduces hepatic first pass metabolism and improves bioavailability. Chondroitin sulfate is a kind of glycosaminoglycan found in the extracellular matrix of the cartilage. Softening and erosion of cartilage the characteristic early lesions of OA occur in sites of decreased chondroitin sulfate concentration. Considering all these attributes, Diacerein loaded SLNs were prepared and thereafter it was indirectly targeted to joint cartilage using chondroitin sulfate as carrier. On these accounts, in the present study chondroitin sulfate was estimated in OA experimental rat model after oral administration of two different formulation of diacerein and compared with control.

**Methods:** Adult wistar rats (200±20g) were divided in three different groups (n=8) for the study. Prior approval from institutional Animal Ethics committee of Banaras Hindu University, Varanasi, India was taken. The three groups were treated orally with 0.2% sodium CMC suspension of pure drug (group 1), prepared SLNs without & with chondroitin sulfate (group 2 & 3) respectively. OA was induced chemically in the right knee of animals using 2mg/joint intra-articular injection of sodium iodoacetate before treatment. Treatment was started after 21 days of intra-articular injection. Left knee of all animals were used as control. After four weeks of treatment all animals were sacrificed and articular cartilage was obtained from knee at autopsy using a scalpel blade to cut as deeply as possible without removing underlying bone. Samples were removed from the posterior surface of the patella or from the condyles and patellar groove of the femur. Ethanol fractions were prepared from cartilage samples digestion in phosphate buffer pH 7 containing trypsin for 16 to 20 hrs at 37°C and then homogenized for 1-2 minute. Insoluble debris was removed by brief centrifugation. Supernatant was brought to 40%, 50% & 80% ethanol respectively by addition of ethanol and aqueous 20% potassium acetate solution. At each step supernatant was centrifuged. All precipitates were pooled, dissolved in water and were used for determination of chondroitin sulfate. Before treatment 6 animals (2 animals / group) were considered for arthritic control. Chondroitin sulfate was indirectly estimated by decreased in absorbance of methylene blue on complexation with chondroitin sulfate using spectrophotometry.

**Results:** Chondroitin sulfate concentration in articular cartilage of Normal control, arthritic control, group1, group 2 and group 3 were found to be 12.43±4.3, 5.53±2.6, 6.51±2.5, 7.5±2.2 and 8.87±2.2 µg/mg wet weight of cartilage respectively. All the values were average of six.

**Conclusions:** It was revealed from the study that chondroprotective activity of diacerein can be enhanced by administration of drug in the form of SLN and it can be further improved by delivering it with chondroitin sulfate as carrier.