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### A NEW MODEL TO STUDY THE EFFECTS OF ADVANCED GLYCATION ENDPRODUCTS ON THE PROGRESSION OF SPONTANEOUS OSTEOARTHRITIS

T.L. Willett<sup>1,2</sup>, A. Decroos<sup>1</sup>, N. Avery<sup>3</sup>, R. Kandel<sup>1,2</sup>, M. Grynaps<sup>1,2</sup>. <sup>1</sup>Mount Sinai Hosp., Toronto, ON, Canada; <sup>2</sup>Univ. of Toronto, Toronto, ON, Canada; <sup>3</sup>Univ. of Bristol, Bristol, United Kingdom

**Purpose:** To determine if advanced glycation endproducts (AGEs) alter the extent of spontaneous osteoarthritis (OA) progression in the male Hartley Guinea Pig (HGP). Some investigators hypothesize that accumulation of AGEs (due to ageing or disease) may play a role in OA pathogenesis by various mechanisms (biochemical, biomechanical). DeGroot et al. (Arth. & Rheum, 2004) reported that a 5-fold increase in AGE content of articular cartilage due to intra-articular injections of PBS containing ribose lead to greater osteoarthritis in a dog ACL injury model of secondary OA. However, that study did not address the possibly more insidious role AGEs may play in primary, idiopathic OA development. Therefore, we determined to study the effect in HGPs – the gold standard model for spontaneous OA.

**Methods:** Fifteen male retired-breeder (~1 year) HGPs received 100 µl intra-articular injections in the right knee [PBS (n=5), PBS+0.6M ribose (n=5) or PBS+2.0M ribose (n=5)] once a week for four weeks. Contralateral (left) knees acted as controls. Femoral cartilage and menisci were harvested for biochemical assays and proximal tibiae were processed for histological grading using Safranin-O staining and histological scoring based on the scheme of Kraus et al (Osteoarthritis & Cartilage, 2010). HPLC was used to quantify pentosidine concentration, an established AGE biomarker, to quantify the extent of AGE accumulation in the cartilage.

Subsequently, in a long-term study allowing for significant OA progression in HGP knees, twenty male 3-month old HGPs received intra-articular injections in the right knee [PBS (n=10), PBS+0.6M ribose (n=10); left knees were controls] for twenty-four weeks (9 months old). Additionally, four non-injected controls HGPs were euthanized at 3-months and another four at the end of the experiment. The tissues were harvested and prepared as described above.

**Results:** In the first study, increased pentosidine content was detected in the menisci of the 0.6M and 2.0M ribose groups compared to the PBS controls. A dose response was confirmed and pentosidine levels reached those of mature human cartilage in the 2.0M treated group (3.5±1.0 mmol pentosidine per mol collagen versus 0.63±0.17 mmol pentosidine per mol collagen in control menisci). Histological examination of the tibial plateau confirmed no acute reaction to the injections and did not demonstrate a difference in disease state between the groups (Kraus score ~16±2), likely because the disease had already progressed significantly in the retired breeders by the start of the study. During the 24-week study, the ribose injected group unexpectedly gained less mass, ranging between 6–8% less between weeks 12 and 24. This suggests that the ribose treated group may have experienced greater pain/inflammation. 0.6M ribose allows for a slower accumulation of AGEs during the treatment period so that levels remain physiological but reach levels similar to 80-year old human cartilage (approx. 8 mmol pentosidine per mol collagen) by 24 weeks. During the period of 3 to 9 months of age, OA typically develops to a moderate level in this model (Kraus score increasing from less than 2 to 12).

**Conclusions:** An in vivo model to test the effect of AGEs on the gradual progression of idiopathic knee osteoarthritis has been established. AGE content reaches levels found in aged cartilage.

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### INCREASED CARTILAGE DAMAGE IN PAPAIN- AND COLLAGENASE-INDUCED OSTEOARTHRITIS IN LRP5<sup>-/-</sup> MICE WITH LOW BONE MASS DENSITY

L. Lodewyckx, F.P. Luyten, R.J. Lories. *KULeuven, Leuven, Belgium*

**Purpose:** Low-density lipoprotein receptor-related protein 5 (LRP5) is a WNT co-receptor, important in the canonical WNT signaling pathway and with a critical role in bone homeostasis. Mutations in the human *LRP5* gene are the cause of osteoporosis-pseudoglioma syndrome (OPPG) characterised by severe juvenile onset osteoporosis. Gain-of-function mutations on the other hand result in abnormally high bone density disorders. Recently, an osteoarthritis susceptibility locus was found on chromosome 11q12–13, including the *LRP5* gene and a significantly higher osteophyte formation score was found in

postmenopausal Japanese women carrying a *LRP5* low bone density polymorphism. We used the *Lrp5*<sup>-/-</sup> mice, in two mouse models of osteoarthritis.

**Methods:** Osteoarthritis was induced in male wild-type or *Lrp5*<sup>-/-</sup> mice at 8 weeks of age by intra-articular injection in the right knee of 10 µl of 2 U/µl collagenase type VII or 10 µl of 1% papain/0.03M L-cystein. The left knee was injected with 10 µl PBS as a negative control. 11 and 9 mice/group were induced for the papain- and collagenase-induced osteoarthritis model respectively. After 7 days mice were sacrificed and knees were isolated for histology. Knee sections were stained with haematoxylin-eosin and safranin O and severity of arthritis was scored.

**Results:** Both papain- and collagenase-induced osteoarthritis resulted in increased cartilage damage in *Lrp5*<sup>-/-</sup> mice as compared to the wild-type mice. Inflammation scores (infiltration, hyperplasia and fibrosis) were also significantly higher in collagenase-induced *Lrp5*<sup>-/-</sup> mice, but not in the papain-induced osteoarthritis model.

**Conclusions:** In this study we show that *Lrp5*<sup>-/-</sup> mice, characterised by low bone mass density, are more susceptible to cartilage degradation induced by papain- and collagenase-induced osteoarthritis. These effects could be caused by either loss of WNT signaling in the joint or by changes in the bone-cartilage biomechanical unit secondary to the low bone mass in *Lrp5*<sup>-/-</sup> mice.

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### CHARACTERISATION OF TEMPORAL SUBCHONDRAL BONE CHANGES IN A RAT MODEL OF LOW-DOSE MONOSODIUM IODOACETATE INDUCED OSTEOARTHRITIS: AN IN VIVO MICRO-CT STUDY

G. Mohan<sup>1,2</sup>, E. Perilli<sup>1,2</sup>, J. Kuliwaba<sup>1,2</sup>, I. Parkinson<sup>1,2</sup>, J. Humphries<sup>1,2</sup>, N. Fazzalari<sup>1,2</sup>. <sup>1</sup>Bone and Joint Res. Lab., Surgical Pathology, SA Pathology and Hanson Inst., Adelaide, SA, Australia; <sup>2</sup>Discipline of Anatomy and Pathology, Sch. of Med. Sci., The Univ. of Adelaide, Adelaide, SA, Australia

**Purpose:** Animal models of osteoarthritis (OA) are important tools to understand the pathology of OA. Moreover, knowledge of early pathological changes is essential for early treatment options and to develop better therapeutic agents to modify the disease progression. Although OA involves the whole joint, there is increasing evidence that changes in the subchondral trabecular bone are also an underlying cause. The purpose of this study was to perform a comprehensive longitudinal *in vivo* characterisation of a rat model of low-dose monosodium iodoacetate (MIA)-induced OA that closely mimics progressive human OA, using non-invasive *in vivo* micro-CT imaging.

**Methods:** Twelve young adult male Wistar rats (200–230g) received a single intra-articular injection of 0.2 mg MIA in the right knee joint and sterile saline in the left knee joint. The animals were scanned *in vivo* by micro-CT (Skyscan 1076, Skyscan, Kontich, Belgium) at 2, 6, and 10 weeks post-injection, analogous to early, intermediate, and advanced stages of OA, to assess architectural changes in the tibial subchondral trabecular bone. An incapacitance tester was used to determine changes in hind paw weight distribution at day -1, 1, 3, 5, 7, 9, 12, and 14 post-injection, as an index of joint discomfort in the OA knee. A blood sample was collected at 2, 6, and 10 weeks, from each animal to measure the serum bone turnover marker C-terminal telopeptide of type I collagen (CTX-I), and the cartilage turnover marker cartilage oligomeric matrix protein (COMP). The articular cartilage changes in the tibia were assessed macroscopically and histologically at 10 weeks post-injection.

**Results:** Micro-CT analysis of tibial subchondral trabecular bone in the MIA-injected knee showed significant bone loss at 2 weeks (p=0.032 for bone volume fraction). This was followed by an increase in bone volume associated with significantly increased trabecular thickness and separation at 6 weeks (p<0.001, p=0.001, respectively) and 10 weeks (p=0.002, p=0.001, respectively); whereas trabecular number was decreased at all time points compared to control tibiae (p=0.009, p=0.012 at 2 and 10 weeks respectively). At 10 weeks, histology revealed chondrocyte necrosis, chondrocyte clusters, cartilage fibrillation, and delamination (Fig. 1). The OARSI score of the medial tibial plateau of the MIA-injected knee at 10 weeks was 17±3 (mean ± SD). Subchondral trabecular bone sclerosis, cysts, osteophytes (Fig. 2) and subchondral plate breach were observed in the tibia of the MIA injected knee at 10 weeks. The control tibiae showed no OA-like changes.

The weight distribution on the right limb was significantly reduced on days 1, 3, and 5 compared to baseline (p<0.05, p<0.001, and p<0.05, respectively). From day 7, the weight distribution returned to normal. The serum CTX-I, and COMP levels showed significant negative correlation