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Creation of monosodium iodoacetate-induced model of osteoarthritis in rabbit knee joint

Sir,

Osteoarthritis (OA), commonly known as degenerative joint disease, is the most common type of arthritis. Knee OA is one of the most common disabling diseases[1]. The aetiology of OA is partly known, and the precise pathological mechanism responsible for cartilage loss and degradation is not fully understood. The inability to reproduce an animal model that can simulate both the symptoms and histopathology of knee OA has hampered the understanding of the disease. The rabbit models of knee OA are broadly of two types, namely, mechanical (transection of anterior cruciate ligament in the knee joint[2,3]) and chemical (intra-articular injection of chymopapain and collagenase[4-6]). Intra-articular injection of monosodium iodoacetate (MIA) has been described for the creation of OA animal model[2,3,7]. Unlike the other models, the stage of OA can be controlled with the MIA by titrating the dose injected intra-articularly. Although this model (chemical model) has been described in rat knees[7-10], it has not been described in rabbit knees. This study describes the creation of various stages of OA in rabbit knees using titrated doses of MIA.

Approval for this study was obtained from the Institutional Review Board (Christian Medical College, Vellore) and the Institutional Animal Ethics Committee. The study was conducted at the College Animal House Facility and Centre for Stem Cell Research, Christian Medical College, Vellore. The inclusion criteria for the study were as follows: male New Zealand white (NZW) adult rabbits above 7±1 months of age, weighing ≥1.5 kg with normal weight-bearing functions and veterinarian health inspection clearance. From a source population of 15, three adult NZW rabbits (five knees) were randomly selected. The rabbits were monitored closely, maintained at standard conditions and were allowed to move freely with free access to food and water. The duration of the study for the purpose of standardization of the ideal dose for creation of OA in the knee model was over three months (November 2015 to January 2016). The rabbits were anaesthetized using intramuscular (IM) injection of 50 mg/kg ketamine and 4 mg/kg of two per cent xylazine. Under sterile aseptic precautions, a single intra-articular injection of MIA reconstituted in sterile water was given. The dose and reconstitution are shown in Table.

Injection meloxicam (0.2 mg/kg) was given subcutaneously for post-procedure analgesia immediately after the procedure and was repeated.

Following sedation, the animals were euthanized with a lethal dose of IM ketamine on the 28th day post-MIA injection. Under sterile conditions, arthrocentesis was performed, where 150 µl of sterile water for injection was injected into the knee joints and synovial fluid was aspirated. The biological sample was immediately stored at −80°C for S100A12 protein analysis. The knee joints were removed, fixed with buffered formalin, decalcified in formic acid and embedded in paraffin. Five-micrometre sections in the transverse plane were prepared and stained with haematoxylin and eosin and safranin O.

Synovial fluid S100A12 is a specific biomarker to diagnose OA and also predict the severity of OA[11-13]. The

Table. Dose of monosodium iodoacetate (MIA) given in rabbit knee joint

Knee joint	MIA dose (mg)	Volume of sterile water (µl)
Left knee joint: Rabbit 1	Nil	250
Right knee joint: Rabbit 1	2.5	250
Left knee joint: Rabbit 2	3	50
Right knee joint: Rabbit 2	4	50
Right knee joint: Rabbit 3	4	250

level of S100A12 was measured using commercially available rabbit protein S100A12 ELISA kit according to the manufacturer's protocol (MyBioSource, Cusabio, China). OA severity assessment was done using the Osteoarthritis Research Society International (OARSI) OA cartilage histopathology assessment system and expression of synovial fluid S100A12 protein[12-15]. According to the OARSI OA cartilage histopathological system, the control knee without MIA (Fig. A1 and A2), 2.5 mg of MIA/250 µl and 3 mg of MIA/50 µl showed no change. A dose of 4 mg MIA in 50 µl of sterile water in adult rabbit (Fig. B1 and B2) showed Grade 1 changes. The changes included an intact surface with focal superficial fibrillations. The chondrocytes, chondron columns were arranged regularly in superficial, mid and deep zones with the tide mark appearing in the mid zone. The subchondral bone showed irregular trabeculae. The 4 mg dose of MIA in 250 µl sterile water showed Grade 3 changes (Fig. C1 and C2). The changes included surface discontinuity, difficulty in appreciating superficial and mid zone, deep fibrillations extending into the deep zone, empty lacunae with hypocellularity and disorientation of chondron columns, loss of subchondral bone density and safranin O stain depletion into the lower two-thirds of the cartilage.

The corresponding synovial fluid analysis of S100A12 protein biomarker showed comparable expression levels. Four milligram MIA in 250 µl showed 4×10^3-fold expression of S100A12 as compared to control. The other doses of MIA in variable volumes showed results similar to the control joint. Although 4 mg/50 µl showed Grade 1 changes on histology, it did not show S100A12 expression greater than the control levels.

The results obtained in this study showed that the stage of OA in rabbit knee joint could be successfully reproduced by intra-articular injection of MIA. The commonly described animal model of OA involves transection of anterior cruciate ligament in the knee joint (mechanical model) and the ensuing mechanical instability results in cartilage damage[2,3]. The treatment of the mechanical OA model with cell-based therapy does not address the persistent mechanical instability due to the transaction of cruciate ligament.

In rabbits, the other chemical induced models of knee OA include intra-articular injection of chymopapain and collagenase[4-6]. In

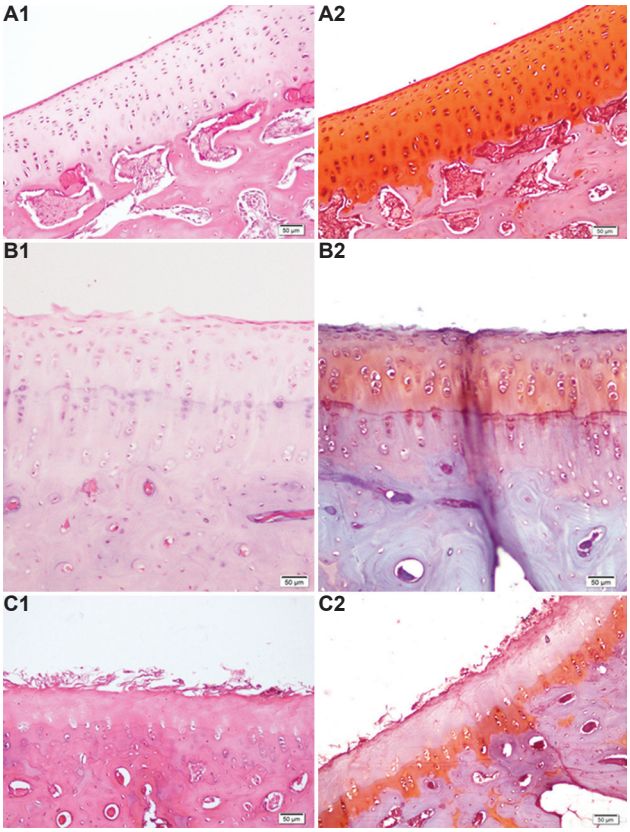

Figure. Haematoxylin-eosin (**A1**, **B1** and **C1**) and safranin O (**A2**, **B2** and **C2**) staining of articular cartilage of rabbit knee, (scale bar=50 µm). **A1** and **A2** showing normal articular cartilage from control rabbit knee (Grade 0 as per Osteoarthritis Research Society International grading). **B1** and **B2**, 28-day post-injection of 4 mg of monosodium iodoacetate in 50 µl of sterile water showing Grade 1 changes as per Osteoarthritis Research Society International grading. **C1** and **C2**, 28-day post-injection of 4 mg of monosodium iodoacetate in 250 µl of sterile water showing Grade 3 changes as per Osteoarthritis Research Society International grading.

chymopapain-induced OA, the mechanism of cartilage degeneration remains unclear requiring high doses. Collagenase has been reported to show dose-dependent OA changes, but its ability to damage other joint structures such as tendons, ligaments and menisci results in joint instability. The presence of collagenase inhibiting factors *in vivo* also limits its ability to produce a stable OA model[5]. MIA has been described for creation of OA in the temporomandibular joints of rabbits[2,3,7], but not for rabbit knees. Our results showed dose-dependent histopathological changes. This chemical model of MIA-induced OA was dependent both on the dose and the final reconstituted volume used for injection. The volume of vehicle seemed to play a vital role probably by causing a uniform distribution of the chemical, thus increasing its availability to induce

the inflammatory changes. Synovial fluid S100A12 protein, which is a specific biomarker to diagnose OA and predict the severity of OA, was also elevated[12].

The limitations of our study included the sample size and minimal volume of retrievable synovial fluid which was just sufficient for protein analysis. Although the number of rabbits used for each arm was less, this pilot study showed that MIA could be used to create an effective model of OA in rabbit knee joints. Further studies can create the stages of OA by titrating the dose based on our preliminary results. This chemical model also offers a rapid and minimally invasive method of creating a rabbit knee model of early OA, a stage in disease condition where targeted research could offer prospective results. In conclusion, intra-articular injection of MIA into rabbit knee joints can be used to create an experimental model of OA. This model can be used to study more about the pathogenesis of OA and develop effective treatment options for the same.

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