Collagenase and Sodium Iodoacetate-Induced Experimental Osteoarthritis Model in Sprague Dawley Rats

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Abstract: The objective of this study was to apply and compare two different experimental osteoarthritis (OA) methods in the rat, namely: Collagenase induced OA (CO) and Monosodium iodoacetate induced OA (MIA) models. The assessment of OA development and progression were performed through three different periods (2, 4 and 6 weeks). Intra-articular injection of either 4 mg joint−1 CO type II or 3 mg joint−1 MIA, were administered to the adult male Sprague Dawley rats, into their right knee joints. Evaluation of OA changes in the knees was achieved with both histopathology score system and radiography approach. Gross results revealed earliest changes such as swelling and redness of the right knee joints of all rats injected with either CO or MIA. Joint dissection revealed distinct thickening of the joint capsule in MIA-injected rats than in CO group. Present finding revealed early development of radiographical as well as histopathological changes in MIA injected group. However, both OA injected groups resulted in a chronic joint degeneration, measured by cellular changes, matrix degradation, subchondral changes and marginal osteophyte formation. Present findings showed significantly higher histopathological score in MIA injected group than those of CO in each of the three selected periods for OA induction. In conclusion, present results demonstrated that MIA can induce OA changes in a shorter period of time than CO in the Sprague Dawley rat. Radiography approach could be a useful tool to evaluate osteoarthritic changes in the knee joints.

Key words: Osteoarthritis, cartilage, rat, MIA, collagenase, histopathology

INTRODUCTION

Osteoarthritis (OA) the most common form of arthritis is a chronic disease characterized by slow degradation of the cartilage, pain and increase disability (Witter and Dionne, 2004). The disease is a disturbance in the balanced mechanism of articular cartilage synthesis and degradation, related to age and factors up regulated by cartilage and synovium, such as: cytokines, growth factors, aggrecanases and matrix metalloproteinases (Moreland, 2003). Radiographical features of OA are marginal osteophytes, subchondral sclerosis, cyst formation, reduced joint space and joint misalignment (Fernihough et al., 2004). It was considered that any reduction in the chondrocytes for any reason will lead to commence establishment of this joint disease (Lee et al., 2005).

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The disease is widely encountered in both humans and animals. Recent survey of
the World Health Organization (WHO) revealed 10% of world population average age 60 years
suffers osteoarthritis pain (McDougall, 2006). Similar problem is encountered in small and
large animals. Sixty percent of equine lameness is primarily caused by OA which lead to
economic losses in equine industry (Williams, 2007). Reports of Pfizer Animal Health
indicated that OA is a progressive degenerative condition affecting an estimated 20% of
adult dogs (North American Veterinary Conference, 1998). In cats, patellar luxation is
considered as the main cause of feline OA with a very high incidence of the disease
(Taylor and Robertson, 2004).

In order to understand OA better, various animal models were designed. The OA
lesions can be caused by either matrix degradation of articular cartilage using enzymatic
means i.e., papain and CO (Kikuchi et al., 1998) or by disturbing chondrocyte metabolism
i.e., MIA (Bove et al., 2003; Dumond et al., 2004; Pomonis et al., 2005) or by surgical
interventions i.e., anterior cruciate ligament transaction (Pelletier et al., 1995; Stoop et al.,
2001; Janusz et al., 2002; Galois et al., 2004) or partial meniscectomy (Kobayashi et al.,
2002; Smith et al., 2002; Appleyard et al., 2003; Oakley et al., 2004; Moore et al., 2005;
Young et al., 2005).

Earlier studies reported that chondrocyte metabolic inhibitor MIA have inhibitory effect
on the activity of glyceraldehyde-3 phosphate dehydrogenase in chondrocytes resulting in
disruption of glycolysis and eventually in cell death. Subsequently the histological changes
occurred in the articular cartilages of the knee joint being closely resembling to human OA
(Beyreuther et al., 2007).

Specific chemical factors which are produced from joint tissues are involved in the
progression of OA. A larger amount of collagenase was detected in OA cartilage than in
normal (Pelletier et al., 1983). Cytokines such as interleukin-1 and tumor necrosis factor which
are secreted from inflammatory cells and synovial cells of OA, stimulate the production of
proteolytic enzymes such as collagenase and stromelysin. Such catabolic enzymes are
closely related to the destruction of articular cartilage and subsequent OA progression.
Accordingly many studies used enzymatic factor CO to induce OA similar to the
spontaneous one (Choi et al., 2002; Blom et al., 2004; Hattori et al., 2005).

The value of animal models is limited by the extended time required for the development
of lesions similar to those observed in the spontaneous OA. Therefore, a model that rapidly
reproduces the clinical and pathological features of OA will be attractive.

This study was undertaken to apply and compare two formerly used animal models of
OA: (1) collagenase (CO) and (2) monosodium iodoacetate (MIA) models. Evaluation of their
OA development was performed through three different periods.

MATERIALS AND METHODS

Animals

Thirty six, twelve weeks old male Sprague Dawley rats, weighing between 380-400 g was
used in this study. These rats were kept in an air-conditioned animal room at 22°C, one rat
per cage and were given tap water and basal diet. The rats were left for 2 weeks for
acclimation before use. All animal experiments conducted were according to guideline for
animal handling and welfare in our facilities. This research project was conducted from
April 2008 to May 2009 at Faculty Veterinary Medicine, University Putra Malaysia. The study
was approved by Animal Care and Use Committee (ACUC), Faculty of Veterinary Medicine,
University Putra Malaysia, on 4th August 2008. Ref., UPM FPV/PS/3.2.1.551/AUP-R44.
Induction of OA

The rats were divided into three groups consisting of 12 rats per group. Each group was subdivided into three subgroups. The first subgroup was euthanized post 2nd week of OA induction (first duration). The second and third subgroups were euthanized post 4 and 6th week of OA induction, respectively (2nd and 3rd durations, respectively). The right knee joints of the first group were injected with 50 μL of normal saline solution and served as a normal control rats. Animals from the second group were injected with CO [Clostridium histolyticum, type II], obtained from Sigma (St. Louis, MO, USA)].

This enzyme (553 units mg⁻¹) was dissolved in saline in a dose of 4 mg mL⁻¹ and filtered with a 0.22 μm membrane. Fifty microliter of CO solution was injected intra-articularly into the right knee joints. The injection was performed twice, on days 1 and 4 of the experiment (Hattori et al., 2005). Finally, the third group was injected with MIA (Sigma, USA). Fifty microliter of MIA diluted with saline at a concentration of 60 mg mL⁻¹ were intra-articularly injected into the right knees, so that the dose of MIA in this group will be 3 mg joint⁻¹ (Kobayashi et al., 2003). Prior to inducing OA, all rats were anesthetized with a mixture of ketamine (50 mg mL⁻¹) and xylazine (20 mg mL⁻¹) at a ratio of 2:1, 1 mL kg⁻¹ b. wt. were injected intramuscularly into the gluteal muscle after knee joints had been shaved and sterilized (Hattori et al., 2005). Following this intra-articular injection of CO or MIA or saline were done through the patellar ligament using a 27 gauge, 0.5 inch needle.

Histological Grading

Four rats from each group were euthanized after the 2nd, 4th and 6th week post CO, MIA or normal saline administration, by intraperitoneal injection of 500 mg kg⁻¹ sodium phenobarbital. Radiography of the injected knee joints were performed before and after the OA induction. Whole right and left knee joints were dissected free from all soft tissues. The patella was removed from each knee to facilitate thorough fixation using standard procedures. Joints prepared for light microscopy were fixed in 4% paraformaldehyde in 0.1 M phosphate buffered saline (pH 7.4) for at least 24 h and subsequently decalcified with Rapid Decalcifiant Osseux (RDO, Apex, USA) approximately 72 h (Galois et al., 2004), then dehydrated through a descending series of ethanol using an automated tissue processing apparatus. After embedding in paraffin, joints were divided into two halves, separating the medial condyle and medial plateau from the lateral corresponding side (Janusz et al., 2002). Serial sectioning of 6 μm thickness was done for all halves and slides were numbered sequentially. Three slides from each medial and lateral condyle and plateau were selected for histopathological examination. Three slides selected represent three different sites (lateral, middle and medial parts) of the articular cartilages. Sections were stained with hematoxylin and eosin (H and E) or safranin-O fast green stain. Changes of OA lesions in the articular cartilage and subchondral bone were evaluated. We applied the previously published histological grading scheme (Kobayashi et al., 2003) for each rat which was expressed simply by the summation of individual grade (no changes: 0, mild: 1, moderate: 2 and severe: 3) for each observation. Observations are structural changes in the articular cartilage such as cellular density, surface irregularity, Safranin-O fast green stain reduction of intercellular matrix, subchondral changes and marginal osteophyte formation (Table 1).

Statistical Analysis

The data collected was scored according to Kobayashi et al. (2003) histopathology scoring system, which was non-parametric in nature. Accordingly, it was analyzed with
Table 1: Different histopathological changes in collagenase and MIA injected joints in time dependent manner (5 durations for OA induction)

<table>
<thead>
<tr>
<th>Observations</th>
<th>Score grades</th>
<th>2nd week (1st duration)</th>
<th>4th week (2nd duration)</th>
<th>6th week (3rd duration)</th>
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<tr>
<td></td>
<td></td>
<td>Collagenase</td>
<td>MIA</td>
<td>Collagenase</td>
</tr>
<tr>
<td>Chondrocyte loss</td>
<td>+</td>
<td>0/4</td>
<td>9/4</td>
<td>4/4</td>
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<td></td>
<td>++</td>
<td>0/4</td>
<td>4/4</td>
<td>0/4</td>
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<tr>
<td></td>
<td>+++</td>
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<td>0/4</td>
<td>0/4</td>
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<tr>
<td>Average pathology</td>
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</tr>
<tr>
<td>Chondrocyte cloning</td>
<td>+</td>
<td>1/4</td>
<td>4/4</td>
<td>3/4</td>
</tr>
<tr>
<td>and hypertrophy</td>
<td>++</td>
<td>0/4</td>
<td>0/4</td>
<td>1/4</td>
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<td></td>
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<td>0/4</td>
<td>0/4</td>
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<td>1.25</td>
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<tr>
<td>Chondrocyte</td>
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<td>2/4</td>
<td>2/4</td>
</tr>
<tr>
<td>Disorganization</td>
<td>++</td>
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<td>2/4</td>
<td>1/4</td>
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<td></td>
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<td>Surface irregularity</td>
<td>of articular</td>
<td>+</td>
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<td>cartilage</td>
<td>++</td>
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<td>Safranin O stain</td>
<td>reduction</td>
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<td></td>
<td>++</td>
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<td>2/4</td>
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<td>0/4</td>
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<td>Degeneration/necrosis</td>
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<td></td>
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<td>+++</td>
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<td>0/4</td>
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<td>0/4</td>
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<tr>
<td>formation</td>
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<td>0/4</td>
<td>0/4</td>
<td>0/4</td>
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<td></td>
<td>+++</td>
<td>0/4</td>
<td>0/4</td>
<td>0/4</td>
</tr>
<tr>
<td>Average pathology</td>
<td>score</td>
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<td>0</td>
<td>0.5</td>
</tr>
<tr>
<td>Synovial changes</td>
<td>+</td>
<td>2/4</td>
<td>2/4</td>
<td>1/4</td>
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<td></td>
<td>++</td>
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<td>+++</td>
<td>0/4</td>
<td>0/4</td>
<td>0/4</td>
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<td>Average pathology</td>
<td>score</td>
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<td>0.75</td>
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<tr>
<td>Fibrillation of cartilage</td>
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<td>0/4</td>
<td>0/4</td>
<td>1/4</td>
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<tr>
<td>surface</td>
<td>++</td>
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<td>0/4</td>
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<td></td>
<td>+++</td>
<td>0/4</td>
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<td>0/4</td>
</tr>
<tr>
<td>Average pathology</td>
<td>score</td>
<td>0</td>
<td>0</td>
<td>0.25</td>
</tr>
<tr>
<td>Total averages</td>
<td>pathology</td>
<td>score</td>
<td>1.75±0.069</td>
<td>8.5±0.160</td>
</tr>
</tbody>
</table>
| 0: No change, +: Mild, ++: Moderate, +++: Severe

Kruskal-Wallis to compare between more than two groups which is parallel to ANOVA test in case of parametric data and Mann-Whitney U-tests to compare between two groups only which is parallel to student-test in case of parametric data.

**RESULTS**

**Gross Changes**

Swelling and redness were the earliest changes that were observed in the right knee joints of all rats included in the CO and MIA groups of this study. However, gross swelling was subsided after 7-10 days post OA induction. Caliber measurements revealed joint diameters changes in the injected knees of both MIA and CO but not the saline injected joints. On rats euthanization there were distinct and characteristic thickening of the joint capsule in MIA-injected rats after 2nd weeks of OA induction. However, CO injected rats showed similar changes with lesser extent and thickening was observed at the 6th week of
Fig. 1: Gross observations of intra-articularly injected right knee joints (R). (a) Slight atrophy and congestion of surrounding soft tissues post the 4th weeks of CO-induced knees. (b) Thickening of synovial capsule post the 6th weeks of CO injection. Prominent capsule thickening of MIA injected knee (c and d) after the 4th and 6th week of OA induction, respectively. Note the whitish and glistening capsule of the left normal joints (L).

OA induction (Fig. 1a-d). Clinical observations of the rats gait revealed lameness in the hind limbs in both second and third groups. Abnormal gait was detected after the 4th week in third group whilst detected after the 6th week in the second.

Radiography

The radiographic changes were detected in the right knees injected with either CO or MIA. Changes have been detected predominantly post the 4 and 6th weeks of OA induction. Cranio-caudal and mediolateral radiographs of the right knee joints injected with saline showed normal opacity and absence of periarticular soft tissues swelling. Thickness of the joint space which refers to the region extended between the subchondral bones of opposing weight bearing surfaces revealed smooth articular cartilage surfaces separated with clear radiolucent microfilm of synovial fluid (Fig. 2a, b).

There was increased subpatellar opacity with erosions of the articular surfaces of the joint bones as early as the 2nd week of post-OA induction in the MIA group (Fig. 2c, d). However, no radiographical changes were observed in the CO group. But at the 4th week of post OA induction, CO group revealed an increase in joint mass (Fig. 2e, f). The MIA injected group, showed thinner joint space, osteophyte expressed by new bone formation at intercondylar space and irregular articular cartilage surfaces (Fig. 2g).

Rats at post 6th week post OA induction with CO revealed impaired joint space and chondrodestructrophic changes of the femoral condyles. While the MIA injected group, showed irregular articular surface of both tibia plates and femoral condyles, osteophytes at lateral and medial condyles, complete loss of joint space and the presence of joint mice in both medial and lateral aspects of the joint space (Fig. 2h, i).
Histopathology

Control Group

Figure 3a-d show normal structures of the articular cartilage and subchondral bone of both tibial plateaus and femoral condyles. It present smooth articular cartilage surface with the underneath layer of flattened chondrocytes in the tangential zone. Chondrocytes were normally distributed in parallel rows in the transitional and radial zones of the non-calcified part of the articular cartilage. Inter cellular matrix deeply and uniformly stained with safranin O fast green stain in the non-calcified part and for a lesser extent in the calcified region.
Subchondral bone revealed normal distribution of trabeculae composed of osteocytes and canaliculi surrounding the bone marrows filled with blood forming elements.

**First Duration (Post 2 Weeks of OA Induction)**

No sign of chondrocytes loss was observed in the knees of CO injected group, while those of the MIA group showed mild cellular loss in their articular cartilage. The loss was in the non calcified zone (tangential, transitional and radial zones). Mild changes such as chondrocyte cloning and hypertrophy were also detected in both groups. Cellular disorganization of the chondrocytes appeared mild in the articular cartilages of the CO injected groups, while it was mild to moderate in those of MIA group. No signs of cellular degeneration or necrosis were found in the knees injected with CO and for those of MIA, it showed mild degenerative changes such as chondrocyte shrinkage with hypereosinophilia and nuclear pyknosis. Depletion of proteoglycan component was detected by safranin O fast green stain reduction. The CO group revealed very mild reduction of this stain and was ranged from mild to moderate reduction in case of MIA injected group. Signs of fibrillation were not detected in the articular cartilage surfaces of both groups. Formation of osteophyte was also absent in the CO injected knees, while it was observed in nearly half number of the MIA injected group. Both groups showed subchondral changes characterized by replacement of bone marrow elements with fibrous tissues (Fig. 4a, b).

**Second Duration (Post 4 Weeks of OA Induction)**

This duration revealed mild changes in the following observations: Chondrocyte loss, chondrocyte cloning and hypertrophy, as well as safranin O fast green stain reduction in the knee articular cartilages of CO group, while there were moderate in the MIA injected rats.

The irregularity of the articular surfaces ranged from mild in CO group into moderate in case of MIA group. Signs of cellular degeneration/necrosis, characterized by chondrocyte shrinkage, nuclear pyknosis and hypereosinophilia of their cytoplasm were varied from mild to moderate in CO group and into moderate to severe in MIA injected group (Fig. 5a, b).
Fig. 4: (a, b) First duration (post 2nd week of OA induction). Left panel: CO injected joint revealed replacement of subchondral bone marrow elements with fibrous tissues (black arrows). Safranin O stain, X200. Right panel: MIA injected joint revealed subchondral fibrosis (thick arrow). Safranin O Stain, X40.

Fig. 5: (a, b) Second duration (post 4th week of OA induction): CO injected joint (left panel) exposed loss of chondrocytes in its tangential zone with mild Safranin O fast green stain reduction (black arrow). MIA injected joint (right panel) revealed chondrocytes loss in the tangential zone, degeneration with prominent pyknotic nuclei (white arrows) in both transitional and radial zones and moderate safranin O stain reduction. Safranin O fast green stain, X200

Osteophyte formation was absent in all rats included in the CO group, while present mild in those of the MIA group. Subchondral changes were found in both groups, but in case of MIA it was detected in both tibial and femoral bones. Similarly to the previous duration of OA induction, fibrillation of the articular surfaces was not detected.

Third Duration (Post 6 Weeks of OA Induction)

Generally, chondrocyte loss was mild in CO group, whilst moderate to severe in the MIA group. All rats showed moderate chondrocyte cloning and hypertrophy in the CO group, while mostly severe in the MIA group. Disorganization of the chondrocytes was varied from mild to moderate in the CO group, but there were mostly moderate in the MIA. Articular surface irregularities were mild and moderate in both CO and MIA groups, respectively. Depletion of proteoglycan synthesis in the intercellular matrix was detected with safranin O stain reduction. In this duration the reduction of this stain was mild to moderate in case of CO group and being moderately reduced in the MIA injected group. The
CO injected group revealed mild to moderate chondrocyte degeneration/necrosis. Same changes were severe in the knees of MIA injected group (Fig. 6a, b).

Similarly to the previous duration, no osteophyte formation was detected in CO group. Mild to moderate marginal osteophyte were observed in the tibial bone of the knees of MIA group (Fig. 7). Both groups revealed mild fibrillation in the surfaces of their knees articular cartilages.

**Fig. 6:** (a, b) Third duration (post 6th week of OA induction): CO injected joint (left panel) exposed chondrocyte loss of the tangential zone and fibrillation (short arrow), chondrocytes cloning and degeneration in both transitional and radial zones (long arrow) in the non calcified zone (doubled-head arrow). H and E, X100. MIA injected joint (right panel) revealed cloning of chondrocytes (black arrow), moderate cellular loss in the non calcified zone (doubled arrow) of articular cartilage. H and E, X200

**Fig. 7:** MIA injected knee joint, post 6th week of OA induction revealed marginal osteophyte formation (thick arrow) and replacement of bone marrow elements with fibrous tissue stroma (long black arrow) in the subchondral bone (S) under the degenerated articular cartilage (A) of the tibial plateau. Safranin O fast green stain, X100
Fig. 8: Differences between collagenase and MIA means (0-2.5) of their histopathological scores for the three consecutive durations of OA induction

Histopathological observations shown in Table 1 for both collagenase and MIA injected groups have been analyzed with non-parametric tests. In collagenase injected group, Kruskal-Wallis test revealed significant differences between the first and both 2nd (p<0.01) and 3rd (p<0.01) durations, while no significant differences were found between the 2nd and 3rd durations (p>0.05). In fact these results reflect the delayed progression of OA lesions in this group with subsequent well development in the later periods. In contrary the analysis of the MIA injected group revealed early higher score than collagenase group with no significant difference between the first and 2nd durations (p>0.05) and between the 2nd and 3rd duration (p>0.05). But first duration was significantly different than the 3rd of this group (p<0.05). Such results clarify the earliest OA development with subsequent gradual progression (Fig. 8). Mann-Whitney test revealed good differences between collagenase and MIA group for the 3 durations of OA induction (p<0.05).

DISCUSSION

In the present study, we investigated the effects of two different agents that are used to induce OA, namely CO and MIA to demonstrates structural histological changes in the knee joints of the Sprague Dawley rats. Histopathological changes of the right knee joints for 2nd and 3rd groups revealed osteoarthritic changes due to either CO or MIA injected substances but with different severities due to 2 different reasons.

- Time dependent manner, in which changes have been progressed from mild to moderate or severe with the more prolonged duration period of OA induction for each injected substance
- Marked and characteristic differences in the articular cartilage and subchondral bone changes in the right knees injected with MIA than those injected with CO

Such differences may be due to their different mode of action in the knee joints. Present results suggest that different time frame is needed to establish OA in the knee joints of the rat using either CO or MIA. Accordingly, we found rapid onset of OA development in the MIA group compared to those in the CO. The rapid development of OA

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(changes in the articular cartilage) in the MIA group was as early as fifteen days post-MIA injection and 6 weeks period of intra articular injection of MIA showed severe OA changes. These facts will be of importance to evaluate new anti-osteoarthritis therapies or drugs.

Injection of the metabolic inhibitor MIA into the knee joints inhibits glyceroldehyde-3-phosphate dehydrogenase activity in chondrocytes resulting in disruption of glycolysis and eventual cell death. Accordingly, the progressive cellular loss of the articular cartilage results in its histological and morphological changes (Williams, 2007). Apart from this, delayed onset of OA in CO group can be due to its action. The CO does not directly damage the articular cartilage but damage other structures within the joint (tendons, ligaments and menisci), which will eventually destruct the articular cartilage (Van der Kraan et al., 1990). This can be the reason why destruction of cartilage was observed only in the later stage, that is on the 4th week compared to the 2nd week in the MIA injected group.

Cellular changes and matrix degradation that had been recognized in the tibial plateau of CO group were similar to those formerly observed in the mice induced knee OA with collagenase (Van der Kraan et al., 1990). However, recent findings stated that CO enzyme may directly digest the collagen in the cartilage and induce the primary degeneration of the articular cartilage of the lateral aspect of the femur and tibia in rabbits more than medial aspect of the joint (Guzman et al., 2003). The CO and MIA injection induced various histopathological changes e.g., degeneration, cloning and disorganization of articular cartilage chondrocytes as well as depletion of proteoglycan contents of its intercellular matrix detected by safranin O stain reduction.

Such changes being closely resembled to human OA and our findings were in agreement with previous investigations concerning animal models of OA induction (Williams, 2007; North American Veterinary Conference, 1998; Kikuchi et al., 1998; Young et al., 2005; Beyreuther et al., 2007).

Evaluation of OA changes will be frequently assessed with histopathological examination of the articular cartilage, synovial membrane and joint capsule (Kobayashi et al., 2003). However, weight bearing was also used to measure the extent and pain of this degenerative joint disease (Williams, 2007). But in the current investigation, radiography approach was found useful mean to compare OA developed by either CO or MIA.

In fact, radiographic evaluation from our study revealed informative and useful morphological changes as early as the 2nd week post-MIA injection, compared to mild changes of the 4th week post OA induced CO group. These changes are due to the direct action of MIA on the cellular component of the articular cartilages of the joint. In conclusion, the overall results of the current study indicated that MIA model will be more preferable in OA studies in comparison with CO.

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