Osteoarthritis and Cartilage xxx (2016) 1–8

Osteoarthritis and Cartilage

OARSI OSTEOARTHRITIS RESEARCH SOCIETY INTERNATIONAL

Monoiodoacetic acid induces arthritis and synovitis in rats in a dose- and time-dependent manner: proposed model-specific scoring systems

M. Udo †, T. Muneta †, K. Tsuji §, N. Ozeki ‡, Y. Nakagawa †, T. Ohara †, R. Saito †, K. Yanagisawa †, H. Koga †, I. Sekiya ‡ *

† *Department of Joint Surgery and Sports Medicine, Graduate School, Tokyo Medical and Dental University, Tokyo, Japan*
‡ *Center for Stem Cell and Regenerative Medicine, Tokyo Medical and Dental University, Tokyo, Japan*
§ *Department of Cartilage Regeneration, Graduate School, Tokyo Medical and Dental University, Tokyo, Japan*

ARTICLE INFO

Article history:
Received 8 August 2015
Accepted 12 February 2016

Keywords:
Monoiodoacetic acid
Cartilage degeneration
Synovitis
Rat
Osteoarthritis
Scoring system

SUMMARY

Objective: In a rat monoiodoacetic acid (MIA)-induced arthritis model, the amount of MIA commonly used was too high, resulting in rapid bone destruction. We examined the effect of MIA concentrations on articular cartilage and infrapatellar fat pad (IFP). We also established an original system for "macroscopic cartilage and bone score" and "IFP inflammation score" specific to the rat MIA-induced arthritis model.
Design: Male Wistar rats received a single intra-articular injection of MIA in the knee. The amount of MIA was 0.1, 0.2, 0.5, and 1 mg respectively. Articular cartilage was evaluated at 2–12 weeks. IFP was also observed at 3–14 days.
Results: Macroscopically, low MIA doses induced punctate depressions on the cartilage surface, and cartilage erosion proceeded slowly over 12 weeks, while higher MIA doses already induced cartilage erosion at 2 weeks, followed by bone destruction. MIA macroscopic cartilage and bone score, OARSI histological score, and Mankin score increased in a dose- and time-dependent manner. The IFP inflammation score peaked at 5 days in low dose groups, then decreased, while in high dose groups, the IFP score continued to increase over 14 days due to IFP fibrosis.
Conclusions: Punctate depressions, cartilage erosion, and bone destruction were observed in the MIA-induced arthritis model. The macroscopic cartilage and bone scoring enabled the quantification of cartilage degeneration and demonstrated that MIA-induced arthritis progressed in a dose- and time-dependent manner. IFP inflammation scores revealed that 0.2 mg MIA induced reversible synovitis, while 1 mg MIA induced fibrosis of the IFP body.

Introduction

Osteoarthritis (OA) is one of the most prevalent joint diseases, especially in women[1]. To explore the pathological mechanisms behind OA and develop new treatments, it is essential to establish optimal OA animal models. Inflammation as well as mechanical stress triggers OA and affects its progression[2–4]. Monoiodoacetic acid (MIA) is commonly used to induce arthritis in rats, primarily in studies of arthritis-related pain[5,6]. However, in most cases the large amount of MIA for these studies resulted in bone destruction beyond cartilage inflammation within a short period of time[5,7,8]. We made a hypothesis that low dose of MIA induced mild inflammatory features of the cartilage. The first aim of this study was to describe cartilage characteristics during the progression of arthritis induced by various amounts of MIA in rats.

To properly evaluate arthritis progression, a quantitative evaluation tool to describe whole features is required to compare

* Address correspondence and reprint requests to: I. Sekiya, Center for Stem Cell and Regenerative Medicine, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo, 113-8510, Japan. Tel: 81-3-5803-4017; Fax: 81-3-5803-0192.
E-mail addresses: udo.orj@tmd.ac.jp (M. Udo), muneta.orj@tmd.ac.jp (T. Muneta), tsuji.orj@tmd.ac.jp (K. Tsuji), ozeorj@tmd.ac.jp (N. Ozeki), ynakagawa.orj@tmd.ac.jp (Y. Nakagawa), ohara.orj@tmd.ac.jp (T. Ohara), saito.orj@tmd.ac.jp (R. Saito), yanagisawa.orj@tmd.ac.jp (K. Yanagisawa), koga.orj@tmd.ac.jp (H. Koga), sekiya.arm@tmd.ac.jp (I. Sekiya).

http://dx.doi.org/10.1016/j.joca.2016.02.005

disease progression in numerous specimens across multiple studies. For this reason, there are several scoring systems to evaluate cartilage degeneration macroscopically in each study that used arthritis models[7,9–12]. However, cartilage inflammation can vary with each model, especially in its early phase, and current scoring systems may not be precise enough to fully categorize each phase of disease progression. The second aim of this study was to establish an original scoring system to quantify arthritis of the knee joint specifically for the rat MIA-induced arthritis model.

Synovitis is an important pathological condition in arthritis and OA, and the infrapatellar fat pad (IFP) is a useful tissue to evaluate synovitis. There are some scoring systems to evaluate synovitis[13,14], however, these systems only evaluate the cell lining layers, ignoring the body of the IFP, though fibrosis of the IFP is an important pathological condition related to synovitis[15]. The third aim of this work was to establish an IFP inflammation score capable of evaluating both the surface and body of the IFP and to quantify synovitis in the rat MIA-induced arthritis model.

Materials & methods

Animals

This study was approved by the Animal Committee of Tokyo Medical and Dental University. All animal care and experiments were conducted in accordance with the institutional guidelines of our Animal Committee. One hundred and twenty male Wistar rats (Charles River, Japan) at 8 weeks of age, 270–285 g in weight, were used for the study.

Preparation of MIA arthritis model for cartilage evaluation

Monosodium iodoacetate (Sigma–Aldrich, St. Louis, MO) was dissolved in saline and used as MIA. Under anesthesia by isoflurane inhalation and intraperitoneal injection of tribromoethanol, the right knee joint had a single intra-articular injection of MIA in 50 μl of sterile saline. The dose of MIA was 0.1, 0.2, 0.5, or 1.0 mg/50 μl and 20 rats were used for each dose. The left knee joint received an injection of saline. The left knee of rats which had a 0.1 mg injection in the right knee was used as control. The knee fixed at 90° and MIA or phosphate buffer saline (PBS) was injected through patellar tendon. After the injection, the animals were returned to their cages and kept under a 12/12 h light/dark cycle with food and water.

Macroscopic observation

The knee joints were harvested at 2, 4, 6, 8, and 12 weeks after injection (Fig. 1). The tibial plateau was carefully separated from the femoral condyle. Macroscopic pictures of the femoral and tibial condyles were taken using a ZIESS Stemi 2000C microscope (Zeiss, Oberkochen, Germany) on a dedicated medical photography platform. Quantification of the size of the cartilage legion was performed using AxioVision Rel 4.8 software (Zeiss). The cartilage degeneration and bone destruction of the femoral and tibial condyles were evaluated using a macroscopic score on a scale of 0–5 points (Table I).

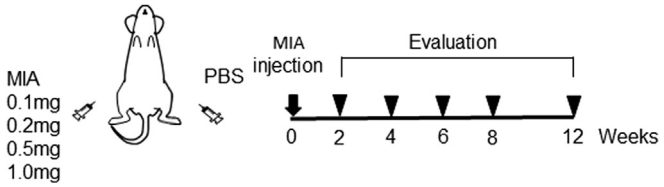

Fig. 1. Study schema. Rats had a single monoiodoacetic acid (MIA) injection in the right knee and phosphate buffer saline (PBS) in the left knee. The knees were evaluated at 2, 4, 6, 8 and 12 weeks after injection. For the control, the left knee of rats which had a 0.1 mg injection in the right knee, was used.

Histological examination

Proximal tibias were fixed in 4% paraformaldehyde for 7 days, decalcified in 20% EDTA solution for 21 days, and then embedded in paraffin wax. The specimens were sagittally sectioned at 5 μm and stained with safranin-o and fast green. Histological sections were visualized using an Olympus BX53 microscope (Olympus, Tokyo, Japan). The cartilage degeneration of the medial tibial plateau was evaluated using OARSI score[16] on a scale of 0–24 points and Mankin score on a scale of 0–14 points[17].

Immunostaining

Paraffin-embedded sections were deparaffinized in xylene, rehydrated in graded alcohol, and washed in PBS. All subsequent incubations were performed in a humidified chamber. The section was pretreated with proteinase K (Dako, Glostrup, Denmark) in Tris HCl buffer for 15 min at room temperature for optimal antigen retrieval. Then endogenous peroxidases were quenched using 0.3% hydrogen peroxidase in methanol for 15 min. Primary antibodies for human anti-type II collagen (Kyowa Pharma Chemical, Toyama, Japan) were applied to sections and incubated at room temperature for 1 h. After extensive washes with PBS, the sections were incubated in the biotinylated horse anti-mouse IgG for type II collagen. Immunostaining was detected with Vectastain ABC regent (Vector, Burlingame, CA) followed by diaminobenzidine staining. The sections were counterstained with hematoxylin.

Evaluation of synovitis

For the evaluation of synovitis, 40 rats were divided into two groups and 0.2 or 1.0 mg of MIA in 50 μl of sterile saline was injected into the right knee joint. The whole knee joint was harvested at 0, 3, 5, 7, and 14 days after the injection and prepared for histological evaluation as described previously (Fig. 5). The slides were stained with hematoxylin and eosin (HE), and the synovitis was evaluated using the IFP inflammation score on a scale of 0–6 points (Table II).

Statistical analysis

The scores were evaluated by three independent observers, where two observers evaluated in a blinded manner. For histology, one representative slice was evaluated. Interclass correlation

Table I
Macroscopic cartilage and bone scoring (0–5) for rat arthritis induced by monoiodoacetic acid (MIA)

Points	Findings
0	Intact articular surface
1	≦10 punctate depressions per condyle*
2	>10 punctate depressions per condyle*
3	Erosion (≦50% of joint surface)
4	Erosion (>50% of joint surface)
5	Bone destruction

Both lateral condyle and medial condyle were evaluated separately, and the higher point value was selected for femur and tibia respectively.
* Condyle; lateral femoral condyle, medial femoral condyle, lateral tibial condyle or medial tibial condyle.

Please cite this article in press as: Udo M, et al., Monoiodoacetic acid induces arthritis and synovitis in rats in a dose- and time-dependent manner: proposed model-specific scoring systems, Osteoarthritis and Cartilage (2016), http://dx.doi.org/10.1016/j.joca.2016.02.005