Effects of Pregnancy on Progression of Osteoarthritis Induced by Monosodium Iodoacetate in Rats

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Abstract

Hormones, particularly estrogen, are known to play a role in the development of osteoarthritis (OA). In relation to this, the impact of pregnancy on OA remains unknown. Pregnancy leads to decreased expressions of both synthetic and degradation genes of articular chondrocytes, as determined using *in vitro* tests, but no *in vivo* studies have been performed exploring pregnancy and OA simultaneously. The present study investigates the effect of pregnancy on the progression of OA in the rat joint degeneration model using an intra-articular injection of monosodium iodoacetate (MIA). Twenty Wistar rats were divided into four groups: A-Pregnancy and intra-articular injection of MIA; B-MIA injection in naive rats; C-Pregnancy, and D-Sham injection of saline in naive rats. Articular cartilage was evaluated 18 days after the detection of vaginal plug in female rats using Janusz's macroscopic score, equilibrium partitioning of an ionic contrast agent via microcomputed tomography, and glycosaminoglycan (GAG) quantification. Results show that there was no significant difference in GAG content, attenuation, volume, and thickness of articular cartilage between pregnant and non-pregnant groups. Contrary to our initial hypothesis, pregnancy does not have observable effects on MIA-induced OA in rats at 3 weeks.

Keywords: Osteoarthritis, Pregnancy, Cartilage, Microcomputed tomography (μCT)

1. Introduction

Osteoarthritis (OA) is a progressive joint disease characterized by loss of cartilage, sclerosis of subchondral bone, formation of osteophytes, and inflammation of synovial membrane due to imbalance in the production and degradation of the cartilage extracellular matrix [1]. Approximately 9.6% of men and 18.0% of women aged over 60 years suffer from symptomatic OA globally [2]. In the United States, an estimated 27 million people are affected by OA and this value is predicted to increase to 60 million individuals by 2020 [3,4]. Treatment strategies for disease-modifying agents for OA are a focus area for research as current treatments mainly target symptomatic relief.

Multiple factors have been proposed to be involved in the initiation and progression of OA, such as injury, age, genes, sex, and obesity [1]. Local factors, such as the curvature and lubrication of articular cartilage, load bearing, and alignment of lower limbs prostheses, have been well studied, while systemic factors have yet to be well investigated due to their complexity. Systemic factors such as age, genes, and sex have been proposed to regulate age-associated pathologies [5]. These factors have been proposed to be implicated in OA. Genetic factors are also influenced by sex. The candidate genes associated with OA vary between men and women [6]. The incidence of OA disease peaks at ages > 30 in men and > 50 in women [7]. Menopause in women has also been co-related with a higher incidence of OA prevalence [7]. It is associated with significant systemic physiologic changes, such as regulation of immune response and changes in hormones (estrogens, progesterone, prolactin, and androgens).

Pregnancy involves significant changes in hormones, metabolism, and gene expression. Pregnancy has been shown to restore debilitated regenerative capacity of aged livers through the activation of the Akt/mTOR pathway [8]. The proliferation of neuronal progenitors and pancreatic β cells was also found to be upregulated during pregnancy [9,10]. Pregnancy has also been reported to suppress the expression of both synthetic and degradation genes, including type II

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collagen, biglycan, transforming growth factor beta (TGF-β), collagenase, tissue Inhibitor of Metalloproteinase (TIMP-1), interleukin-1 beta (IL-1β), tumor necrosis factor alpha (TNF-α), Inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2) [11,12]. Based on these studies, it is reasonable to hypothesize that pregnancy, as well as subsequent physiological changes, may affect the progression of OA. The effect of pregnancy on the progression of OA has not been previous studied. The present study examines the effect of pregnancy on the progression of OA in a rat joint degeneration model.

OA can be induced through various methods, such as surgery, chemical injection, and mechanical overuse, or through spontaneous genetic OA models. The injection of monosodium iodoacetate (MIA) for chemically inducing OA is well characterized and has been extensively used in relation to examining joint pain in rats [13]. MIA is an inhibitor of glyceraldehyde-3-phosphate dehydrogenase, which restrains glycolysis in chondrocytes, leading to chondrocyte death. Previous data show that a high-dose injection of 1 mg MIA causes macroscopically visible cartilage damage in rats in 3 weeks [14]. Due to the extensive damage caused at 3 weeks after a high dose, studies have utilized a low dose of MIA to limit the global damage caused to the articular surface [15]. In the present study, OA was induced in Wistar rats using a low dose of MIA (0.3 mg). Equilibrium partitioning of an ionic contrast agent via microcomputed tomography (EPIC-μCT) was used to quantitatively analyze cartilage morphology and structure [14]. EPIC-μCT is a three-dimensional (3D), high-throughput, non-destructive method for quantitatively evaluating the articular cartilage surface. It can measure cartilage thickness and volume as well as cartilage attenuation, which is an indicator of proteoglycan content. This technique has been previously used to characterize a high-dose MIA model in Wistar rats [14]. EPIC-μCT is an efficient analysis technique compared to traditional methods such as glycosaminoglycan (GAG) quantification.

2. Materials and methods

2.1 Animals

All animal experiments were performed at the Animal Center of Peking University, and all procedures were approved by the Association for Assessment and Accreditation of Laboratory Animal Care (AALAC). Twenty female Wistar rats aged 12 weeks and weighting 280-300 g were divided into four groups. Five adult male rats were used to mate with the female rats. All the animals were housed in sterile animal rooms with controlled temperature and humidity. The animals were divided into four groups: A-Pregnancy and intra-articular injection of MIA; B-MIA injection in naive rats; C-Pregnancy, and D-Sham injection of saline in naive rats. MIA (I2512, Sigma-Aldrich, St. Louis, MO, USA) solution was injected into the intra-articular joint space of the female rats on the day of detection of vaginal plug. The rat knee joint of group A was injected with 0.3 mg of MIA in 50 μL saline with a 27 gauge

needle after pregnancy was confirmed. Group B received the same dose of MIA. Group C (only pregnancy) and group D (sham group) received 50-μL saline injections. All the rats were euthanized on day 18 via CO_2 inhalation.

2.2 Macroscopic score

Rat limbs were harvested after sacrificing the rats and the left legs were fixed in 10% neutral buffered formalin for 48 h. The rat joint was dissected and surrounding tissue was excised. Images were taken using a digital camera (DSC-H10, Sony) and evaluated by two people in a blind evaluation. The cartilage was assessed using a scoring system with a range of 0-4. Increasing score represented increasing severity of OA (0 represented normal appearance; 1 represented slight yellowish discoloration on the surface; 2 represented little erosions in load-bearing zone; 3 represented large erosions extending to the subchondral bone; and 4 represented large erosions with subchondral bone exposure) [15]. The scores were added and the mean was calculated for different groups.

2.3 Micro-computed tomography

For EPIC-μCT, the proximal end of each tibia was immersed in 2 ml of 30% Hexabrix 320 contrast agent (Covidien, Hazelwood, MO, USA) and 70% ion-free phosphate-buffered saline (PBS) at 37 ℃ for 30 min, an incubation period known to result in equilibration of the agent [16,17]. Proximal tibiae were blotted dry and scanned using a μCT 40 system (Scanco Medical, Brüttisellen, Switzerland) at 45 kVp, 177 μA, 200-ms integration time, and a voxel size of 16 μm [16].

Scanco evaluation software was used to assess the 3D morphology and composition. Raw scan data were automatically reconstructed into two-dimensional grayscale tomograms. These were rotated to sagittal sections, and the cartilage was contoured to separate it from trabecular bone and surrounding air [14,17]. The cartilage was segmented using a threshold value of 175-225 and then 3D images were generated. The attenuation, volume, and thickness of tibial cartilage were calculated for the tibial articular cartilage. Cartilage attenuation is a measure of the proteoglycan content and there is an inverse relationship between attenuation value and proteoglycan content.

2.4 GAG quantification

For quantification of the GAG content, fresh tibial cartilage from the right knees of the rat joints was harvested using a scalpel, weighed, and flash-frozen using liquid nitrogen. The lyophilized sample was digested with 0.5 mg/ml proteinase K (P2308, Sigma) in 100 mM K_2HPO_4 at pH 8.0 and 56 ℃ overnight. The digested sample was added to 1, 9-dimethylmethylene blue solution (DMMB, 341088, Sigma), shaken for 30 min, and then centrifuged at 10,000g to separate the precipitate. The precipitate was dissolved with a dissociation reagent and the absorbance was measured at a wavelength of 656 nm in a microplate reader (680, Bio-rad, Japan). The standard of GAG content was constructed using chondroitin sulfate (C4384, Sigma-Aldrich (Shanghai) Trading

Co., Ltd). The GAG content of each group was normalized by its weight.

2.5 Statistical analysis

All the data are presented as mean ± standard deviation (SD). SPSS V13.0 (SPSS Inc., IL, USA) was used to analyze the data, and one-way analysis of variance (ANOVA) with least significant difference (LSD) analysis was used to compare the differences between the four groups. The statistical significance was set at a 95% confidence interval, and p values indicate statistical significance compared to group D. (p < 0.05).

3. Results

3.1 Macroscopic score

There were no signs of infection in any experimental animals during the entire experiment. The tibial plateaus were smooth without any observable damage in the rats from groups A (Fig. 1(a)), B (Fig. 1(b)), C (Fig. 1(c)), and D (Fig 1(d)). No chondral erosions were observed and no erosions appeared in the load-bearing areas. No statistical significance was found between groups A, B, C, and D based on macroscopic scoring.
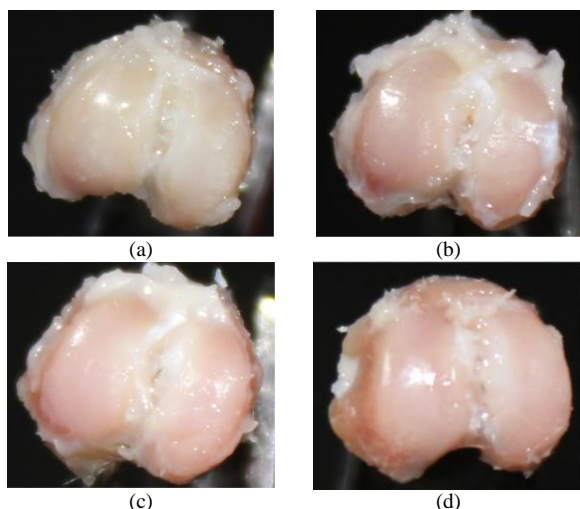

Figure 1. Macroscopic images of tibial cartilage for groups (a) A, (b) B, (c) C, (d) and D. No chondral erosions were observed on the cartilage surface. No statistical significance was detected between groups based on macroscopic scoring.

3.2 Micro-computed tomography

EPIC-μCT was used to measure cartilage attenuation, volume, and thickness. Cartilage attenuation, which is an indicator of proteoglycan content, was significantly higher in groups A (420.26 ± 17.94 mgHA/cm^3, p = 0.001) and B (420.39 ± 12.73 mgHA/cm^3, p = 0.001) compared to that in C (361.67 ± 17.34 mgHA/cm^3, p = 0.889) and D (369.32 ± 18.80 mgHA/cm^3) (Fig. 2). However, there was no significant difference in cartilage volume and thickness between the groups. The average values of volume were 1.466 ± 0.408 mm^3 (p = 0.831), 1.337 ± 0.199 mm^3 (p = 0.296), 1.494 ± 0.106 mm^3 (p = 0.974), and 1.499 ± 0.085 mm^3 for groups A-D, respectively (Fig. 3). The average values of thickness were

0.126 ± 0.020 mm (p = 0.850), 0.121 ± 0.008 mm (p = 0.395), 0.130 ± 0.007 mm (p = 0.774), and 0.129 ± 0.013 mm for groups A-D, respectively (Fig. 4). Data from each group are shown in Table 1.
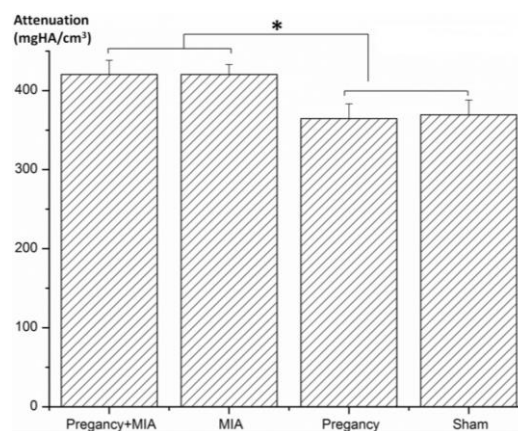

Figure 2. Cartilage attenuation for groups A, B, C, and D as calculated from EPIC-μCT. Cartilage attenuation is an indirect indicator of proteoglycan content. Attenuation was significantly higher in groups A and B compared to that in groups C and D. Pregnancy did not have a significant effect on cartilage attenuation. Data are expressed as mean ± SD. * = p < 0.05.
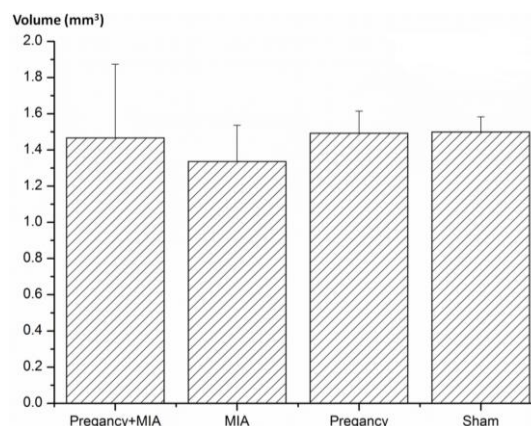

Figure 3. Cartilage volume for groups A, B, C, and D as calculated from EPIC-μCT. No significant differences were observed between groups. Pregnancy did not have a significant effect on cartilage volume. Data are expressed as mean ± SD.
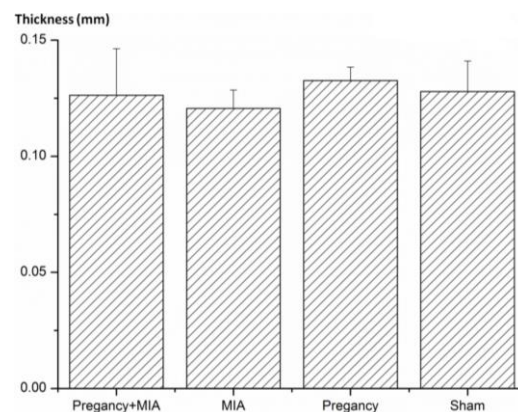

Figure 4. Cartilage thickness for groups A, B, C, and D as calculated from EPIC-μCT. No significant differences were observed between groups. Pregnancy did not have a significant effect on cartilage thickness. Data are expressed as mean ± SD.

Table 1. Raw data for cartilage attenuation, volume, and thickness for each group calculated from EPIC-µCT. p values indicate statistical significance compared to group D.

Group	Attenuation (mgHA/cm^3)	Mean ± SD	p value	Volume (mm^3)	Mean ± SD	p value	Thickness (mm)	Mean ± SD	p value
Pregnancy +MIA	410.3498			1.7478			0.1349		
	414.099			1.1294			0.1082		
	425.0345	420.26 ± 17.94	0.001	1.5964	1.466 ± 0.408	0.831	0.1418	0.126 ± 0.020	0.850
	402.8511			1.9046			0.1449		
	448.9884			0.9519			0.1015		
MIA	418.6815			1.4087			0.1247		
	434.6161			1.2437			0.11		
	422.2226	420.39 ± 12.73	0.001	1.6065	1.337 ± 0.199	0.296	0.1304	0.121 ± 0.008	0.395
	426.1801			1.3543			0.1223		
	400.2474			1.0697			0.1156		
Pregnancy	346.299			1.5742			0.1281		
	359.6299			1.4101			0.1274		
	361.5045	361.67 ± 17.34	0.889	1.6183	1.494 ± 0.106	0.974	0.1391	0.130 ± 0.007	0.774
	390.5617			1.3677			0.1358		
	350.3608			1.4973			0.121		
Sham	350.5691			1.4051			0.1247		
	360.0465			1.5787			0.1367		
	362.4419	369.32 ± 18.80	---	1.4283	1.499 ± 0.085	---	0.1171	0.129 ± 0.013	---
	374.1064			1.4898			0.115		
	399.4143			1.5907			0.1458		

3.3 GAG Quantification

MIA injection reduced the GAG content of cartilage in the rat OA model. GAG content of cartilage in group A (15.53 ± 1.31 µg/mg, p = 0.001) and group B (14.72 ± 2.01 µg/mg, p = 0.001) was significantly lower compared to that in group C (25.74 ± 3.17 µg/mg, p = 0.981) and group D (25.20 ± 2.80 µg/mg) (Fig. 5).
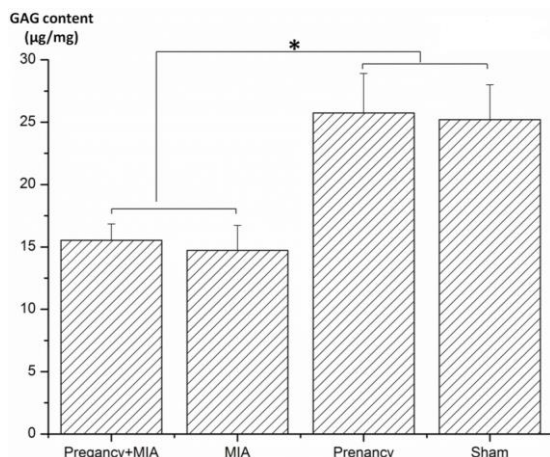

Figure 5. GAG content as measured by DMMB assay. GAG content was significantly higher in C and D groups compared to that in A and B groups. Pregnancy did not have a significant effect on GAG content. Data are expressed as mean ± SD. * = p < 0.05.

4. Discussion

Pregnancy is a complex physiological process accompanied with changes in hormonal levels. It has been implicated in playing a role in the regenerative capacity of certain tissues and cells [8]. In this study, the focus was to examine the effect of pregnancy on the progression of OA.

Multiple factors are involved in the initiation and progression of OA, with estrogen being an important one. Estrogen receptors have been found in cartilage, immortalized chondrocyte cell lines, and primary chondrocytes [18]. This suggests that cartilage is a potential target for estrogen. However, findings on the relationship between estrogen and OA are inconsistent. Some studies found that there was no direct relationship between estrogen and OA. Estrogen treatment did not have an effect on the severity of OA in ovariectomized animals and deletion of estrogen receptors did not cause overt cartilage damage [19-21]. In contrast, some results have indicated that estrogen has protective effects on OA. OA was elevated with estrogen deficiency and estrogen therapy preserved cartilage [22,23]. On the other hand, some results suggest that estrogen has negative effects on OA. Estrogen treatment caused synovial estrogen and estradiol receptor bindings to be increased, which induced cartilage degeneration and cell death, leading to the development of knee OA [24,25]. The relationship between estrogen and OA is thus still unclear.

As the amount of estrogen is elevated during pregnancy in mammals, pregnancy may be related to the metabolism of cartilage and OA. The gene expression of normal articular cartilage can be influenced by pregnancy. The gene expression of type II collagen, biglycan, collagenase, TIMP-1, IL-1β, TNF-α, TGF-β, iNOS, and COX-2 has been reported to be suppressed in pregnant rabbits [11]. It was also found that the gene expression levels changed variably in cartilage surfaces between lateral and medial femoral condyles, lateral and medial tibial plateaus, and the femoral groove during pregnancy compared to non-pregnancy [12]. Besides affecting cartilage, pregnancy could also affect the metabolism and improve the

regenerative capacity of some cells and tissues. Prolactin, a hormone secreted during pregnancy mediates neurogenesis in the adult female forebrain during pregnancy [9]. The ovariectomized mice injected with prolactin exhibited proliferation of neuronal progenitors in the forebrain subventricular zone [9]. Pregnancy also stimulates the proliferation of maternal pancreatic islet β-cells. The maternal β-cell mass was two-fold that prepartum. The process was controlled by menin, a protein regarded as an endocrine tumor suppressor, whose Men1 gene expression is regulated by prolactin [10]. Pregnancy also restores debilitated regenerative capacity of aged livers by switching from the Akt to the mTOR pathway, which transforms aged livers from proliferation to regeneration [8]. Although pregnancy could improve the regenerative capacity of some tissues, the relationship between pregnancy and OA cartilage has not been explored *in vivo*.

Various animal models have been established to mimic human OA to study the pathology of the disease, including surgical/physical, chemical, and spontaneous models. Experimental OA model mainly include physical/surgical models, such as anterior cruciate ligament transection (ACLT), and chemical induction of OA through MIA. Spontaneous and surgical models take a longer time to induce OA compared to that required for chemical models, and the natural/spontaneous model is limited to certain strains of animals. Considering the gestation period of rats (around 20 days), MIA was chosen here to induce OA due to its rapid effect [15]. MIA induces OA through restraining glycolysis of the chondrocytes by inhibiting glyceraldehyde-3-phosphate dehydrogenase. It is different from the natural OA process, as inflammatory mediators such as IL-1β and TNF-α are increased. It is important to note that an animal model cannot represent all aspects of human OA [26]. The physical behavior of human OA varies widely *in vivo* [27]. Since this study focused on the morphology of arthritic cartilage, the MIA-induced OA model was chosen as it is efficient and reproducible. A low-dose MIA model was used to elucidate the changes in the pregnant rat. A low-dose model prevents severe articular damage from taking place but results in lower proteoglycan content.

In our results, the attenuation of cartilage as detected by EPIC-μCT was higher in MIA groups compared to that in non-MIA groups. This indicates lower proteoglycan levels in MIA groups, which is a characteristic of OA. The results show that there was no significant effect of pregnancy on attenuation, volume, thickness, and GAG content. However, compared with chemical quantitative methods, such as DMMBassay, EPIC-μCT provides a non-destructive and quantitative method to evaluate articular cartilage while yielding similar results.

In this study, as no significant effects were found, it is difficult to speculate on any long-term effects of pregnancy on OA. One of the reasons for no changes could be due to the short gestation period of rats compared to other mammals. It may be possible that if an animal with longer gestation is analyzed, significant changes could be observed. To comprehensively study the effect of pregnancy on cartilage, additional parameters, such as gene expression, protein levels, and synovial fluid analysis are needed.

5. Conclusion

The effect of pregnancy on the progression of OA in the rat joint degeneration model using an intra-articular injection of MIA was investigated. EPIC-μCT was used to analyze articular cartilage in terms of attenuation, volume, and thickness. The relationship between pregnancy and OA is complex, and the results show that there was no significant different in attenuation, volume, and thickness between pregnant and nonpregnant groups. No significant pregnancy effect on OA progression was found, contrary to our initial hypothesis. Comprehensive studies on genes, proteins, and synovial fluid components should be performed to elucidate the effects of pregnancy on OA.

Acknowledgement

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