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Intra-articular injection of xanthan gum reduces pain and cartilage damage in a rat osteoarthritis model

Huarong Shao[a,b], Guanying Han[a,b,c], Peixue Ling[a,b,*], Xiqiang Zhu[b], Fengshan Wang[a], Lijuan Zhao[a,b], Fei Liu[b], Xia Liu[b], Guilan Wang[a,b], Yong Ying[d], Tianmin Zhang[a]

[a] Key Laboratory of Chemical Biology of Natural Products (Ministry of Education), Institute of Biochemical and Biotechnological Drugs, School of Pharmaceutical Sciences, Shandong University, Jinan 250012, China
[b] Post-doctoral Scientific Research Workstation, Institute of Biopharmaceuticals of Shandong Province, Jinan 250101, China
[c] School of Pharmaceutical Sciences, Liaoning Medical University, Jinzhou 121001, China
[d] Drug Safety Evaluation Center of Shandong Institute of Pharmaceutical Industry, Jinan 250101, China

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ABSTRACT

The objective of this study was to evaluate the alleviative effect of intra-articular (IA) injection of xanthan gum (XG) on pain and cartilage degradation in a model of monosodium iodoacetate (MIA)-induced knee osteoarthritis (OA). The rheological study and hyaluronidase (HAse) degradation analysis of XG injection were presented. The effect of pain relief was determined by measurements of paw withdrawal threshold and weight bearing by hind limbs. The protective effect on the cartilage was evaluated by gross morphological observation and histological evaluation of knee joints. The effect was investigated in two protocols: a therapeutic treatment protocol, and a prophylactic treatment protocol. Our results showed that HAse had no effect on the rheological properties of XG injection. Local XG administration in both protocols could reduce OA pain and alleviate the joint cartilage degradation induced by MIA. IA injection of XG might be an effective method for OA treatment in human.

1. Introduction

Osteoarthritis (OA) is a chronic disease characterized by structural and functional degradation of the joints. Associated with the remodeling of bone and destruction of cartilage, chronic pain is the most prominent and debilitating symptom which can cause a significant aggravation of joint dysfunction (Fernihough et al., 2004). The OA pathogenesis mechanisms are complicated (Lotz, 2012; Suri & Walsh, 2012), however, the main goals of current pharmacological therapies of OA continue to be the alleviation of pain and protection of cartilage degeneration (Plaas et al., 2011; Sofat, Ejindu, & Kiely, 2011).

Intra-articular (IA) injection of sodium hyaluronate (SH) is an effective and safe therapy for OA (Bannuru, Natov, Dasi, Schmid, & McAlindon, 2011; Boettger, Kummel, Harrison, & Schaible, 2011; Mihara, Higo, Uchiyama, Tanabe, & Saito, 2007). IA injection of SH can reduce the joint nociceptor discharge and protect the joint cartilage predominantly because of the role of SH as a viscoelastic filler, which is attributed to its rheological properties (García-Abuín, Gómez-Díaz, Navaza, Regueiro, & Vidal-Tato, 2011; Gomis, Miralles, Schmidt, & Belmonte, 2007; Gomis, Miralles, Schmidt, & Belmonte, 2009; Pozo, Balazs, & Belmonte, 1997). SH offers good analgesic effects for those who are suffering from OA pain (Balazs, 2003; Chevalier et al., 2010; Kolarz, Kotz, & Hochmayer, 2003; Neustadt, 2003). However, SH is not stable and is quickly degraded *in vivo* through hydrolytic or enzymatic reactions (Zhong et al., 1994). Therefore, a compound with similar function compared to SH but bearing a longer effect in the joints is needed to avoid frequent injections.

Xanthan gum (XG) is a microbial extracellular heteropolysaccharide produced by the bacterium *Xanthomonas campestris.* It is similar to SH in viscosity and rheology (Bewersdorff & Singh, 1988; García-Abuín et al., 2011). However, compared with SH, XG is likely to be more stable and not easily degraded in vivo (Han, Wang, et al., 2012). Our previous studies demonstrated that IA injection of XG could protect the articular cartilage in a rabbit OA model induced by papain. Fewer injections were needed than SH to achieve the same treatment results (Han, Shao, et al., 2012; Han, Wang, et al., 2012). In order to further develop this potential therapeutic method, it is of great importance to know whether XG can reduce OA pain. In the present study, we measured the paw withdrawal threshold (PWT) and weight bearing distribution (Fernihough et al., 2004) together with the cartilage structural changes of the injured knee in a rat OA model induced by monosodium iodoacetate (MIA) to evaluate the antinociceptive efficacy and cartilage protective effect

* Corresponding author at: Institute of Biopharmaceuticals of Shandong Province, Jinan 250101, China. Tel.: +86 053 181 213 002; fax: +86 053 188 524 738.
E-mail addresses: lpx@sdfmg.com, lingpeixue23@yahoo.com.cn (P. Ling).

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of IA injection of XG (Guingamp et al., 1997; Guzman, Evans, Bove, Morenko, & Kilgore, 2003; Kalbhen, 1987).

2. Materials and methods

2.1. Materials

MIA (Sigma, cat#12512) was dissolved in 0.9% (w/v) saline (3 mg in 50 μL). The solution was filtrated with 0.22 μm membrane before administration. The XG preparations (1%, w/v) were prepared according to the method described by Han, Wang, et al. (2012). The 1% (w/v) SH injection ($M_W \approx 1500$–2500 kDa, Lot. 101019034) was supplied by Bausch & Lomb-Freda Pharmacutical Co. (Jinan, China). Hyaluronidase (HAse, Sigma, cat# H3506) was dissolved in 0.9% (w/v) saline (1 mg in 100 μL).

2.2. Rheological study and enzymatic degradation analysis of XG

Rheological study of the XG and SH was performed using a Kinexus rheometer (Malven Instruments Ltd., Worcestershire, UK), equipped with a stainless steel cone and plate geometry (40-mm-diameter cone with a 2° cone angle) at 37 °C (Bhuanantanondh, Grecov, & Kwok, 2010). Steady-state viscosity was determined as a function of the shear rate covering the range from 0.1 to 1000 s^{-1}. Dynamic experiments were performed in the liner viscoelastic region over a frequency range of 0.1–10 Hz. For the HAse degradation analysis, 1 mL of the polymers preparations (1%, w/v) was added with 100 μL of HAse solution or saline, respectively (Picotti et al., 2012). The mixture was incubated at 37 °C. The rheological analysis was made after the SH mixture was incubated for 2 h, and the XG mixture was incubated for 72 h.

2.3. Experimental animals

All animal experiments were implemented according to the internationally accredited guidelines with the approval of the Institutional Animal Care and Use Committee of Drug Safety Evaluation Center of Shandong Institute of Pharmaceutical Industry (Jinan, China). Experiments were performed on 100 male Wistar rats (250–300 g). Rats were housed in groups of 3 per cage under the same environmental conditions (temperature of 19–21 °C, relative humidity of 50%–60%), with access to filtered water and a standard pellet diet ad libitum. Animals were acclimatized for 7 days before the experiments were initiated.

2.4. Induction of OA

Rats were deeply anaesthetized with an isoflurane/O_2 gas mixture until the flexor withdrawal reflex was abolished. Left knees were injected intra-articularly with 50 μL of MIA using a 0.5-in. 27-gauge needle, while the contralateral knees received an IA injection of 0.9% saline. The PWT and weight bearing distribution were tested before OA induction with the results being presented as day 0 and on days 3, 7, 10, and 14 after MIA injection. Significant changes of the PWT and weight bearing onto the ipsilateral limb would indicate that the OA model was successfully induced.

2.5. Treatment regimens

2.5.1. Treatment after OA induction

The 48 rats with induced OA on the left knees were randomly divided into four groups: an XG-treated twice group, a SH-treated twice group, a SH-treated five times group, and a saline control group. For the animals of the XG-treated twice group and SH-treated twice group, XG or SH injections were given into the left knees on days 14 and 32, respectively, while saline was given on days 21, 28 and 42. SH or saline were, respectively, injected into the left knees of animals in the SH-treated five times group and the saline control group on days 14, 21, 28, 35 and 42 after MIA injection. Saline was administered into the right knees of OA rats while the left knees were given XG, SH or saline treatment. The injection volumes of XG, SH and 0.9% saline were 0.2 mL/kg. The antinociceptive efficacy and cartilage protective effects of XG were compared with those of SH and saline treatment groups. Pain related behaviors were tested on days 3, 7, 14, 21, 28, 32, 35, 42 and 49, double-blindly. Rats were sacrificed on day 49 by overdose anesthesia and the knee joints were collected for subsequent analysis.

2.5.2. Pre-treatment before OA induction

Forty rats were randomly divided into four groups of 10 each: a sham group, a saline pre-treatment group, an XG pre-treatment group and a SH pre-treatment group. The left knees of rats were injected with saline, XG or SH 48 h before MIA administration, respectively, and saline was given into the contralateral knees at the same time. Injection volumes of the XG, SH and 0.9% saline were 0.2 mL/kg. Pain related behaviors were tested on days 3, 7, 14, 21, 28 and 35 after MIA injection, double-blindly. Rats were sacrificed on day 35 by overdose anesthesia and the knee joints were collected for further analysis.

2.6. Assay of weight bearing

An incapacitance tester (Yiyan Science, Jinan, China) was employed for the determination of hind paw weight distribution between the left and right limbs (Bove, 2003). Rats were placed in an angled Plexiglas chamber positioned so that each hind paw rested on a separate force plate. Each measurement reported was the average of 3 separate measurements of 5 s duration. Results were presented as the weight bearing of left limbs, calculated as follows:

$$\text{Weight bearing by ipsilateral limb}(\%) = \left[\frac{(\text{Weight bearing by ipsilateral limb})}{(\text{Weight bearing by ipsilateral limb} + \text{Weight bearing by contralateral limb})}\right] \times 100$$

2.7. Assay of mechanical allodynia

Tactile allodynia was tested using von Frey monofilaments (North Cost Medical, Inc. USA) that ranged in stiffness from 0.4 to 15 g (0.4, 0.6, 1, 2, 4, 6, 8 and 15 g) and the up-down method was adopted (Field, Bramwell, Hughes, & Singh, 1999). Briefly, rats were placed in Plexiglas boxes with mesh flooring and acclimatized for at least 20 min until grooming and exploratory behaviors declined to a level compatible with behavioral testing. Then the von Frey monofilaments were applied to the mid-plantar left or right hind paws initially with a 2 g monofilament. If the rat responded to the 2 g monofilament (a rapid withdrawal of the left hind paw or licking of the paw), the next lower filament was used until the rat stopped emitting a positive response. If the rat did not emit a positive response, the next higher filament was tested until the rat showed a positive response. The PWT was defined as the percent force eliciting a positive response of the ipsilateral paw and determined by the formula below. At each time point the average was

determined by 3 readings taken for each rat (at least 1 min elapsed between each test), and used for the subsequent analysis:

$$\text{Percent force eliciting withdrawal on ipsilateral paw}(\%) = \left[\frac{\text{Force eliciting withdrawal on ipsilateral paw}}{(\text{Force eliciting withdrawal on ipsilateral paw} + \text{Force eliciting withdrawal on contralateral paw})}\right] \times 100.$$

2.8. Evaluation of the cartilage

Femoral condyle and tibial plateau were collected and the surfaces of cartilage were photographed by a digital camera. Macroscopic lesions were graded according to the method described previously (Laverty, Girard, Williams, Hunziker, & Pritzker, 2010). The whole knee joints were prepared for histological evaluation using standard procedures. Samples were fixed with 10% (w/v) buffered formalin for 48 h, and subsequently decalcified in a solution containing 5% formic acid, 8.5% hydrochloric acid and 7% (w/v) $AlCl_3$ for 7 days, dehydrated, embedded in paraffin, and cut into 5 μm-thick serial sections. Serial sections were collected from the same anatomical site and stained with hematoxylin and eosin (H&E) for the analysis of cartilage structure or with safranin O for the analysis of proteoglycans (PGs). The histology was evaluated through double-blind observations following the method described previously.

3. Statistical analysis

All data were presented as mean ± standard deviation (SD). SPSS 16.0 statistical software package was used. Data were initially evaluated for normal distribution. Statistical significance among groups was then tested using a one-way analysis of variance (ANOVA) and differences between groups were further confirmed by the Student's *t*-test. $P < 0.05$ was considered to be significant.

4. Results

4.1. Rheological properties and enzymatic degradation analysis

Fig. 1A shows the dependence of viscosity upon the shear rate variation. At lower shear rates (0.1–2 s^{-1}), the viscosity of XG is higher than that of SH solutions. But at higher shear rates (2–1000 s^{-1}), XG had less viscosity than SH. Fig. 1B shows the mechanical spectra for both solutions inside the liner viscoelastic field. The XG injection showed a gel-like behavior, that the G' exceed G'' over the entire oscillation frequency range. However, the SH solution showed a viscous-like behavior at low frequencies, that the G'' was higher than G' (0.1–1.7 Hz), and a gel-like behavior at higher frequencies (1.7-10 Hz), that the G' overcome G''. Fig. 1C shows that the HAse had no influence on the viscosity of XG until 72 h practically, but it could degrade SH quickly. These results showed that the XG injection exhibited a non-Newtonian shear thinning behavior and viscoelastic behavior. XG was tolerant to HAse.

4.2. Effect of XG treatment after OA induction

Fig. 2 presents a comparison of different treatment groups, illustrating the magnitude and time course of changes in PWT and weight bearing ratios following injections of MIA, XG, SH and saline. Compared with the control group, the post-MIA injected rats exhibited significant decrement in the PWT and weight bearing of the ipsilateral limb ($P < 0.01$), which indicated that the OA model was successfully induced. Changes in hind paw weight distribution over time closely followed the changes seen in PWT, and the hyperalgesia induced by MIA was stable and lasted for the duration of the whole experiment. For the rats in the XG-treated twice and SH-treated five times groups, the PWT of the ipsilateral limb in response to the von Frey monofilaments stimulus increased significantly [Fig. 2(A), $P < 0.01$ XG vs saline group; $P < 0.01$ SH vs saline group] over time after treatments, and similar for the weight bearing distribution [Fig. 2(B), $P < 0.01$ XG vs saline group; $P < 0.01$ SH vs saline group]. There were no significantly alterations of the PWT and weight bearing values after saline injection in the control group. These results indicated a therapeutic anti-hyperalgesic effect of XG in OA rats. However, for the rats in the SH-treated twice

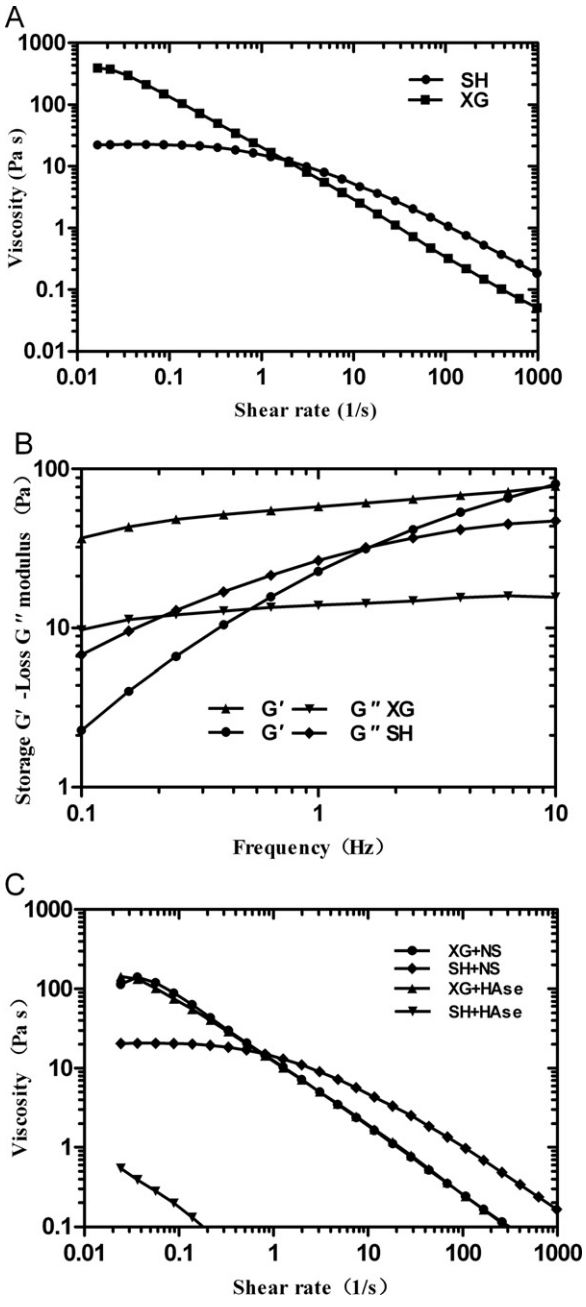

Fig. 1. Rheological properties of XG and SH: (A) flow curves; (B) mechanical spectra; (C) effect of HAse on the viscosity.

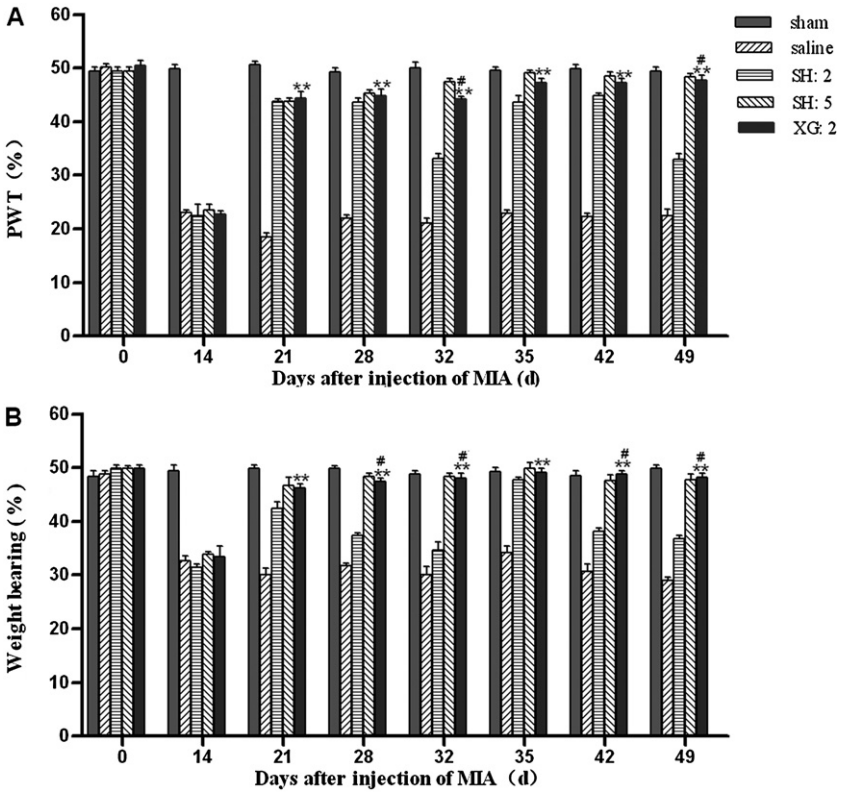

Fig. 2. Time course of changes in paw withdrawal threshold (PWT) (A) and weight bearing distribution (B) over a 49-day period of different treatment groups after osteoarthritis induction. For the rats in the XG-treated twice group (XG: 2) and SH-treated five times group (SH: 5), the PWT and weight bearing distributions of the ipsilateral limbs significantly increased, sustaining over 49 days. For the rats in the SH-treated twice group, the efficacy declined on days 28 and 32 and restored by SH injection thereafter. Data were presented as means with error bars equal to SD. $^{**}P < 0.01$ compared with saline control group values. $^{*}P < 0.05$ compared with saline control group values. $^{\#}P < 0.05$ compared with SH-treated twice group values.

group, the efficacy declined on days 28 and 32, but was restored by SH injection thereafter.

The macroscopic observations of the tibial plateaus and femoral condyles are presented in Fig. 3. The articular cartilage surface of the control group showed integrity and was smooth [Fig. 3(A) and (F)]. In the saline group, MIA injection resulted in full depth cartilage erosion in both tibial plateaus and femoral condyles [Fig. 3(B) and (G)]. The cartilage surfaces of the XG-treated twice group [Fig. 3(E) and (J)] and SH-treated five times groups also appeared to be uneven and damaged [Fig. 3(D) and (I)], but the erosion severity of which was milder those of saline group and SH-treated twice group [Fig. 3(C) and (H)]. The gross morphological scores of articular cartilages are shown in Table 1. The score of the XG-treated twice group was lower than that of the saline group ($P < 0.01$) and the SH-treated twice group ($P < 0.05$). However, no significant difference was observed between the XG-treated twice group and SH-treated five times group.

Fig. 4 shows the knee from the control group with normal articular cartilage overlaying the subchondral bone plate and cancellous bone, of which the superficial layer was smooth with integrity. Chondrocytes were flattened and arranged in neat rows, and the cartilage matrix was well stained with safranin O. However, in the saline group, the anterior surface of the joint cartilage showed erosion of the articular cartilage with marked loss of safranin O staining intensity. Compared with saline group, XG and SH groups showed significant improvements of fissures, disorganization and loss of chondrocytes, and an increment of the safranin-O unstained area. The scores of the XG-treated twice group were lower than that of the saline group ($P < 0.01$) and SH-treated twice group ($P < 0.05$). However, no significant difference was observed between the XG-treated twice group and SH-treated five times group (Table 1).

Table 1
Gross morphological and histological scores of articular cartilage in treatment groups.

Groups ($n = 12$)	Morphological observation		Histological evaluation	
	Fermoral condyle	Tibial plateau	Fermoral condyle	Tibial plateau
Sham	0.10 ± 0.12	0.13 ± 0.22	0.15 ± 0.11	0.3 ± 0.24
Saline	2.7 ± 0.51	3.0 ± 0.71	9.70 ± 0.37	10.30 ± 0.46
SH: 2	1.6 ± 0.53	2.10 ± 0.63	6.30 ± 0.49	8.10 ± 0.68
SH: 5	1.1 ± 0.42	1.35 ± 0.45	4.30 ± 0.57	5.80 ± 0.89
XG: 2	1.3 ± 0.51**	1.42 ± 0.35**	3.80 ± 0.55**	5.60 ± 0.70**

Values are the means ± SD. Comparisons considered significantly when $P < 0.05$.
** $P < 0.01$ vs saline group.

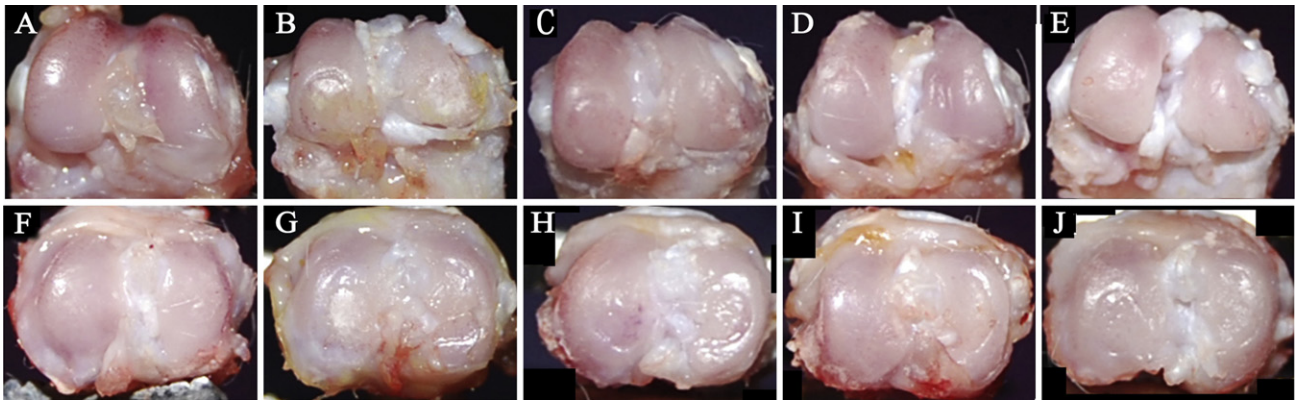

Fig. 3. Gross morphological observation of femoral condyle and tibial plateau from different treatment groups after osteoarthritis induction. Femoral condyle: (A) sham group; (B) saline group; (C) SH-treated twice group; (D) SH-treated five times group; (E) XG-treated twice group. Tibial plateau: (F) sham group; (G) saline group; (H) SH-treated twice group; (I) SH-treated five times group; (J) XG-treated twice group. Cartilage surfaces were normal in (A) and (F), slight lesion were seen in (C), (D), (E), (H), (I) and (J), and the most significant erosion in (B) and (G).

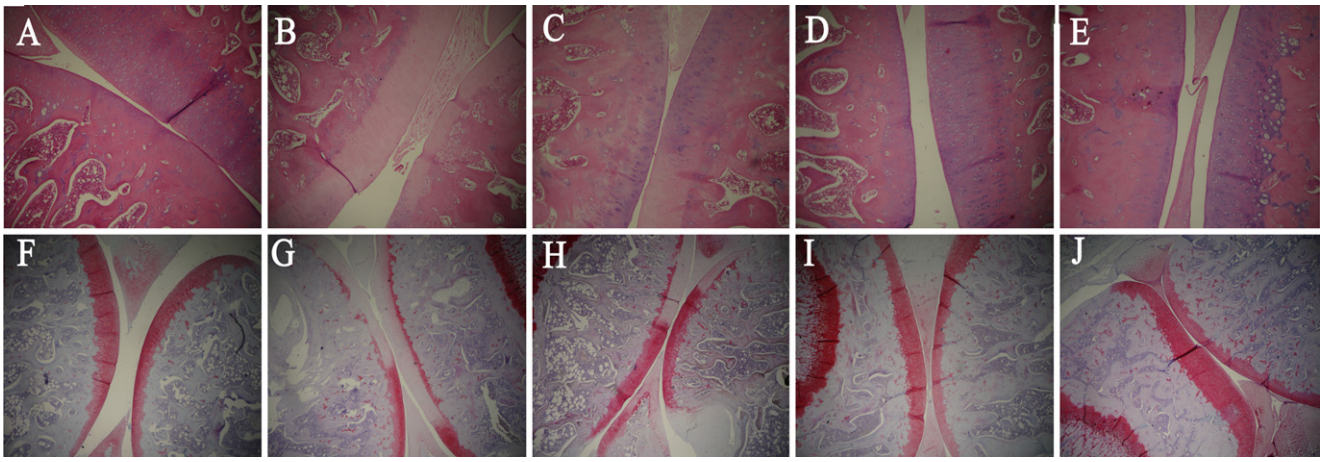

Fig. 4. Histological evaluation of cartilage from different treatment groups after OA induction (H&E, 40× magnification): (A) sham group; (B) saline group; (C) SH-treated twice group; (D) SH-treated five times group; (E) XG-treated twice group (safranin O, 100× magnification): (F) sham group; (G) saline group; (H) SH-treated twice group; (I) SH-treated five times group; (J) XG-treated twice group. Normal cartilage structure was seen in (A). The most significant erosion of the articular cartilage was seen in (B). Mild disorder was seen in (C), (D) and (E). Normal safranin O staining intensity was seen in (F). The most serious loss of staining intensity was seen in (G), and mild loss of staining intensity was seen in (H), (I) and (J).

4.3. Effect of XG pre-treatment before OA induction

Compared with the saline pre-treatment group, local XG and SH pre-treatments could increase both PWT and weight bearing of the limb applied to MIA (Fig. 5, $P < 0.01$) on days 7, 10 and 14, indicating the anti-hyperalgesic effects of XG and SH pre-treatment. In the XG pre-treatment group, the improvement of pain-related behaviors lasted for about 4 weeks, much longer than the approximately 2 weeks of the SH group.

Fig. 6 shows the knee of the saline group, with the anterior surface of the joint cartilage showing erosion of the articular cartilage with marked loss of safranin O staining intensity. Compared with the saline group and the SH group, XG pre-treatment significantly improved the fissures, disorganization and loss of chondrocytes, and increased the safranin-O unstained area. The score of the XG pre-treatment group was lower than that of the saline group and the SH group ($P < 0.01$) (Table 2).

5. Discussion

The current study assessed the alleviative effects of XG preparations on OA pain and cartilage degradation. The rat OA model was induced through IA injection of MIA. The antinociceptive efficacy of XG preparations was evaluated by measurements of the PWT and weight bearing. Gross morphological observations and histological evaluation of the knee joints were performed to evaluate the protective effect of XG on cartilage.

Single IA injection of MIA can inhibit glyceraldehyde-3-phosphate dehydrogenase activity in chondrocytes, and result in disruption of chondrocyte metabolism and eventual cell death (Kalbhen, 1987; van der Kraan, Vitters, van de Putte, & van den Berg, 1989). The progressive loss of chondrocytes results in a reliable and reproducible model, closely resembling the pathological and behavioral features associated with human OA (Guzman et al., 2003; Janusz et al., 2001; McDougall, Watkins, & Li, 2006). Previous studies (Fernihough et al., 2004; Guingamp et al., 1997) and

Table 2
Histological scores of articular cartilage in pre-treatment groups.

Groups ($n = 10$)	Fermoral condyle	Tibial plateau
Sham	0.18 ± 0.31	0.36 ± 0.35
Saline	10.1 ± 0.43	10.80 ± 0.79
SH	7.10 ± 0.64	8.40 ± 0.58
XG	5.90 ± 0.71**	6.50 ± 0.47**

Values are the means ± SD. Comparisons considered significantly when $P < 0.05$.
** $P < 0.01$ vs saline group.

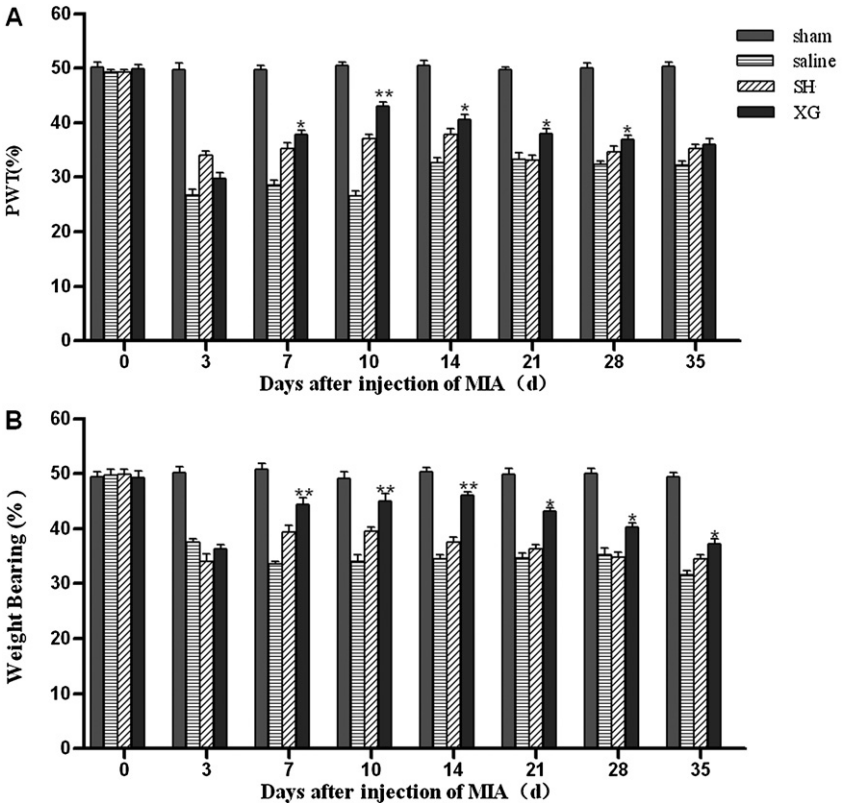

Fig. 5. Time course of changes in paw withdrawal threshold (PWT) (A) and weight bearing distribution (B) over a 35-day period in the pre-treatment osteoarthritis induction groups. Compared with saline group, the XG and SH pre-treatment significantly inhibited the shifts of PWT and weight bearing distribution of the ipsilateral limbs on days 7, 10 and 14. In the XG pre-treatment group, the improvement of pain-related behaviors lasted for about 4 weeks, much longer than SH group with approximately 2 weeks. Data were presented as means with error bars equal to SD. $^{**}P < 0.01$ compared with saline control group values. $^{*}P < 0.05$ compared with saline control group values.

our preliminary experiments (data not shown) demonstrated that MIA injection produced a time and concentration dependent shift in weight bearing and PWT of the injured hind limb, as well as the reduction in cartilage density. Measurements of the changes in the hind paw weight distribution and PWT have been utilized by a number of investigators to test pharmacological agents for their ability to alleviate MIA induced OA pain (Kalff et al., 2010; Schuelert et al., 2011). The dose of MIA utilized in the present study was chosen based on the degree of joint comfort and the histological changes. When MIA administered at 3 mg per joint, changes in weight bearing and PWT were found to be maintained over 49 days duration and significant erosion was observed in the surfaces of the tibial plateaus and femoral condyles. Therefore, the rat OA model induced by 3 mg MIA per joint was appropriate and reliable to evaluate the abilities of pharmacologic agents to alleviative the joint discomfort and preserve cartilage structure.

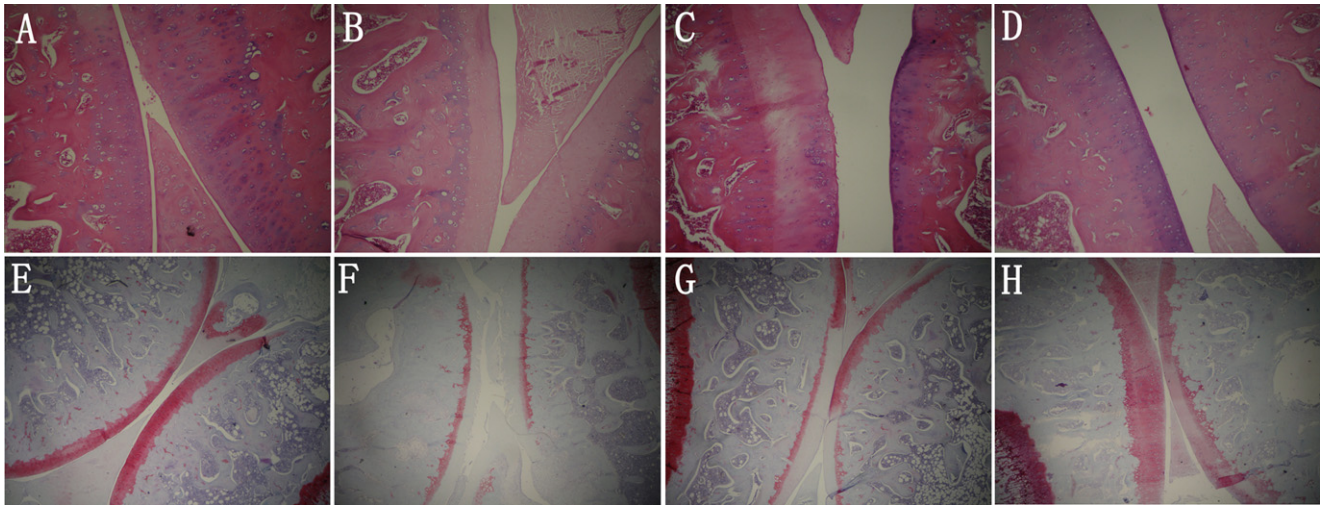

Fig. 6. Histological evaluation of cartilage from different pre-treatment groups (H&E, 40× magnification): (A) sham group; (B) saline group; (C) SH pre-treatment group; (D) XG pre-treatment group (safranin O, 100× magnification): (E) sham group; (F) saline group; (G) SH pre-treatment group; (H) SH pre-treatment group. Normal cartilage structure was seen in (A). Significant erosion of the articular cartilage was seen in (B) and (C). Mild disorder was seen in (D). Normal safranin O staining intensity was seen in (E). Significant loss of staining intensity was seen in (F) and (G), and mild loss of staining intensity was seen in (H).

MIA injection caused initial acute inflammatory response, which had largely subsided by day 7 (Bove, 2003; Kalff et al., 2010). Accompanying the remodeling of bone and destruction of cartilage, the OA-like knee pain could be observed from post-day 14 onward (Fernihough et al., 2004; Guingamp et al., 1997; Guzman et al., 2003). In our study, the injection volumes and the treatment period were determined according to the clinical injections volumes and therapeutic regimen in human. Therefore, the XG injection was given on day 14, and the therapeutic effect could be observed until day 49 post-MIA injection.

For the rats in the XG-treated twice group and the SH-treated five times group, the PWT and weight bearing of the MIA injured limb increased over time after the onset of pain, and no decrease of the antinociceptive effect similar to that of the SH administrated twice group was observed. IA injection of XG could also reduce the erosion of cartilage surfaces, and preserve the proteoglycans of femoral condyle and tibial plateau, which act as a mechanical shock absorbing system especially in the joint. Furthermore, no significant differences between the XG-treated twice group and the SH-treated five times group were observed, which is in agreement with our previous findings (Han, Wang, et al., 2012). Therefore, XG could alleviate OA pain and protect cartilage, and required fewer injections than SH to produce the same treatment results under the current treatment regimen.

In the pre-treatment study, XG could produce a consistent pain relief effect when it was administered before the appearance of pain-related behaviors. The presence of a lag in effect on day 3 might be explained by the initial acute inflammatory response caused by the MIA injection. An important finding is that the antinociceptive effect of 1% XG preparations could persist for about 4 weeks in the MIA-induced OA model. XG pre-treatment significantly improved the fissures, disorganization and loss of chondrocytes, and increased the safranin-O unstained area. Due to the longer effect of XG injections, this potential therapeutic method could avoid frequent injections for human OA patients.

The mechanisms underlying the beneficial effects of XG in OA pain are not understood. One hypothetic proposal is that IA injection of XG could make up for the loss of viscoelasticity of the synovial fluid, thus acting as a mechanical protector for the joint. The results showed that XG injection exhibited a non-Newtonian shear thinning behavior and viscoelastic behavior. IA injection of XG may be able to restore the rheological homeostasis of the synovial fluid. XG was tolerant to HAse, and may have a longer residence time within the joint cavity than SH. Thus, we speculate that the efficacy of XG was longer than SH in the present research due to its longer residence time in the joint cavity. Furthermore, due to the properties of the XG preparation, it might be capable of protecting sensory nerve endings and decreasing their sensitivity to mechanical stimulus and inflammatory mediators. The inflammatory mediators might be entrapped in the viscous XG preparation, thereby unable to reach the respective receptors in an adequate concentration. Considering the possible mechanisms discussed here for the effects of XG on OA, it is highly important to know how long the preparation can actually remain in the joint cavity before being cleared. In this respect, XG was less easily cleared than SH from the joint cavity because of its solution stability over wide ranges of pH, ionic concentration and temperature, and the fact that the degradative lyase for XG is produced primarily by bacteria (Dário, Hortêncio, Sierakowski, Neto, & Petria, 2011; Moreland, 2003; Nankai, Hashimoto, Miki, Kawai, & Murata, 1999). However, IA injection of XG producing a longer antinociceptive effect might be the result of some physiological effects similar to SH (Moreland, 2003). Besides, the duration of the pain relief effect could be closely related to the particular OA model employed.

IA injection of XG could reduce OA pain and alleviate joint cartilage degradation induced by MIA, and seemed to be an effective therapeutic means for the treatment of OA. A valuable finding of our study was that the antinociceptive efficacy period for XG preparations was much longer than that of SH under the current treatment regimen. However, it is essential to study the pharmacokinetics and metabolism of intra-articularly administered XG, the dose/response effects of XG injection, as well as to conduct further research to develop deeper insights into its mechanism of in vivo degradation and loss.

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