A Pathophysiological Validation of Collagenase II-Induced Biochemical Osteoarthritis Animal Model in Rabbit

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Abstract

BACKGROUND: Current dilemma working with surgically-induced OA (osteoarthritis) model include inconsistent pathological state due to various influence from surrounding tissues. On the contrary, biochemical induction of OA using collagenase II has several advantageous points in a sense that it does not involve surgery to induce model and the extent of induced cartilage degeneration is almost uniform. However, concerns still exists because biochemical OA model induce abrupt destruction of cartilage tissues through enzymatic digestion in a short period of time, and this might accompany systemic inflammatory response, which is rather a trait of RA (rheumatoid arthritis) than being a trait of OA.

METHODS: To clear the concern about the systemic inflammatory response that might be caused by abrupt destruction of cartilage tissue, OA was induced to only one leg of an animal and the other leg was examined to confirm the presence of systemic degenerative effect.

RESULTS: Although the cartilage tissues were rapidly degenerated during short period of time upon biochemical induction of OA, they did not accompanied with RA-like process based on the histology data showing degeneration of articular cartilage occurred only in the collagenase-injected knee joint. Scoring evaluation data indicated that the cartilage tissues in non-induced joint remained intact. Neutrophil count transiently increase between day 8 and day 16, and there were no significant change in other complete blood count profile showing a characteristics of OA disease.

CONCLUSION: These study shows that biochemically induced cartilage degeneration truly represented uniform and reliable OA state.

Keywords Animal model · Osteoarthritis · Cartilage · Regeneration · Inflammation

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1 Introduction

Recent progress in regenerative medicine focuses on aging-related degenerative diseases [1]. Validating the efficacy of treatment regimen involves examination with animal model. However, aging-related disease models representing true disease mechanism are hard to establish. For osteoarthritis (OA), there are several animal models. Spontaneous OA models include the models developed by natural aging and the transgenic animals that are susceptible to OA disease [2]. Among these, OA model generated by natural aging can serve as best model for human OA condition because they produce most similar pathological degeneration found in human OA. However, long-term following to obtain the disease state can be a critical demerit of naturally induced OA model because of expensive housing cost and lengthy period of procedure limits flexibility of experimental design.

Animal model for OA can also be induced by surgical or biochemical method, which results in rapid progression compared with the model induced by natural aging. Among the surgically induced OA model, most commonly used model include transection of anterior cruciate ligament (ACL) and partial meniscectomy. After surgically-induced transection of ACL or meniscectomy, they need exercise session to fully induce cartilage degeneration after the surgery and this exercise session induce compromised structural integrity that leads to destabilized joint and articular cartilage degeneration. Although uniform extent of cartilage degeneration is needed for test of regeneration therapy, OA models induced by surgical damage on ACL or meniscus results in wide variety of degrees in the induced OA state.

Biochemically induced OA involves the injection of protease such as collagenase or papain directly into the cavity of articular joint [3]. In contrast to surgically induced OA model, biochemically induced OA model have several advantageous point in a sense that it can be established even with small animals such as rats and mice, and involves only a simple procedure, which is less invasive. Compared with surgically induced OA model, biochemically induced model shows high reproducibility and results in relatively homogeneous degree of pathological state. Also, biochemically induced OA can be more practically applied to large scale study because it is much easier to establish the model.

Even with those merits, critical concern existed about the pathophysiology of biochemical model for they might induce significant amount of inflammation and also may not represent naturally occurring OA state found in human [4]. Therefore, biochemical OA model mostly used to investigate the mechanism of arthritis pain [5]. In line with

the concern that the biochemical model do not recapitulate human OA, characterization of biochemical model reported by Kikuchi [3] showed that the articular cartilage underwent gradual degeneration up to 6 weeks after collagenase injection, which might imply involvement of chronic inflammation resulting in rheumatoid arthritis (RA)-like pathophysiology. As animal model for RA is induced by systemic injection of type II collagen to provoke immune response [6], abrupt degradation of articular cartilage during short period of time in Kikuchi's model might increase degradation fragments of type II collagen in circulation which mimic the RA model that might leads to immune response.

Discerning the difference of pathophysiology in animal model is very important because research focus of regenerative medicine puts weight not only on reconstructive therapeutic regimen [7–10] but also preventive strategy before or at the onset of disease progression [11–17]. Therefore, understanding the subtle difference of disease mechanism and realization of rightful animal model is very important in the investigation of the therapy. In regenerative therapy for OA, focus should be made for reconstruction of physically damaged cartilage and true OA model should not accompany with systemic degenerative effect coming from inflammatory state. In this study, we aimed to clear the concern whether the biochemically induced OA model accompany systemic inflammation, and proved that degeneration of articular cartilage by collagenase injection resulted in only local tissue damage without acute systemic inflammatory response.

2 Methods

2.1 Materials

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise indicated.

2.2 Biochemically induced rabbit OA model

Female New Zealand white rabbits (weighting 2.0–2.5 kg; Central Laboratory Animal Inc., Seoul, Korea) were used for this study. Biochemical rabbit OA model was induced as described previously [3, 18]. Briefly, each rabbits were anesthetized and intraarticularly injected with 250 μ l volume of saline or 4 mg/ml collagenase II in saline as indicated in the results. The injection was repeated after 3 days as indicated in Fig. 1A. Rabbits were sacrificed at the indicated interval to examine the induced OA state. The knee thickness was measured with Vernier calipers at the thickest point of the knee joint—thickness between medial and lateral epicondyle of femur and tibia—before

Fig. 1 Experimental design to induce **A** biochemical Osteoarthritis (OA) model and **B** knee thickness after OA injection. Statistical significance was analyzed by two way ANOVA, and the knee

dissecting knee joint. Blood were withdrawn from portal vein to test complete blood count (CBC) with cell counter analyzer MS9-5V (Melet Schloesing Laboratories, Osny, France). Normality of each blood cell count was determined based on previous literatures [19–21].

2.3 Histological analysis

Knee joint tissues fixed with 4% paraformaldehyde in PBS were subjected decalcification using 0.25M EDTA. EDTA solution was changed every day for the first week and then changed every other day until all bones were decalcified. Scalpel was used to test the softness of bone tissue. Decalcified knee joints were dissected, and medial side of the femoral condyle was collected for tissue processing. Paraffin-embedded tissues were subjected to tissue section in 4–6 μm thickness. Sectioned tissues were stained with hematoxylin and eosin (H&E) to examine tissue morphology.

2.4 Immunological staining

For immunological staining, deparaffinized sections were rehydrated with PBS before quenching endogenous peroxidase activity with H_2O_2 . Sections were blocked with 20% normal goat serum (Dako, Gostrup, Denmark) in PBS and incubated with primary antibodies against type I collagen (Abcam, Cambridge, UK) and type II collagen (Abcam) overnight at 4 °C. After primary antibody binding, sections were washed three times with PBST and incubated with secondary antibodies conjugated with horseradish peroxidase (Bio-Rad Laboratories, Hercules, CA). Horseradish peroxidase-conjugated antibodies were detected by incubating the sections with 3,3'-

thickness between saline-injected group and collagenase-injected group showed significant difference ($p < 0.0001$)

diaminobenzidine tetrahydrochloride (DAB) using DAB substrate kit (Vector Laboratories, Burlingame, CA) with nickel for dark color development. Sections were counterstained with hematoxylin and observed under light microscope (Olympus, Tokyo, Japan).

2.5 Evaluation of cartilage degeneration by histological grading

For histological grading, tissue sections immunologically stained against type I and type II collagen as well as H&E stained sections were subjected to blind evaluation by 3 investigators using the histological grading scale as presented in Table 1. The histological grading criteria for evaluation of articular cartilage degeneration were modified from the histological grading scale described by Wakitani et al. [22] to examine the disease progression in OA animal model disregarding uncollectable component such as pain. The histological grading scale comprises 4 categories, which determines the intactness of tissues in terms of multi-layer structure, cellularity, regularity of cartilage tissue surface, and presence of cartilage-specific matrix molecules. Each category was compared separately to investigate the tissue state after OA induction.

2.6 Statistical analysis

Differences among multiple groups were analyzed using analysis of variance (ANOVA) in conjunction with a Tukey–Kramer multiple comparisons test (InStat; Graph-Pad Software Inc., San Diego, CA). Differences between two data points were analyzed by t test. Statistical significance was accepted at p values < 0.05 . All experiments were repeated at least three times.

Table 1 Histological grading criteria for evaluation of articular cartilage tissue degeneration

Category	Criteria for grading	Score
Multi-layer structure	Intact 3 layers	6
Superficial, middle, and deep zone structure (– 2 grades each for missing structure)	Superficial layer missing	4
	Presence of radial layer	2
	No layer is remaining	0
	Every layer shows cellularity	3
Presence of cells	No cells at all	0
Superficial, transitional, radial zone (– 1 grades for each acellular structure)		
Surface regularity	Smooth	3
	Moderate	2
	Irregular	1
	Severely irregular	0
Cartilage-specific matrix	Pattern and intensity OK	3
	Pattern or intensity	2
	No pattern low intensity	1
	Extremely low expression	0
Total maximum		15

3 Results

3.1 Injection of collagenase II induced local tissue damage without degenerative effect in other tissues

Collagenase II was injected only on the right-side knees of rabbits and saline was injected to the left side knees to induce OA in only one leg of each rabbits. Four days after the final injection, occurrence of edema was evident on collagenase II-injected right-side knee, while saline-injected left side knee didn't show any indication of edema. OA-induced right side knee was average $9.05 \pm 2.38\%$ thicker compared with the saline-injected knee at all time point as shown in Fig. 1B.

To check if there was degenerative inflammatory response induced by collagenase injection, histological morphology of joint cartilage collected from saline injected knee and collagenase injected knee was compared. The collagenase II injection caused its degenerative effect on the articular cartilage of the knee joint, and the degenerative effect was shown only in the injected site. Eight days after the OA induction, increased surface roughness was evidently shown in the histology of femoral articular cartilage in collagenase-injected knee, while no difference was seen in the saline-injected knee of each same rabbits ($n = 3$). The degree of surface roughness in collagenase-injected knee kept increasing along the time course up to 21 days upon OA induction, while there was no evident degeneration seen in saline-injected knee (Fig. 2).

3.2 Type I collagen-positive superficial layer was degenerated in biochemically induced OA while no tissue-destructive effect was shown in saline-injected knee

To identify the degree of degeneration caused by collagenase injection, tissue sections of femoral condyle parts were subjected to immunohistochemical staining against type I collagen and type II collagen. In articular cartilage, distribution of each extracellular matrix (ECM) molecules comprise distinct depth-dependent structure. Type I collagen is highly expressed in the superficial layer comprising outermost fibrous barrier of articular cartilage. Maintaining intact type I collagen-positive superficial layer is important to keep healthy cartilage tissue from degeneration, because compromised superficial fibrous layer can lead to further damage of articular cartilage. In collagenase-injected knees, type I collagen-positive outermost layer was disappeared at day 8 after the OA injection, while saline-injected knees showed typical thin outermost expression pattern of type I collagen (Fig. 3A). At 16 and 21 day after the OA induction, type I collagen expression was slightly seen in outermost region of articular cartilage in collagenase-injected knees. However, cellular structure indicate that flattened fibrous morphology of outermost cells are still absent and the middle layer is exposed in collagenase-injected knees (Fig. 3A). The normal expression pattern of type II collagen spans through all the layers in articular cartilage, and it shows gradual decrease near the deep zone. The expression of type II collagen in saline-injected left-side knee showed normal expression pattern, while in

Fig. 2 Comparison of cartilage tissue histology between saline-injected and collagenase-injected femoral condyle tissue section. In a rabbit, left-side knee was injected with saline and right-side knee was injected with collagenase to induce OA model and confirm the effect of OA progression on systemic catabolic state of cartilage tissues in remote site ($n = 3$, bar = 100 μm)

collagenase-injected right-side knee showed drastically decreased expression (Fig. 3B).

3.3 Comparative evaluation of cartilage tissue degeneration

The articular cartilage tissue in each rabbit were meticulously compared between collagenase-injected right-side knee and saline-injected left-side knee using the histological grading criteria for evaluation of cartilage degeneration state. The grading criteria was composed giving weight on the intactness of multilayer structure to distinguish the

tissues based on the degradation state. The scores of saline-injected left-side knee indicated normal tissue composed of 3 layers—superficial layer, middle zone, and deep zone—while that of collagenase-injected right-side knee showed significantly low scores indicating severely degenerated cartilage tissue. The scores indicating multi-layer structure (Fig. 4A) indicate that the cartilage tissues from the collagenase-injected knee showed more than superficial layer missing and part of middle zone was also degenerated (Figs. 3, 4A). In the collagen-injected knees, the number of layers showing normal cellularity was less than 2 layers (Fig. 4B), surface regularity was significantly irregular

Fig. 3 Immunohistochemical analysis on expression of type I and type II collagen. In each rabbit, left-side knee and right-side knee was injected with saline and collagenase, respectively. The articular

cartilage was subjected to immunohistochemical staining against **A** type I collagen and **B** type II collagen to check the state of tissue degeneration ($n = 3$, bar = 100 μm)

Fig. 4 Comparison of the histological grading scores of saline-injected knee and collagenase-injected knee in each rabbit at indicated time points. The grading criteria is presented in Table 1. **A** Scores in multi-layer structure covers from the state no layer is remaining (score = 0) to intact 3 layers remaining (Superficial, transitional, and radial layer; score = 6). The statistical difference between groups were analyzed by two-way ANOVA with p value less than 0.0001. **B** Scores in the criteria for presence of cells covers from the state no cell is remaining (score = 0) to every layer showed cellularity (score = 3). The statistical difference between groups were

analyzed by two-way ANOVA with p value less than 0.0001. **C** Scores in the criteria for surface regularity covers from the state all the surface is severely irregular (score = 0) to all the layer is smooth (score = 3). The statistical difference between groups were analyzed by two-way ANOVA with p value less than 0.0001. **D** Scores in the Cartilage-specific matrix covers from the state showing extremely low expression of matrix (score = 0) to the state showing normal pattern and intensity of depth-dependent structure (score = 3). The statistical difference between groups were analyzed by two-way ANOVA with p value less than 0.0005

(Fig. 4C), and the cartilage-specific matrix was impaired both in their expression pattern and intensity (Fig. 4D). These results indicate that induction of OA by local injection of collagenase worked only on the local tissue without affecting remotely located tissues.

3.4 Complete blood count analysis after biochemical OA induction do not indicate systemic inflammation

Complete blood count (CBC) was analyzed to confirm the presence of systemic inflammatory condition. The count of platelet and white blood cells (WBC) normally ranges between 150–450 and 4–10 K/ μ l, respectively [20]. As presented in Fig. 5A, B, the count of platelet was slightly

on the upper limit of normal ranges and WBC was within the normal range. The lower and upper limit of monocytes are 3–4 and 7–10%, respectively, and the count of monocytes are within the normal ranges (Fig. 5C). Considering the fact that count of neutrophils is normally within 45–62% of WBC, the neutrophil count was slightly lower than normal range (Fig. 5D). Basophiles and lymphocytes were within normal range, which are 0–2 and 16–33% of WBC, respectively (Fig. 5E, F). The blood cell count results indicate that there was no significant change in WBC profile except transient and slight increase in neutrophil at day 8 and day 16, and this coincides with characteristics of OA disease. These study shows that biochemically induced OA do not accompany RA-like phenomenon.

Fig. 5 Blood count profile of **A** platelet, **B** WBC, **C** monocyte, **D** neutrophil, **E** basophil, and **F** lymphocyte after induction of OA model. K/uL means thousand count per microliter. % means percent of WBC. **A**, **B**, **C**, **E**, **F** No statistical difference was found between

time points for each blood components by ANOVA. **D** Difference along the time point was found with t-test in neutrophil count (* $p < 0.05$)

4 Discussion

Selecting rightful animal model to validate the efficacy of therapeutic agent has massive importance. If the therapeutic regiment aims on regeneration of damaged tissues, the animal model should represent end stage of tissue degeneration. However, if the therapeutic agent aims on prevention of tissue degeneration at its early state of disease onset, the animal model's tissue degeneration mechanism should be appropriate to the treatment strategy.

In the previous report describing biochemically induced OA model, cartilage tissue showed gradual degeneration up to 6 weeks [3]. Since the activity of injected collagenase might have lasted for only a short period of time, it was suspected that inflammation was caused by initial collagenase injection and then caused further tissue damage. Traditionally OA has been defined as a non-inflammatory pathological process. However, it is more probable that onset of articular cartilage damage should accompany certain amount of inflammation during brief period [23]. However, if the massive breakdown of cartilage tissue induced by biochemical method accompany chronic and systemic inflammatory response, the model represents RA-like state and might not suitable to be used for examining tissue engineering approach.

The results in this study showed that the occurrence of edema and the cartilage tissue degeneration were localized only to the collagenase-injected knees along the time course up to 6 weeks. The other side of knee was injected

with saline to test the presence of degenerative effect, which might be caused by RA-like systemic inflammatory response. The knee joint from saline-injected side did not show any sign of edema or cartilage tissue degeneration implying that there was no systemic degenerative effect. The blood cell count in the CBC analysis did not indicate inflammatory condition nor it did not increase along the time course. Far from being rheumatoid arthritis-like condition. Our results showed that cartilages were rapidly degenerated during short period of time by biochemical mechanism, and they did not accompany with RA-like process based on the fact that the cartilage tissues on non-induced side remained intact.

Therefore, biochemically induced OA model can be safely applied to test efficacy of regenerative therapy. However, care should be taken because the OA model induced by local injection of collagenase produce only half-thickness wound on articular cartilage at its most. It is desirable to use biochemically induced OA model to investigate the efficacy of therapeutic agent aimed on prevention of cartilage degeneration during early onset of OA disease. In this study, the pathological state of biochemically induced OA model was investigated and was confirmed as not involving RA-like immune response. Our results based on histological analysis and CBC profile show that biochemically induced cartilage degeneration truly represent reliable OA state in reproducible manner.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no potential conflicts of interest.

Ethical approval All animal studies were reviewed and approved by the Institutional Animal Care and Use Committee of the Kyung Hee University (KHMC-IACUC-2017-19) and all procedures including animal care were performed according to NIH guideline.

References

- van der Kraan P, Matta C, Mobasheri A. Age-related alterations in signaling pathways in articular chondrocytes: implications for the pathogenesis and progression of osteoarthritis: a mini-review. *Gerontology*. 2017;63:29–35.
- Van den Berg WB. Osteoarthritis year 2010 in review: pathomechanisms. *Osteoarthritis Cartilage*. 2011;19:338–41.
- Kikuchi T, Sakuta T, Yamaguchi T. Intra-articular injection of collagenase induces experimental osteoarthritis in mature rabbits. *Osteoarthritis Cartilage*. 1998;6:177–86.
- Little CB, Smith MM. Animal models of osteoarthritis. *Curr Rheumatol Rev*. 2008;4:175–82.
- Lampropoulou-Adamidou K, Lelovas P, Karadimas EV, Liakou C, Triantafillopoulos IK, Dontas I, et al. Useful animal models for the research of osteoarthritis. *Eur J Orthop Surg Traumatol*. 2014;24:263–71.
- Hong HS, Son Y. Substance P ameliorates collagen II-induced arthritis in mice via suppression of the inflammatory response. *Biochem Biophys Res Commun*. 2014;453:179–84.
- Appleton CT, Usmani SE, Pest MA, Pitelka V, Mort JS, Beier F. Reduction in disease progression by inhibition of transforming growth factor alpha-CCL2 signaling in experimental posttraumatic osteoarthritis. *Arthritis Rheumatol*. 2015;67:2691–701.
- Nam S, Cho W, Cho H, Lee J, Lee E, Son Y. Xiphoid process-derived chondrocytes: a novel cell source for elastic cartilage regeneration. *Stem Cells Transl Med*. 2014;3:1381–91.
- Usmani SE, Ulici V, Pest MA, Hill TL, Welch ID, Beier F. Context-specific protection of TGFalpha null mice from osteoarthritis. *Sci Rep*. 2016;6:30434.
- Sun MM, Beier F, Pest MA. Recent developments in emerging therapeutic targets of osteoarthritis. *Curr Opin Rheumatol*. 2017;29:96–102.
- Pest MA, Pest CA, Bellini MR, Feng Q, Beier F. Deletion of dual specificity phosphatase 1 does not predispose mice to increased spontaneous osteoarthritis. *PLoS One*. 2015;10:e0142822.
- Poulet B, Beier F. Targeting oxidative stress to reduce osteoarthritis. *Arthritis Res Ther*. 2016;18:32.
- Bay-Jansen AC, Henrotin Y, Karsdal M, Mobasheri A. The need for predictive, prognostic, objective and complementary blood-based biomarkers in osteoarthritis (OA). *EBioMedicine*. 2016;7:4–6.
- Mobasheri A, Henrotin Y. Biomarkers of (osteo)arthritis. *Biomarkers*. 2015;20:513–8.
- Mobasheri A, Batt M. An update on the pathophysiology of osteoarthritis. *Ann Phys Rehabil Med*. 2016;59:333–9.
- Musumeci G, Castrogiovanni P, Trovato FM, Weinberg AM, Al-Wasiyah MK, Alquhtani MH, et al. Biomarkers of chondrocyte apoptosis and autophagy in osteoarthritis. *Int J Mol Sci*. 2015;16:20560–75.
- Murphy CL. HIF-2alpha: a mediator of osteoarthritis? *Cell Res*. 2010;20:977–9.
- Hur JE, Baek YH, Ryu SR, Lee JD, Choi DY, Park DS. Efficacy and mechanism of action of KHBj-9B, a new herbal medicine, and its major compound triterpenoids in human cartilage culture and in a rabbit model of collagenase-induced osteoarthritis. *Int Immunopharmacol*. 2009;9:230–40.
- Roland L, Drillich M, Iwersen M. Hematology as a diagnostic tool in bovine medicine. *J Vet Diagn Invest*. 2014;26:592–8.
- Park SK. An interpretation on abnormal finding of CBC. *Korean J Med*. 2010;78:531–9.
- von Vietinghoff S, Ley K. Homeostatic regulation of blood neutrophil counts. *J Immunol*. 2008;181:5183–8.
- Wakitani S, Goto T, Pineda SJ, Young RG, Mansour JM, Caplan AI, et al. Mesenchymal cell-based repair of large, full-thickness defects of articular cartilage. *J Bone Joint Surg Am*. 1994;76:579–92.
- Rahmati M, Mobasheri A, Mozafari M. Inflammatory mediators in osteoarthritis: a critical review of the state-of-the-art, current prospect, and future challenges. *Bone*. 2016;85:81–90.