Therapeutic Effects of Intra-articular Ultra-purified Low Endotoxin Alginate Administration on An Experimental Canine Osteoarthritis Model

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Disclosures:

Introduction: Osteoarthritis (OA) is the most common among degenerative joint diseases. For conservative treatment of OA, hyaluronic acid (HA) solution is widely available as intra-articular injection agents [1] [2]. An intra-articular administration of HA is known to have analgesic and anti-inflammatory effects, however, its efficacy is still controversial [3]. Alginate is a naturally abundant and anionic polysaccharide with homopolymeric blocks of (1-4)-linked β-D-mannuronate and its C-5 epimer α-L-gluronate residues. Although alginate is known to enhance chondrogenesis of stem cells and progenitor cells [4] [5], alginate includes the problem that mitogenic and cytotoxic impurities inducing a foreign body reaction or heavy pericapsular fibrosis in a living body. To overcome this drawback, we have developed an ultra-purified low endotoxin alginate (UPLE-alginate), highly purified biocompatible alginate material, which can drastically reduce the endotoxin level [4]. Our previous study showed that intra-articular administration of the UPLE-alginate was effective in preventing articular cartilage degeneration and on improving joint lubrication in a rabbit OA model[6]. As a next step for the clinical application to the human, the evaluation of the UPLE-alginate effect is required in larger animal model such as canine. The aim of this study was to clarify the therapeutic effect of intra-articular administrations of UPLE-alginate on arthritis in canines.

Methods: Ethics Statement.
All experimental protocols of canines were in compliance with the rules and regulations of Animal Care of Rakuno Gakuen University.

Materials.
An UPLE-alginate (AL), which had 1,000 kDa molecular, was prepared (Mochida Pharmaceutical Co. Ltd., Tokyo, Japan). This material was highly purified, high viscosity and quite a low endotoxin level of 5.76 EU/g (commercial grade 75950 EU/g).

Experimental canine OA model.
We used mix beagle dogs weighting less than 20 kg. All animals were anesthetized by an intravenous injection of pentobarbital, followed by sevoflurane in oxygen and nitric oxide gas anesthesia. After a 3 cm longitudinal paralateral incision in the lateral aspect of the knee, the patella was everted through a lateral parapatellar approach. OA was induced by anterior cruciate ligament transaction (ACLT) on each dog’s left knee, and sham surgery served as control on each dog’s right knee.

Experimental protocols.
The dogs were divided into two groups, control group and therapeutic group. The control group was administrated weekly with 0.7 ml of saline during 0-3 week after ACLT or sham operation (control group). The therapeutic group was administrated weekly with 0.7 ml of 0.5% AL during 0-3 week after ACLT or sham operation (AL group). At 14 weeks after ACLT, all dogs were sacrificed, and all knees were analyzed.

Morphological assessment.
Gross morphological assessment of the knee (five knees in each group) was performed. The articular cartilage surface was stained with a solution of India ink diluted with phosphate buffered saline (1:5). Macroscopic findings were assessed according to the previous report [7]. We use this score for femur and tibial plateau.

Histological assessment.
The distal femur and proximal tibia from each knee (five knees in each
group) were fixed with 10% paraformaldehyde, decalcified with ethylenediamine tetraacetic acid (EDTA), and embedded in paraffin. Sagittal sections were obtained. The sections were stained with safranin-O. Cartilage degeneration in each sample was quantitatively assessed using the scoring system described by Kikuchi et al [8]. We use this score for femur and tibial plateau.

**Statistical analysis.**

Data are expressed as mean ± SEM. Differences between groups were analyzed by Mann-Whitney U test. A P value of less than 0.05 was considered statistically significant.

**Results:** The macroscopic findings tended to be severer in the femoral condyle than those in the tibial plateau. The macroscopic findings of bilateral femoral condyles in control group were severer than those in AL group (Fig. 1). In the tibial plateau, the macroscopic scores of both groups were similar (data not shown) whereas bony spurs in control group tended to be larger than those in AL group (Fig. 1). The femoral macroscopic scores in control group were higher than those in AL group (Fig. 1). In addition, the femoral histological findings and scores in control group were also severer than those in AL group (Fig. 2). The histological scores of tibial plateau in both groups were almost equal (data not shown). The macroscopic and histological findings in sham groups were intact (data not shown).

**Discussion:** Similar UPLE-alginate effect against degenerative cartilage is confirmed compared with our previous results in rabbit model [5]. Our study showed that intra-articular administrations of UPLE-alginate had the therapeutic effect in not only in rabbit model but in large animal model. A limitation of this study, the mechanism of the therapeutic effect of UPLE-alginate is not clarified, so we need research this mechanism.

**Significance:**

This is the first attempt to clarify that intra-articular administrations of UPLE-alginate have the therapeutic effect of OA using large animals. Although future studies are required to elucidate the role of UPLE-alginate in preventing OA progression, UPLE-alginate has promising potential as an effective agent in the treatment of OA.

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