Intra-articular Administration of Cyclic Phosphatidic Acid (cPA) Suppresses Pain, Swelling, and Articular Cartilage Degeneration in Knee Joints with Rabbit Experimental Osteoarthritis.

Ikuko Masuda, MD, PhD\(^1\), Kodo Okada, PhD\(^2\), Hisashi Yamanaka, MD, PhD\(^1\), Shigeki Momohara, MD, PhD\(^1\).
\(^1\)Inst. of Rheumatology, Tokyo Women's Medical University, Tokyo, Japan, \(^2\)SANSHO Co. Ltd., Tokyo, Japan.

Disclosures:

Introduction: Cyclic phosphatidic acid (cPA) is one of bioactive lipid, has been implicated as a mediator of various biological effects\(^1\) including i) antiproliferative effect on eukaryotic cell cycle, ii) regulation of Ca\(^{++}\) release, iii) regulation of actin rearrangement, iv) inhibition of tumor cell invasion. Furthermore, on human skin fibroblasts, cPA stimulates high molecular hyaluronic acid (HA) production through up-regulating HA synthase (HAS). cPA also has shown to have antinociceptive effect on animal models of acute and chronic pain\(^2\).

The aim of this study was to evaluate the effects of cPA on articular chondrocytes HA synthesis in vitro, and its in vivo effect using a rabbit model of osteoarthritis.

Methods: In vitro studies were performed using human osteoarthritic chondrocytes obtained at joint replacement surgery. cPA 0-50 microM was added to chondrocyte cultures and effects of cPA on chondrocyte HA metabolism was assessed at various time points (0-48hrs). Newly synthesized HA from chondrocytes in cultured media was measured by sandwich ELISA using bovine nasal HA binding protein. HAS expression in chondrocytes was examined by real time PCR using specific primers to HAS1, HAS2, and HAS3. Beta-actin was used as endogenous expression control. In vivo experimental OA was induced in the knee joints of 12 mature rabbit knee joints by partial lateral meniscectomy. After the surgery, they were divided into two groups, and cPA (10 micro gram/rabbit) or saline were injected intra-articularly twice a week immediately after the surgery. General health, weight, pain score (weight bearing %= (weight bearing of right leg) / (right + left leg) \times 100), and swelling score (swelling %= (circumference of right knee - left knee) / (left knee+ left knee) \times 100) of rabbits were observed once a week. At 42 days after surgery, animals were painlessly sacrificed and degenerative changes in their femoral and tibial cartilages were graded histopathologically.

Results: cPA stimulated endogenous HA synthesis from articular chondrocytes as time and dose-dependent manner in vitro. 50 microM cPA increased HA production three times more than control at 48 hours (Figure 1). HAS2 gene was up-regulated as dose-dependent manner and kept increased through the time. HAS1 and HAS3 up-regulated temporally at 2 hours after addition, but down at 4 hours. There was no difference from control on HYAL expression.

Since intra-articular administration of HA has been considered as a viscosupplementation and an anti-inflammatory therapy for osteoarthritis, we examined the in vivo effect of cPA in rabbit knee joint with OA. In vivo study, cPA-treated rabbit showed significant less pain score (20% more weight bearing than control) (Figure 2a), and dramatic reduce of swelling score than control (p=0.0164) (Figure 2b). The histological score of cartilage degeneration was significantly less with cPA administration (cPA 8.33±2.51 vs. control 14.33±2.33; p=0.0649) (Figure 3ab).

According to the compelling results from in vivo pilot studies, we have carried additional in vivo studies using 0,5,50,500 microgram/kg body weight cPA once a week administration for the same length of experiment. Up to 50 microgram of cPA, the suppression of pain was dose-dependent, as well as histological grade, confirming that cPA can be a therapeutic molecule for osteoarthritis.

Discussion: The in vitro results confirmed that cPA had stimulatory effects on endogenous HA synthesis by articular chondrocytes. Also stimulation of HA synthesis was shown in normal rabbit knee joint in dose-dependent manner.

In vivo study using rabbit experimental OA model indicated that cPA not only had stimulatory effects on endogenous HA synthesis, but also might have anti-nociceptive and anti-inflammatory effect, and was very effective in suppression of cartilage degeneration in early OA. Since the duration of experiment and amount of production of HA from chondrocytes is unlikely to be a direct contribution factor for above in vivo effects, we think cPA may play direct role on anti-inflammatory and cartilage degeneration process on arthritis. One possible mode of anti-inflammatory action of cPA may be through antagonizing LPA/Autotaxin (ATX) pathway. cPA is naturally occurring analog of lysophosphatidic acid (LPA), which is a potent inflammation mediator, however cPA has distinct/overing biological functions from those of LPA. Recent mouse studies showed that LPA receptor-1 played important role in developing arthritis\(^3\). Inhibiting LPA/ATX pathway could also contributing inhibit chronic and acute inflammation-induced C-fiber stimulation, which has been shown as antinociceptive effect of cPA\(^4\).

Molecular mechanism of cPA to prevent cartilage degeneration remains to be elucidated, however, further study should be warranted for cPA as a novel candidate for therapeutic agent of OA.

Significance: OA is the most common form of arthritis. Despite many people have suffered this painful and disabling condition, pharmacological treatment options are limited. We here investigated effects of bioactive lipid, cPA, on articular chondrocytes/cartilage, knowing that cPA has stimulatory effect on HA synthesis on human fibroblasts, has shown here that cPA...
has significant anti-pain, anti-swelling, and chondroprotective effect on rabbit experimental OA knee joints. Further study may elucidate the role of bioactive lipid on developing arthritis and cPA could be a promising therapeutic agent for OA.

Acknowledgments: Authors thank to Ms Kaori Arai for her technical assistance.


Figure 1. cPA stimulates HA synthesis of OA articular chondrocytes.
Figure 2a. Pain score (weight distribution ratio)

![Graph showing pain score over post-operation days for saline and cPA groups.](image1)

**p=0.0053

Figure 2b. Joint swelling score

![Bar chart showing ratio of diameter of right hind leg to left.](image2)

**P=0.00164
Figure 3a. Pathology: Femoral medial chondyle

Saline group (animal #106)  cPA group (animal #203)

HF  SO

Figure 3b. Pathological score of femoral and tibial chondyle

ORS 2014 Annual Meeting
Poster No: 0146