Study on the Mechanical Lubrication Effects of the Intra-articular Injections of High Molecular Weight Hyaluronic Acid in Rheumatoid Arthritis patients by Confocal Laser Scanning Microscopy (CLSM)

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Summary

To investigate the mechanical tribology effect of intra-articular high molecular weight Hyaluronic Acid (HA) as a lubricant for Rheumatoid Arthritis (RA) joint, especially in the boundary lubrication mode, the articular cartilage surfaces with three kinds of lubricant; normal synovial fluid, RA synovial fluid, and RA synovial fluid added HA, were observed and compared by the Confocal Laser Scanning Microscopy (CLSM) under the high loading condition.

As a result, the articular cartilage surface with normal synovial fluid exhibited two distinct areas under the high loading; one in direct contact area and one with a fluid pooling area. Between these two areas, a third morphological area was observed and suggested the existence of liquid crystal formation as protective macromolecular layer using an optical-isolation technique method. However, in RA synovial fluid lubricant case, little reflected image of protective layer was found as compared with normal fluid case, which meant poor liquid crystal formation. Moreover, adding HA showed the remarkable enhancement of reflected image as compared with RA synovial fluid only.

These results suggested that a high molecular weight HA could form the protective layer on the cartilage surface as well as a synovial fluid, the intra-articular injected HA provide an effective therapy against mild RA joints by not only biochemical contributions but improving the mechanical lubrication system.

Keywords: Rheumatoid Arthritis (RA), Synovial fluid, Hyaluronic Acid (HA), Lubrication, Confocal laser scanning microscopy (CLSM)

1. Introduction

Rheumatoid Arthritis (RA) is a chronic inflammatory joint disease affecting synovial tissue in multiple joints. Although RA is thought to be one of immune-mediated disease, no specific antibody has been found like other collagen disease, its etiology is still uncertain. Therefore the current therapy for RA is mainly allopathy in order to prevent and keep the function of joints, QOL (quality of life).

The destruction of synovitis and joints by RA is mainly due to chronic inflammatory. Recent advance of the
treatment such as disease-modifying anti-rheumatic drugs (DMARDs), nonsteroidal anti-inflammatory drugs (NSAIDs) and the combination with methotrexate (MTX) could lead the RA patient to the control of inflammation and structural damage in early stage, moreover, improve the prognosis and mortality\(^{1,2}\).

However, this approach aims at only control of the inflammation, cannot be expected the recovery of damaged joints. In fact, many patients with mild RA have complaints of joint pain, swelling and limitation of movement in spite of no clinical arthritis sign and normal examination data such as ESR (erythrocyte sedimentation rate), CRP (serum C reactive protein) by using these drugs. In this case, these symptoms are though to be due to the mechanical-lubricative stress on mild destructive or degenerative joints by RA. And these painful symptoms have a great effect on the quality of life in individuals with RA.

Recently, some reports have indicated that the intra-articular administration of high molecular weight Hyaluronic Acid (HA) had excellent pharmacological effects for RA\(^3\). Many biochemical studies on HA effects have already reported that HA affect the proliferation of chondrocytes, synoviocytes, the structure of articular cartilage in RA joints and the inflammatory process\(^6\)-\(^9\). Some of these cellular effects might contribute to the clinical successful response to RA joint, but its mechanical effect is unclear.

To investigate the effect of HA as a lubricant on RA joint lubrication system, especially boundary lubrication, we observed the articular cartilage surface with RA synovial fluid, and added high molecular weight HA by using confocal laser scanning microscopy (CLSM).

2. Materials and Method

2.1 Materials

For CLSM, normal articular cartilage and three kinds of lubricants; normal synovial fluid, RA synovial fluid and high molecular weight HA (mean molecular weight 2.0 \(\times\) 106 daltons) were prepared. Articular cartilage specimens were obtained from rabbits knee joint. After the rabbits were sacrificed, the knee joints were immediately removed en block with the surrounding soft tissue, and frozen and stored in physiological saline. The articular cartilage was cut into pieces immediately before CLSM observation.

Normal synovial fluid was also obtained from the rabbit’s knee joint. The RA fluid was gained from the patient’s knee joint with mild RA (stage I and II in ARA (American College of Rheumatology)). The HA preparations were obtained from Denki Kagaku Kogyo (Tokyo, Japan).

2.2 Method

To perform CLSM, a confocal optical system with a laser beam source is usually used. This system provides high quality images of specimens without the need for pre-treatment such as metal-shadowing or freeze-fracture, as required for Scanning Electron Microscopy (SEM). CLSM and optical microscopy are performed using similar methods. In our study, we used CLSM (® 1 LM21 real-time CLSM, Lasertec Co, Japan) as shown in Fig-1.

Fig-1. Schematic diagram of real-time 1LM21 confocal laser scanning microscopy (CLSM).

Each specimen was washed with physiological saline and placed on the specimen table. After the addition of lubricant to the specimen’s surface, a glass plate (0.15mm thick) was placed on specimen, and a load (12N = 1.2 Kg) was applied to cartilage contact area. This load corresponded to physiological loading condition. The behavior of the specimen surface with each lubricant, which were normal synovial fluid, RA synovial fluid and 0.3 ml HA added RA synovial fluid, was observed (Fig-2). The arrangement and observation of each specimen took about 2 minutes.
including the focus and scan time (10 sec).

3. Results

3.1 Normal synovial fluid
The compression of articular cartilage with normal synovial fluid by a glass plate, which was equivalent to physiological loading, caused the cartilage surface to exhibit two distinct areas; one in direct contact with the glass plate and one with a fluid pool between the cartilage and the plate. Between these two areas, a third morphological area was observed, as shown in Fig-3.

In the area in direct contact with the glass plate, surface depressed in the articular cartilage disappeared due to compression. In the area of the fluid pool, the presence of a fluid membrane between the articular cartilage and glass plate was confirmed, but the state of the cartilage was unclear. In the third area, a fringe-like pattern was observed around the contact area at high magnification.

In addition, the observation of these areas by an optical-isolation method (the technique of observing specimens by 90° rotation of polarization by pulling the quarter-wave plate), revealed a reflected image that corresponded to the contact area and third area, as shown in Fig-4. This finding indicates that a liquid crystal or some crystal structure is composed in these areas.

3.2 RA synovial fluid
Fig-5 shows CLSM image of cartilage surface with RA synovial fluid. CLSM image using ordinary technique revealed the third area between direct contact area and liquid pool area, had no significant difference from that with normal fluid (Fig-5(a)). However, in CLSM image using an optical-isolation method, little reflected image was found as compared with normal fluid case, which meant poor liquid crystal formation (Fig-5(b)).
Fig-5. (a) CLSM image of natural articular cartilage with Rheumatoid arthritis (RA) synovial fluid (×2000). (b) CLSM image with optical isolator technique about this area. A less reflection image was obtained in the third area and contact area than normal synovial fluid case (×2000).

3.3 RA synovial fluid + High molecular weight HA

Fig-6 shows CLSM image of cartilage surface with RA synovial fluid plus HA added. While CLSM image using ordinary technique was similar to the surface with other lubricants, CLSM image using an optical-isolation method revealed the remarkable increase of reflected image in these areas as compared with RA synovial fluid only case.

Fig-6. (a) CLSM image of natural articular cartilage with RA synovial fluid added high molecular weight hyaluronic acid (HA) (×2000). (b) CLSM image with optical isolator technique about this area. The reflection image increased in the third area and contact area as compared with only RA synovial fluid case (×2000).

4. Discussion

The biochemical efficacy of intra-articular injected HA on RA joint has been demonstrated in many experiments. And this therapy has received attention as a treatment for RA, but its mechanical efficacy has not been established yet.

Synovial joint lubrication has mainly two mechanisms; fluid lubrication and boundary lubrication system. In fluid lubrication, synovial fluid acts as a fluid film in physiological motion, high molecular weight HA is also expected to be effective in this lubrication system by its viscoelasticity. However, the role of the viscoelastic properties of lubricants is not generally accepted in boundary lubrication. Therefore the effects of intra-articular injection of HA in the case of boundary lubrication should be also accessed.

We have previously used CLSM to study the lubrication mechanism of synovial joints, and reported that the most remarkable CLSM finding of the natural joint was the presence of not only a solid contact area, but also of fluid pool on the articular cartilage surface that occurred even with marked loading condition. Moreover, between the solid area and the fluid pool area in articular cartilage, a third area containing liquid crystals, which had a ordered structure, was observed. It suggested that fluid film lubrication in fluid pool area and boundary lubrication in solid contact area might operate together as a mixed lubrication.

To discuss the excellent lubrication mechanism of synovial joints in boundary lubrication, we must address the molecular level nature of the protective film layer that covers the articular cartilage surface. Many investigators have already proposed the presence of lamina splendens, lubricin, proteins and a functional macromolecular layer. Our observation indicates that the liquid crystal formation may exhibit a protective function. And we presume that this protective liquid crystal layer is composed of synovial macromolecules such as proteins, phospholipids and hyaluronic acid. Hyaluronic acid and proteins do not have an intrinsic liquid crystal structure, but their molecular structure does exhibit polarity. On the other hand, as Orford reported, the normal cartilage surface is negative charged. These facts may be associated with the protective liquid crystal layer of synovial macromolecule under the high pressure condition. Considering the imaging of the solid contact area and third area in this experiment, the synovial fluid and articular cartilage must form characteristic liquid crystal layer on the articular cartilage surface, which contributes to the excellent lubricative mechanism in boundary lubrication.
inadequately for protective macromolecular layer. According to our hypothesis, it indicates that hyper mechanical loading may often apply to cartilage surfaces directly without protective layer in boundary lubrication. It has been known that the content of component elements of RA synovial fluid were changed; The concentrations of HA, normal protein decreased, while inflammatory cell, leucocyte, enzyme and degenerated protein, glycosaminoglycans increase as a result of RA inflammatory reactions. The shortage of normal proteins and HA might result in the insufficient formation of liquid crystal. CLSM image using an optical-isolation method in the administration of HA showed the increased liquid crystal formation and supported that intraarticular injection of HA improved the lubrication system of RA patient’s joints. However, the reflection image area in RA fluid with HA added was smaller than that in normal synovial fluid. We think it was because the molecular weight of natural HA in normal synovial fluid was higher than HA used in this observation, and other components of synovial fluid except HA also characterize the liquid crystal formation. Kawano et al. have reported that the mixture of phospholipids plus HA showed the significant efficiency in frictional test using osteoarthritis (OA) cartilage as compare with only HA lubricant. It suggested the role of the other macromolecules of synovial fluid except HA should be investigated in order to improve lubrication system of disordered joints. On the other hand, our hypothesis address that the characteristics of articular cartilage surface for bio-chemical and electromagnetic interaction with macromolecules in synovial fluid are also very important to the formation of protective liquid crystal. The severe damaged cartilage could not form the sufficient liquid crystal layer even by normal synovial fluid. In this study, normal rabbits articular cartilage was used because of difficulty of getting the articular cartilage from mild RA patients. Therefore, the CLSM reflection image of RA cartilage might be slight different from our observation results using normal cartilage. However, considering the more suitable mechanical condition for degraded RA cartilage, high molecular weight HA is expected to improve the lubrication mechanism of mild RA joint and prevent further progress of disordered RA cartilage. Present CLSM observation indicated that the intraarticular injected HA provided an effective therapy against mild RA joints by improving the mechanical lubrication system, especially on the boundary lubrication, expect biochemical contributions. To develop more effective clinical lubricant for RA joints, further studies about other macromolecules such as proteins, phospholipids and the synthesis of higher molecular weight of HA are needed in future.

参考文献
1) Gabriel SE, Mortality in rheumatoid arthritis; have we made an impact in 4 decades? J. Rheumatol. 1999; 26: 2529-33.


