

## 3-D CT Imaging of Pathological Bone Changes in a Rat Model of Adjuvant-Induced Arthritis

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### <요약>

CT는 단층촬영을 이용한 의학적인 영상 진단 기법이다. CT는 3차원적인 방사선학적 영상 기법으로 염증의 평가에는 적합하지 않으나, 석회화된 조직을 직접적인 3차원 영상으로 보여주므로 뼈 손상의 평가에는 유용하다. 본 연구는 실험적으로 유도된 랫드의 보조관절염에서 관절의 병리학적 변화와 뼈 파괴의 정량적 분석을 3차원 CT 영상을 통하여 평가하고자 실시되었다. 그 결과 랫드의 보조관절염에서 병변의 파괴성 진행이 3차원 CT 영상을 통해 정량화될 수 있었고, 따라서 관절염 질환의 상태 및 실험적인 치료 약제의 효능 평가에 3차원 CT 영상 기법이 효과적일 것으로 생각된다.

핵심어: 3차원 CT, 보조관절염, 랫드

### <Abstract>

Computed tomography (CT) is a medical imaging method employing tomography. CT is a 3-Dimensional (3-D) radiographic imaging technique, which is not suited for assessment of inflammation, but can be considered a reference method for assessment of bone damage, due to its direct 3-D visualization of calcified tissue. In this study of pathological joint changes in a rat model of adjuvant-induced arthritis (AIA) and quality analysis of bone destructions were performed by 3-Dimensional computed tomography images. These data demonstrate that the destructive progression of disease in a rat AIA model can be quantified using 3-D CT image analysis, which allows assessment of arthritic disease status and efficacy of experimental therapeutic agents.

**Key word:** 3-D CT, adjuvant-induced arthritis, rat

## I. Introduction

Rheumatoid arthritis (RA) is a common chronic disease, characterized by non-suppurative inflammation of synovial joints, frequently associated with a variety of extra-articular manifestations. RA can infect both the peripheral and axial skeleton. Any synovial joint in the limbs may be affected, although the metacarpophalangeal and proximal interphalangeal joints of the hands and feet, the carpal joint are the

most common RA infected areas. Moreover, the distal radioulnar and radiocarpal joints. The acromioclavicular and sternoclavicular joints, the temporomandibular joints, the knees, ankles, hips, elbows, and shoulders may also be affected by RA [1].

The earliest abnormality of RA is synovitis, congestion, and edema of the synovial membrane with accumulation of erythrocytes, polymorphonuclear

leukocytes and lymphocytes. Intra-articular fluid increases, as capsular distension and surrounding soft tissue edema occurs in more advanced stages. These abnormalities lead to an early radiographic finding, namely a swelling of fusiform soft tissue on the infected joints. The chronically inflamed synovial tissue (pannus) applied to the cartilaginous surface causes cartilage destruction, for both its enzymatic activity and the interference with cartilaginous nutrition. Radiography presents diffuse or widespread loss of joint space. Hyperemia caused by synovial inflammation leads to regional or periarticular osteoporosis- the second early radiographic sign of RA. Radiographically, the small areas of discontinuity or gaps in the subchondral bone plate may be seen <sup>[1]</sup>.

Even if plain bone radiography is insufficient for diagnosing early bone loss, conventional radiology can identify an osteopenic status, since the losses of up to 40% can occur before any change is detected. Computed tomography (CT) and magnetic resonance imaging (MRI) may be useful in studying infected regions which are anatomical complex and difficult to show with radiography, that is the carpal tunnel.

As stated above, the radiographic counter part of cartilage destruction is the loss of articular space which is an earliest stage. At a slightly later stage, the pannus spreads across the subchondral bone leading to small bone erosions. The early erosions are marginal, in the synovial pockets and bare bone areas because they lack protective cartilaginous lining. The radiographic appearance of erosions (multiple subchondral lucent area) is well known. In addition, two other types of bone erosion have been described in RA: compressive erosions and superficial surface resorption. In advanced RA, pannus spreading into bones can lead to the destruction of bones, and also cause a the formation of large marginal and central radiolucent cyst-like areas. These central radiolucent cyst-like areas are multiple of symmetric patterns which vary in sizes and have lock of sclerotic margins.

Conventional radiography is the imaging method of choice for detecting both early and late stages of RA bone erosions from a practical clinical viewpoint over conventional radiography.

Radiography depicts the progression of joint and bone damages, A late radiographic progression being a good indicator of the success/failure of long term treatment <sup>[2]</sup>. Several radiographic methods have been proposed to study the progression and extent of rheumatic disease. Unfortunately, the quantitative analysis of changes over time and the reliability of the scoring systems remain weak spots. The Sharp's method (which adds up erosions and articular space changes) seems to be the best in evaluating the progression rate in the earlier years of the illness <sup>[3]</sup>. Nevertheless, the actual relationship of clinical, laboratory and radiologic findings in RA remains controversy. For example, the association between radiologic progression and clinical deterioration has been reported, however, the results are inconsistent.

Although joint destruction may begin early in the course of RA, diagnosis and subsequent initiation of therapy are often delayed <sup>[4]</sup>. Early treatment brings more favorable patient outcomes than does delayed treatment <sup>[5]</sup>. Today, American College of Rheumatism Association (ACR), advocates early diagnosis.

CT imaging provides a unique opportunity to capture 3-D architectural information in bone samples. In this study of pathological joint changes in a rat model of adjuvant-induced arthritis and quality analysis of bone destructions were performed by 3-Dimensional computed tomography images.

## II. Materials and Methods

### 1. Animals

Female Sprague-Dawley rats (Samtako, Korea), 7 weeks of age at the time of adjuvant injection, were used for study. Five rats were housed per cage (43 x

27 x 18 cm) in an air-conditioned environment (room temperature  $23\pm 2^{\circ}\text{C}$ , humidity  $55\pm 5\%$ ) that was illuminated from 6:30 to 18:30. Animals were fed with a commercial diet (Samyang Feed Co., Korea), and divided into two groups, adjuvant-noninjected group (normal group) and adjuvant-injected group (experimental group), and both groups were composed of 10 animals.

## 2. Induction of arthritis

Each rat was injected in the plantar region of the right hind limb with Freund's complete adjuvant (Gibco, USA, Lot No. 1020159) containing 1 mg of *Mycobacterium butyricum* (Difco, USA, Lot No. 138137LA) suspended in 0.1 ml of paraffin oil.

## 3. Computed tomography imaging

All CT imaging of the adjuvant-injected limb in rats were obtained on a CT scanner (CT-HiSpeed Advantage<sup>TM</sup>, USA). One-mm thick CT images were taken at one-mm intervals with a resolution close to 0.25 mm/pixel. The CT images were processed using a custom software (Advantage windows 2.0, SUN, USA) permitting the bone contours to be extracted and converted into mathematics entities and then written into a formatted file. By reading this file from the pre- and post-processing software a 3D reconstruction of adjuvant-injected paw could be obtained by the superimposition of the bone contours segmented into a finite number of parametric cubic curves.

## III. Results

### Assessment of arthritis with computed tomography

The CT images afforded a vivid, nondestructive visualization of the bone changes that occurred over the entire tibiotarsal joint and the hind paw. Coronal and sagittal views of the joints were shown Figures 1

and 2. The various bones that constituted the joint, namely, the femur, distal tibia/fibula, talus, and the calcaneus, were clearly resolved. Bone images of adjuvant-induced rat showed intact joint architecture as well as normal bone surfaces on day 7 (Fig. 1 and 2). On day 14, the joint in adjuvant-induced rat showed mild bone erosion of several bone surfaces, especially at the junction of the distal tibia and fibula and also along the length of the calcaneus (Fig. 1 and 2). Severe bone changes were detected by CT in the femur, distal tibia/fibula, talus, and the calcaneus of the adjuvant-injected leg on day 28 (Fig. 1 and 2).

## IV. Discussion

Rat adjuvant-induced arthritis has been widely used as an experimental model for preclinical screening of treatments for rheumatoid arthritis (RA). The model system is robust, the incidence rate for the disease is 100%, and adjuvant-induced arthritis in rats shares many features with RA in humans, such as inflammation, marked bone resorption, and periosteal bone proliferation<sup>[6-8]</sup>. The aggressive nature of rat adjuvant-induced arthritis, however, must be considered when compared with RA in humans. RA can be difficult to manage, and many patients experience progressive functional deterioration<sup>[9]</sup>. A number of indicators suggest that improved outcomes in RA depend on early diagnosis and prompt, appropriate therapeutic intervention. Evidence that began to appear in the 1970s continues to mount that substantial joint damage occurs early in RA, often during the first 2 years after onset, and that such damage may be deterred by prompt intervention<sup>[10]</sup>. Radiography has been used to demonstrate an abnormal process in the joints in early RA. It has been proposed that during this period of early joint damages, a therapeutic window exists; anti-inflammatory therapies administered during this early phase may be more effective than the same

drugs used later [5].

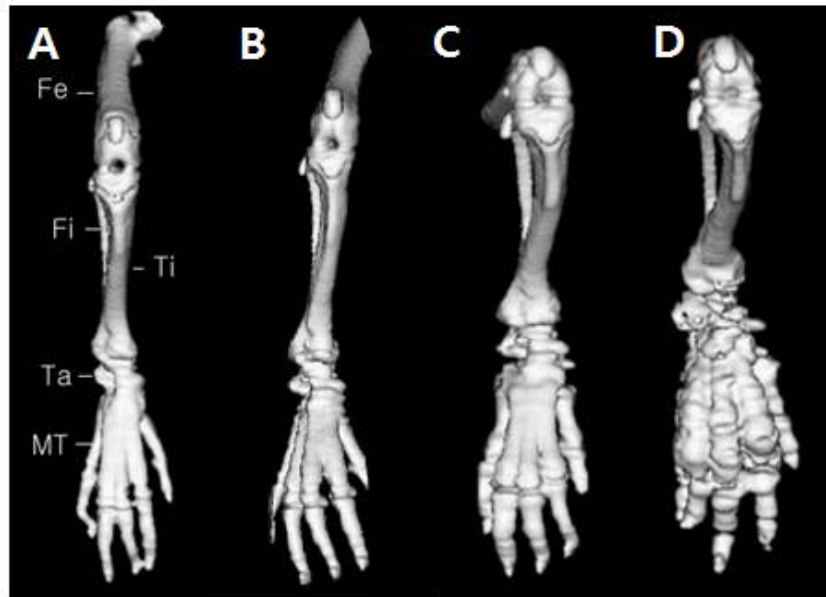


Fig. 1. 3-Dimensional computed tomography images of adjuvant-injected paw in arthritic rat.  
A: Day 7, B: Day, 14 C: Day 21, D: Day 28.  
Femur (Fe), Fibula (Fi), Tibia (Ti), Tarsal (Ta), Metatarsal (MT).

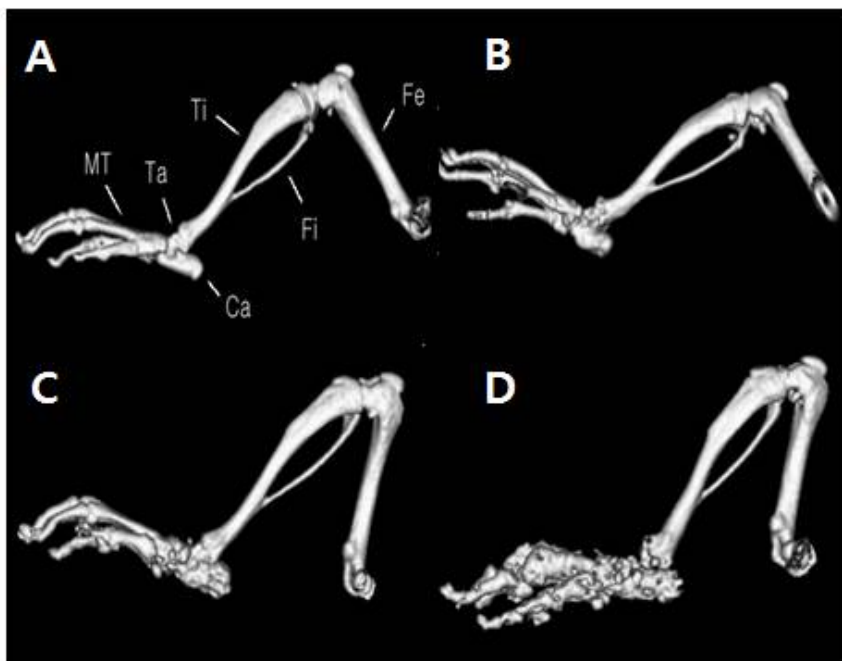


Fig. 2. 3-Dimensional computed tomography images of adjuvant-injected paw in arthritic rat.  
A: Day 7, B: Day, 14 C: Day 21, D: Day 28.  
Femur (Fe), Fibula (Fi), Tibia (Ti), Tarsal (Ta), Metatarsal (MT).

Two clinical trials [5, 11] have provided strong evidence for this hypothesis: in both studies, patients who received disease-modifying anti-rheumatic drugs (DMARDs) immediately after RA was diagnosed had better patient outcomes than patients who received DMARDs later in the course of the disease. The bone erosion in preclinical models of rheumatoid arthritis is valuable for the evaluation of drug treatments. This study introduces a three-dimensional method for bone surface roughness measurement from micro-computed tomographic data obtained from rats subjected to adjuvant-induced arthritis, in which the degree of bone erosion is related to the severity and the duration of the disease. In two studies of rat AIA, the surface roughness of the tarsal and metatarsal bone from following 14 days of disease increased from the normal group and the joint in adjuvant-induced rat showed mild bone erosion of several bone surfaces, especially at the junction of the distal tibia and fibula and also along the length of the calcaneus. Severe bone changes were detected by CT in the femur, distal tibia/fibula, talus, and the calcaneus of the adjuvant-injected leg on day 28. In our previous study [12] had shown that <sup>99m</sup>Tc-MDP scintigraphy, when compared to plain radiography and CT, is a more rapid method to detect bone lesions, and more sensitive in the detection of different degrees of arthritis activity in rat. These conclusions are based upon the observations that adjuvant-induced arthritis was initially observed by bone scintigraphy on day 7, but plain radiography and CT were detected only on day 14. Investigative tools for bone lesion detection and measurement of disease activity in arthritis still have to be fully developed. Nevertheless, 3-D CT is a rapid and effective means of identifying and locating bone damage, particularly in the limbs of patients. Scintigraphic nuclear imaging offers several advantages over higher resolution cross sectional imaging modalities such as CT and MRI, including its ability to image and screen the whole body for sites of

abnormal uptake. We believe that bone scans are the study of choice for initial screening for rheumatoid arthritis, because of their overall high sensitivity, lower cost, availability and ability to assess the entire whole body conveniently. Bone scans employing <sup>99m</sup>Tc-MDP maybe a useful complementary study in patients with equivocal or negative bone radiography findings in the context of high clinical suspicion, or in patients with a positive bone radiography and low clinical suspicion for rheumatoid arthritis. These data demonstrate that the destructive progression of disease in a rat AIA model can be quantified using 3-D CT image analysis, which allows assessment of arthritic disease status and efficacy of experimental therapeutic agents.

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