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Scintigraphic Detection of Adjuvant-induced Arthritis in Rats Using ^{99m}Technetium-methylene Diphosphonate

Seong-Soo Kang, Sung-Soo Koong*, Chun-Sik Bae and Seok-Hwa Choi[1]**

College of Veterinary Medicine and Research Institute of Veterinary Medicine
**Department of Nuclear Medicine, College of Medicine, Chungbuk National University, Cheongju, 361-763, Korea*
***Chonnam National University, Kwangju, 500-757, Korea*

^{99m}Technetium-methylene Diphosphonate의 전신 골격주사술을 이용한 관절염의 핵의학적 진단

강성수 · 궁성수* · 배춘식 · 최석화[1]**

충북대학교 수의과대학 및 동물의학연구소, *충북대학교 의과대학 핵의학교실
**전남대학교 수의과대학

요 약 : 본 연구는 랫드에서 Freund's complete adjuvant를 랫드의 후지에 투여하여 인의적으로 유발된 관절염을 진단하기 위한 방법으로 ^{99m}Technetium-MDP (^{99m}Tc-MDP)을 이용한 골 스캔의 임상적 의의를 찾고자 하였다. 성숙 Sprague-Dawley 암컷 랫드를 대조군과 실험군으로 분리하고, 골의 스캔은 0.05 mCi의 ^{99m}Tc-MDP를 정맥 주사하여 3시간 후에 전신 영상을 얻었다. 골의 스캔과 방사선학적 평가를 비교한 결과 관절염 유발 7일 후부터 관절염의 병변이 관찰되었지만, 방사선학적 소견에서는 14일 후부터 병변이 관찰되었다. 골의 스캔에서 관절염 유발 7일 후에 유의적으로 골의 이상적 섭취 증가가 관찰되었고($p<0.05$), 21일 후에는 전지와 후지의 모든 부위에서 골의 이상적 섭취 증가가 관찰되었지만, 방사선학적 소견에서는 후지에서만 병변 부위가 관찰되었다. 이상의 결과에서 ^{99m}Tc-MDP을 이용한 골의 스캔은 류마티스 관절염을 임상적으로 진단하는데 방사선학적 진단보다 효과적인 방법임을 알 수가 있었고, 골의 스캔은 류마티스 관절염을 초기에 진단하는데 유효한 진단 방법임을 확인하였다.

Key words : adjuvant arthritis, bone scan, ^{99m}Tc-MDP, radiography.

Introduction

Arthritis in rat induced by intradermal injection of mycobacterial adjuvant is widely used as a model for the evaluation of compounds with anti-inflammatory or anti-rheumatic activity[16].

Technetium-99m-labeled phosphates have served as bone scanning agents for the diagnosis of a broad spectrum of pathological conditions affecting the skeleton, with 99mTechnetium-methylene diphosphonate (^{99m}Tc-MDP) being the most widely used today[9].

The theoretical basis for the use of ^{99m}Tc-labeled phosphates in the diagnosis of bone pathology is based on the high affinity of the phosphates for hydroxyapatite[6], enzymes[18] and immature collagen1[3]. The kinetics of ^{99m}Tc-phosphate uptake in bone plays a major role in local vascularity, with marked localization of ^{99m}Tc-phosphates[5].

Scintigraphic nuclear imaging offers several advantages over higher resolution cross sectional imaging modalities, such as CT and MRI, including its rapid ability to image and screen the whole body for sites of abnormal uptake. Another significant advantage of scintigraphic imaging is the ability to quantify non-invasively the amount of injected activity that reaches a particular location in the body.

However, little information is available on the quantitative changes using bone scans during the development of adjuvant-induced arthritis. There are little data on the bony changes which occur during the development of rheumatoid arthritis. The aim of this study was to compare radionuclide bone scans and plain radiographic imaging in detecting adjuvant-induced arthritis. It will be informative to compare the responses of a variety of skeletal sites.

Materials and Methods

Animals

Mature female Sprague-Dawley rats (KRICT, Daejon, Korea), 6 weeks of age at the time of adjuvant injection, were used for study. Five rats were housed per cage (43× 27× 18 cm) in an air-conditioned environment (room temperature 23± 2°C, humidity 55± 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, a negative control group (adjuvant non-injected) and an experimental group (adjuvant injected), and both groups

[1]Corresponding author.
E-mail : shchoi@cbucc.chungbuk.ac.kr

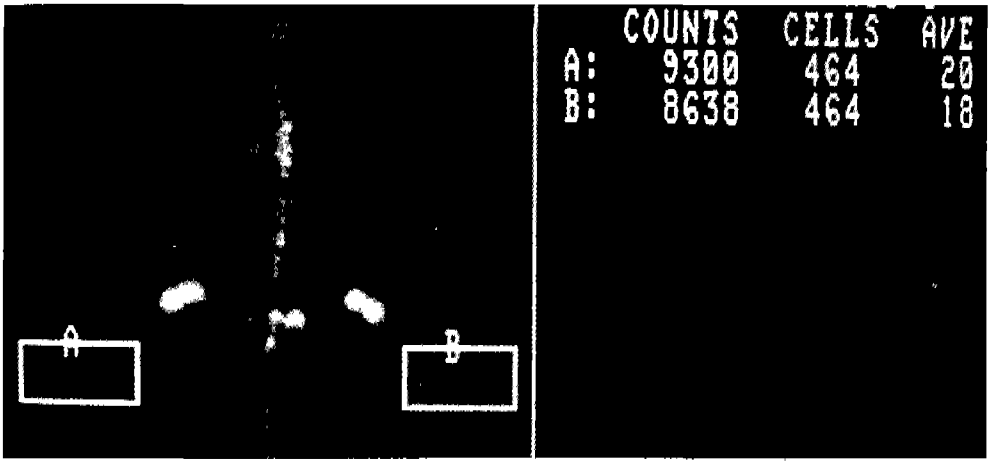

Fig 1. ROIs for regional skeletal uptake measurements in posterioanterior (PA) view. Scintigraphic image obtained at 3 hours after injection of ^{99m}Tc-MDP. Thereafter, regional skeletal uptakes of right (A) and left hindpaw (B) were assessed by evaluating the rectangular ROIs. According to these regular ROIs, ROIs for regional skeletal uptake measurement were assessed by the same method in all animals.

were composed of 6 animals.

Induction of arthritis

On day 0, each rat was subcutaneously injected in the plantar region of the right hind limb with Freund's complete adjuvant (Gibcobrl, USA, Lot No. 1020159) containing 0.6 mg of Mycobacterium butyricum (Difco, USA, Lot No. 138137LA) suspended in 0.1 ml of paraffin oil.

Radiographic assessment of bony changes

Radiographic changes were assessed under blind conditions using a previously described scoring system[14]. Each limb was assessed for osteopenia and bony erosions and graded from 0 to 3 as follows: 0 = no change; 1 = slight change; 2 = moderate change; and 3 = severe change. The right and left hindpaw were graded, and the scores were summed up to a maximum possible score of [3].

This was performed using an X-ray unit (BLD-15RK, Dong-A X-ray, Co., Korea). Exposure was 51 kVp and 7.5 mAs with diagnostic film (medium speed, Kodak Co, USA) of 14"×14". Rats were anesthetized with xylazine HCl (0.1 mg/kg, i.m.) and ketamine HCl (0.2 mg/kg, i.m.) prior to X-raying. Whole bodies were X-rayed using a 90° projection from the dorsal-ventral aspect.

Bone scan

Bone scans were obtained using a large field of view gamma camera, equipped with a parallel-hole, low-energy collimator, 3 hours after intravenous injection of 0.05 mCi/rat (0.4 ml/rat) of ^{99m}Tc-MDP. Images of the entire body were acquired with a gamma camera (SX300, PICKER Co., USA) (set at 140 KeV photoelectric peak, 20% symmetrical window). For the first 1 min, 3 second dynamic images were acquired. For the next 30 minutes, 60 second sequential static images were obtained. Sixty second static whole body dorsal ventral images were also obtained at 1, 2, 3 4, and 5 hours following injection of the radiopharmaceutical. Quantitative data were derived from 300 kcounts static images of the right and left hindpaws in which bone lesions were included.

In experimental and control, ROIs for regional skeletal uptake measurements were assessed in posteroanterior (PA) view. Scintigraphic image obtained at 3 hours after injection of ^{99m}Tc-MDP. Thercafter, regional skeletal uptakes of right and hindpaw were assessed by evaluating the rectangular ROIs (pixel: 464 cells). According to these regular ROIs, ROIs for regional skeletal uptake measurement were assessed by the same method in all animals (Fig 1).

Statistical analysis

All results were expressed as the mean ± standard deviation of the mean. The statistical significance of differences was assessed by the unpaired two-tailed Student's t-test; P values of <0.05 were considered significant.

Results

Bone scintigraph was performed after intravenous injection of 0.05 mCi/rat of ^{99m}Tc-MDP. Images of the entire body were acquired with a gamma camera at 9 second, and there was normal radiopharmaceutical uptake in the kidney. Bone uptake showed at 10 min postinjection because the rat had skeletal bone of small size.

In the experimental animals, the adjuvant-injected paw showed a significant increase bone uptake on day 7 after adjuvant injection ($P<0.05$). On day 14, the increase of bone uptake peaked at 250%, which continued to increase until the end of the experiment (Table 1). On the other hand,

Table 1. ^{99m}Tc-MDP bone uptake and ratio in the adjuvant-induced arthritis of the right hindpaw

	Day 3	Day 7	Day 14	Day 21	Day 28
Control	1,801± 199	1,780± 92	1,857± 660	1,853± 181	1,690± 623
Experimental	1,921± 422	3,172± 505*	5,346± 1,144*	6,645± 680**	,037± 1,202*
Ratio	1.49± 0.44	2.34± 1.18	2.94± 0.51	5.55± 3.29	5.55± 3.45

Ratio : Experimental uptake/ Control uptake
Results expressed as the mean ± standard deviation of the mean.
*$P<0.05$, **$P<0.01$ compared to control group.

Table 2. ^{99m}Tc-MDP bone uptake and ratio in the adjuvant-induced arthritis of the left hindpaw

	Day 3	Day 7	Day 14	Day 21	Day 28
Control	1,730± 154	1,652± 215	1,561± 621	1,609± 160	1,675± 248
Experimental	1,752± 127	2,494± 501	2,207± 265	3,613± 730*	5,141± 1,310*
Ratio	1.18± 0.29	1.91± 0.76	1.77± 1.12	2.80± 1.56	3.17± 1.25

Ratio : Experimental uptake/ Control uptake
Results expressed as the mean ± standard deviation of the mean.
*$P<0.05$ compared to control group.

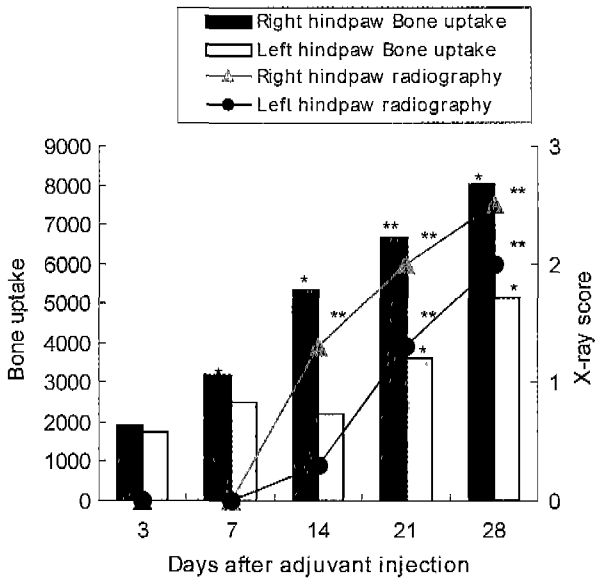

Fig 2. Comparison of ^{99m}Tc-MDP bone uptake and X-ray score in adjuvant arthritis ($n = 6$). *$P<0.05$, **$P<0.01$ compared to control rats.

the non-injected paw showed a significant increase in bone uptake from day 21 after injection (Table 2, $P<0.05$).

The bone scintigram revealed exactly the location of bone lesions which were not detected with plain radiography. Adjuvant-induced arthritis was initially detected by bone scintigraphy on day 7 (Fig 4), but in plain radiography it was not detected until day 14 (Fig 5). Bone uptake showed significant increase from day 7 after adjuvant injection ($P<0.05$). On day 21, all region of the legs were positive by bone scan, and partially positive by plain radiography. The radiological score of the experimental group didn't show any significant difference from day 0 to 7 during the experimental period (Fig 2).

During the experimental period, the main radiological changes were osteoporosis, erosion and osteal reaction in hindpaw after Day 14, especially in the injected hindpaw (Fig 5). Severe bones changes were shown by radiography in all regions of the femur and tibia of the non-adjuvant-injected leg as well as the adjuvant-injected leg after Day 28 (Fig 7), but in bone scan after only Day 21 (Fig 6).

Discussion

A particular breakthrough in bone scanning occurred in the early 1970s when technetium-99m labelled phosphorous compounds made possible the widespread use of this procedure in diagnosing malignant and benign bone disease[15]. The mechanism of localization of radiophosphate compounds is thought to involve rapid chemisorption onto the shells of hydroxyapatite crystals and gradual incorporation into the crystal[6] of newly formed bone. Soft tissue uptake has been detected in a variety of malignant, inflammatory and metabolic disorders[17]. This effect is thought to be due to either increased blood flow or augmented local calcium ion concentration[11], which may also explain the hindpaw uptake observed the adjuvant-induced arthritis in rats, but not control rat. Technetium-99m-labeled phosphates have served as bone scanning agents for the diagnosis of a broad spectrum of pathological conditions affecting the skeleton, with ^{99m}Technetium-methylene diphosphonate (^{99m}Tc-MDP) being the most widely used today[10]. In the recent work of Dick[5], local vascularity plays a major role ^{99m}Tc-phosphate uptake in bone, with marked localization of ^{99m}Tc-phosphates. Hygeian et al[9]. have further suggested that hydrolysis of Tc-

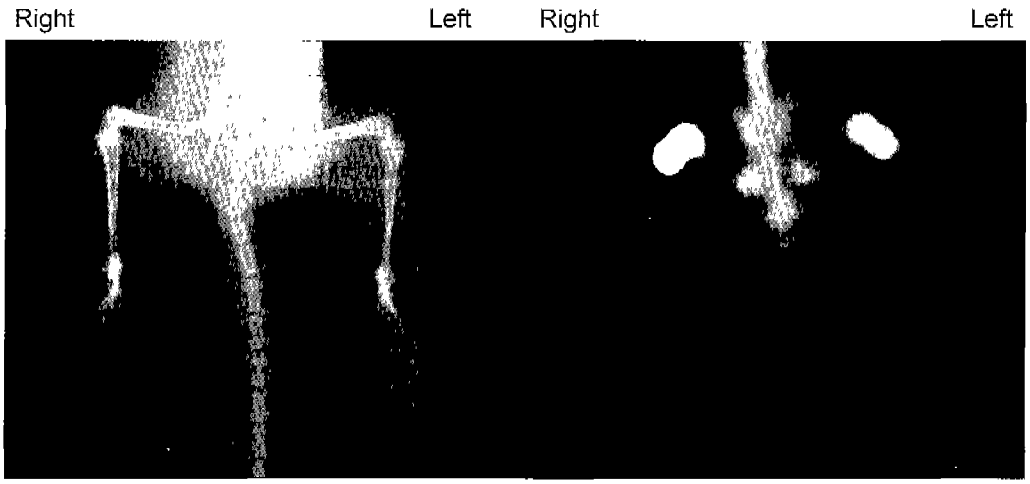

Fig 3. Dorsal-ventral radiographic and bone scan image of whole body of rat with adjuvant arthritis on day 3. These images demonstrate that bone uptake increased in the right hindpaw of a rat (right: scintigraphy), but bone scan lesions were not visible in the plain radiography.

Fig 4. Dorsal-ventral radiographic and bone scan image of whole body of rat with adjuvant-induced arthritis on day 7. Initial detection of bone lesions was observed by bone scintigraphy, but in the plain radiography was not detected.

Fig 5. Dorsal-ventral radiographic and bone scan image of whole body of rat with adjuvant arthritis on day 14. Radiological changes were mild osteoporosis, erosion and osteal reaction in the right hindpaw. Bone lesion (right hindpaw) of radiographic positive was shown by further bone scan image. Bone scan compared to radiography was identifiable by radiography in the same region.

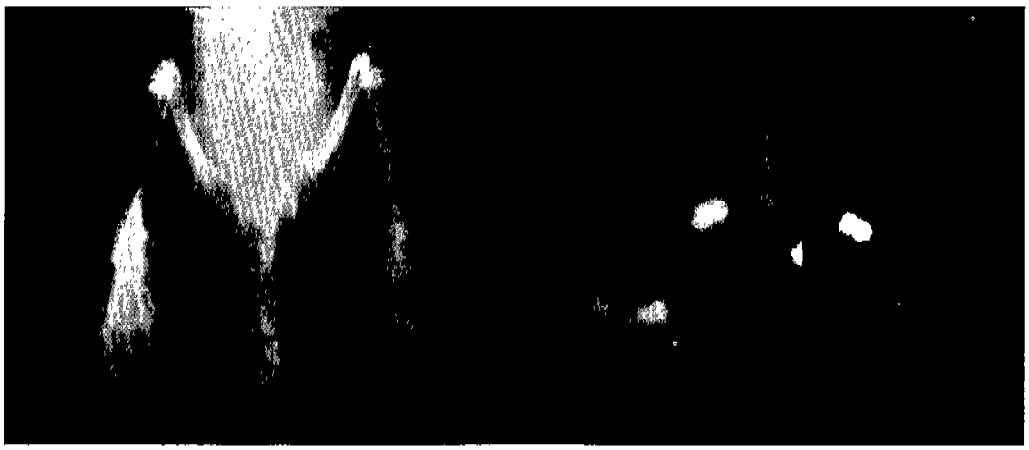

Fig 6. Dorsal-ventral radiographic and bone scan image of whole body of rat with adjuvant arthritis on day 21. All regions of the legs were positive by bone scan, and partially positive by plain radiography.

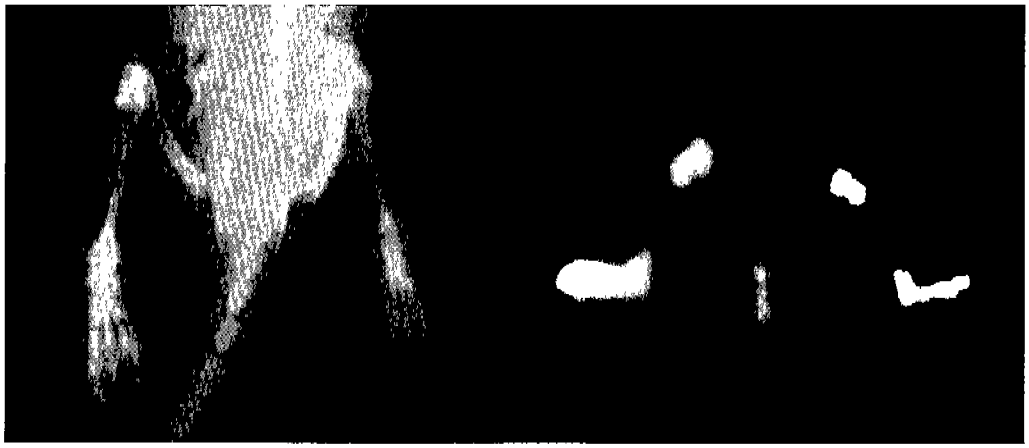

Fig 7. Dorsal-ventral radiographic and bone scan image of whole body of rat with adjuvant arthritis on day 28. Severe bone changes were visible by radiography in all regions of the leg of the non-adjuvant-injected leg as well as the adjuvant-injected leg, in both radiography and in bone scan.

MDP in blood and interstitial fluid can only be minor[8], based on the transition time of Tc-MDP in these tissue, and therefore it is likely that hydrolysis occurs in the bone tissue.

The results of this study have shown that ^{99m}Tc-MDP scintigraphy, when compared to plain radiography, is a more sensitive method to detect bone lesions[1,3,7], 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 (Fig 4), but in plain radiography was detected only on day 14 (Fig 5). Bone uptake showed a significant increase from day 7 after adjuvant injection ($P<0.05$). Investigative tools for bone lesion detection and measurement of disease activity in arthritis still have to be fully developed. Nevertheless, called bone scanning is a rapid and effective means of identifying and locating bone damage, particularly in the limbs and pelvis of patients. Bone scans are primarily performed in situations where the bone injury is minimal and undetectable using conventional methods such as X-rays. This diagnostic modality is most indicated in patients with a relatively acute (short duration), moderate to severe lameness that cannot be localized or diagnosed with a thorough lameness exam and radiographs. In our study, radiographic abnormalities was not present in the acute phase of arthritis, but was observed in the chronic phase of arthritis. Scanning has a high sensitivity for diagnosis of the acute phase of arthritis, but is nonspecific because increased uptake is often present around asymptomatic total knee arthroplasties with normal radiographs. Pauwels et al[12]. showed that scintigraphy provided the best alternative for the detection of focal bone abnormalities. Blandt et al[2]. studied bone scintigraphy in the canine cruciate deficiency model of osteoarthritis. Scintigrams of each dog showed that the predominant increase in radioactivity in the osteoarthritic knee occurred in the distal femur and proximal tibia. A bone scan is performed by injecting a radioactive isotope

into the bloodstream and then scanning a particular area of the body with a gamma camera. Technetium 99m is the radioisotope (radioactive compound) of choice for bone scanning. Technetium 99m is bound to a phosphate compound (^{99m}Tc-MDP; commonly called the "bone seeking agent") which the body incorporates into bone undergoing rapid turnover (fracture, stress fracture, infection, etc.). The Technetium 99m emits gamma rays which are detected by the gamma camera. This results in an image of the bones produced by the gamma camera. Uptake of ^{99m}Tc-MDP is greater in regions of increased bone activity, such as with fractures.

Our results demonstrate a higher sensitivity for bone scan employing ^{99m}Tc-MDP detecting adjuvant-induced arthritis than for plain radiography[4]. 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 ^{99m}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.

Conclusion

The aim of this study was to assess the diagnostic value of ^{99m}Technetium-MDP scintigraphy by means of inducing arthritis in rats by a single injection of Freund's complete adjuvant. Mature female Sprague-Dawley rats were assign to 2 groups, such a control group and an experimental group. Three hours after intravenous injection of approximately 0.05 mCi/rat ^{99m}Tc-MDP, whole body scans were performed using a gamma camera (set at 140 KeV photoelectric peak). Our data showed that adjuvant-induced arthritis was initially observed by bone scintigraphy on day 7, but in plain radiography , was first detected on day 14. Bone uptake showed a significant increase from day 7 after adjuvant injection ($P<0.05$). On day 21, all regions of the legs were positive by bone scan, but only partially positive by plain radiography.

In conclusion, radionuclide bone scan employing ^{99m}Tc-MDP was more sensitive than plain radiographs in the detection on adjuvant-induced arthritis. Therefore, bone scintigraphy appears to be the method of choice for the initial detection of Rheumatoid arthritis.

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