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Comparison of the Effect of ^{99m}Tc-DPD and ^{99m}Tc-MDP on Experimentally-induced Osteoarthritis in the Stifle Joint of the Dog

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Abstract. *This study was designed to evaluate the effect of ^{99m}Technetium-dicarboxypropane diphosphonate (^{99m}Tc-DPD) and ^{99m}Technetium-methylene diphosphonate (^{99m}Tc-MDP) on bone scan image quality and time in dogs with osteoarthritis. The left cranial cruciate ligament (CrCL) in ten healthy adult Beagle dogs was transected under general anesthesia. The dogs were assigned to ^{99m}Tc-DPD-injected or ^{99m}Tc-MDP-injected groups. Stifle joint scintigraphy was performed after intravenous injection of 10 mCi ^{99m}Tc-DPD or ^{99m}Te-MDP. Scintigraphy was conducted before CrCL transection and 2, 4, 6, 8, 10 and 12 weeks after the procedure. There were no significant differences in density, sensitivity and pathological foci between the ^{99m}Tc-DPD and ^{99m}Tc-MDP groups of experimentally-transected CrCL dogs. A comparison of the images obtained with Pinhole type and low energy general purpose type collimators of the stifle joint of normal dogs and after CrCL transaction revealed no significant differences in bone radioactivity. Scintigraphs were obtained 3 h after ^{99m}Tc-MDP and 2 h after ^{99m}Tc-DPD injection. In conclusion, application of ^{99m}Tc-DPD and ^{99m}Tc-MDP in experimentally-induced osteoarthritis of the stifle joint in dogs results in similar effects on radioactive uptake ratio and image quality. ^{99m}Tc-DPD is more efficient than ^{99m}Tc-MDP in reducing the overall time of scintigraphy.*

The radionuclide ^{99m}Technetium (^{99m}Tc) emits gamma radiation with an energy of 140 KeV, ideal for diagnostic purposes. It is particularly useful for gamma cameras with thin crystals that absorb over 90% radiation (1). ^{99m}Tc has excellent physical properties for nuclear medicine imaging due to its application in the gamma camera (2). The short half-life of the radionuclide allows the injection of several mCi of activity, leading to images with high information density (3, 4). The development of ^{99m}Tc-labelled phosphorous compounds has made bone scintigraphy one of the most useful nuclear medicine scintigraphy techniques available (5). Until the introduction of the ^{99m}Tc-tripolyphosphate complex, there was no major choice of radiopharmaceutical for skeletal imaging (6). Radio pharmaceuticals have been utilized for several years, and a number of studies comparing the different ^{99m}Tc-labelled phosphates and diphosphonates for bone imaging have been completed (7, 8). To date, no unanimously preferred radiopharmaceutical is available for skeletal imaging studies. The majority of the present data suggest that ^{99m}Technetium-methylene diphosphonate (^{99m}Tc-MDP) (9), (^{99m}Tc-HMDP) (10) and/or ^{99m}Technetium-dicarboxypropane (^{99m}Tc-DPD) (11) offer the best combination for skeletal localization and soft tissue clearance.

At present, ^{99m}Tc-labelled phosphonate compounds are frequently employed, but their mechanism of bone uptake remains to be determined (12-14). Most of the bone-seeking agents operate on the basis of the P-O-P bond (pyrophosphate) and the P-C-P bond (diphosphonates) (15, 16). ^{99m}Tc-MDP and ^{99m}Tc-DPD contain the P-C-P bond (17, 18). ^{99m}Tc-MDP is currently the most widely used bone imaging agent. Following the recent commercial introduction of ^{99m}Tc-DPD, the clinical importance of bone scintigraphy has increased significantly. In Korea, the frequency of its use is increasing continuously in humans, but not in animals.

Analysis of the early stages of osteoarthritis in humans presents numerous difficulties, since the patient generally does not seek medical attention until pathologic changes are far

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advanced and articular cartilage is already extensively lost (19). Consequently, researchers employ animal models to obtain information about the early changes in the articular cartilage, bone and synovium (20, 21). Among these, the cruciate-deficient dog is the most widely studied model (1, 22). Cranial cruciate ligament (CrCL) transection in the dog serves as a standard experimental representation of osteoarthritis (20). In an osteoarthritis model of the stifle joint of dogs, ^{99m}Tc-labelled phosphorous complexes accumulate mainly at the osteochondral junction of the osteophytes (20, 23-25). The primary objective was the detailed evaluation of the clinical efficacy of ^{99m}Tc-DPD for detecting osteoarthritis in the knee joint, compared to ^{99m}Tc-MDP (7, 26-28).

Care should be taken not to misinterpret abnormalities resulting from technical factors, that is, differences in uptake resulting from poor positioning of the patient, and focal regions of increased activity caused by radioactive contamination (2). The efficacy of single-head Pinhole bone scintigraphy is well established (29), and the technique is increasingly used to diagnose a broad spectrum of skeletal diseases (30). Pinhole magnification delineates both anatomic and pathologic signs in detail and enhances diagnostic efficacy (31, 32).

The main purpose of the present study was to assess bone uptake changes and image quality, using scintigraphy of nuclear medicine, in a dog model of osteoarthritis with experimentally-transected CrCL. In addition, we compared the uptake ratios and image times with ^{99m}Tc-DPD and ^{99m}Tc-MDP in stifle joint scintigraphy with the Pinhole or LEGP collimator.

Materials and Methods

Animals. Ten healthy adult Beagles, weighing between 9 and 12 kg, were employed. Before CrCL transection, all the dogs were screened by orthopedic and radiographic examination. The dogs had not exhibited lameness for the previous three months, nor when examined at walk or trot on a hard surface in a straight line and in a circle. Following the examination, preoperative stifle joint scintigraphy data were collected. The dogs were randomly divided into two groups, specifically, ^{99m}Tc-DPD- and ^{99m}Tc-MDP-injected groups. Each group comprised five animals.

Surgical procedure. The dogs were sedated with atropine (0.5 mg/kg, *i.m.*) and acepromazine (0.05 mg/kg, *i.m.*) 30 minutes before anesthesia. General anesthesia was induced by the administration of thiopental (12 mg/kg, *i.v.*) and maintained with isoflurane in oxygen. Arthrotomy was performed using a lateral parapatellar approach, and the CrCL was identified and extirpated. All animals were walked for 30 minutes per day over 4 weeks to induce osteoarthritis in the stifle joint. The protocols employed in this study were approved by the Animal Care Committee of Chungbuk National University, Korea.

Scintigraphy. The dogs were administered atropine sulphate (0.05 mg/kg, *s.c.*). After ten minutes, anesthesia was induced with xylazine (1.1 mg/kg, *i.v.*) and maintained with ketamine (11 mg/kg, *i.v.*). Images were acquired using a parallel-hole collimator with 1.6x magnification or a Pinhole collimator with no magnification and a 20% energy window over the 140 keV photopeak of ^{99m}Tc, and a 256 x 256 matrix. Stifle joint scintigraphy was performed 4 h after the intravenous injection of 10 mCi ^{99m}Tc-MDP (Daiichi, Japan) and ^{99m}Tc-DPD (CIS Biointernational, France) by means of a large field gamma camera (Picker SX-300, USA) with the Pinhole or LEGP collimator. The left stifle joint image was obtained for a set number of 100*k* counts, and then the right stifle joint image in the study was obtained for the same time as the initial image. Scintigraphy was performed every 2 weeks for 3 months after CrCL transection.

Measurement of radioactivity uptake. All regions of interest (ROI) were mapped to the same size. Quantitative analysis included the calculation of both stifle joint and synovial fluid, and some soft tissue. ROIs were drawn around the left and right stifle joint images, respectively. Ratios comparing the osteoarthritis left stifle joint to the ROIs for the contralateral normal right stifle joint were calculated.

Statistical analysis. Statistics obtained for independent samples were used for the comparison of scintigraphic data between ^{99m}Tc-DPD and ^{99m}Tc-MDP agents. All statistical computations were made using the SAS system. Differences in the distribution of values between ^{99m}Tc-DPD and ^{99m}Tc-MDP were assessed with the Wilcoxon rank sum test. The data are expressed as means±S.D. Probability values of less than 0.05 were considered statistically significant.

Results

Clinical appearance. All experimental dogs recovered well from the anesthesia and surgical interventions, and demonstrated no signs of distress throughout the observation period. In particular, no complications, such as sequels to the surgical interventions, abscess, consequential diseases or infections, were observed throughout the entire study period.

Comparison of the uptakes of ^{99m}Tc-DPD and ^{99m}Tc-MDP. Stifle joint scintigraphs revealed increased uptake in the left transected CrCL dogs as early as 2 weeks. In contrast, no increased uptake was evident in the normal right stifle joint. At 8 weeks after the procedure, more radioactivity uptake was observed in the left stifle joint relative to the non-operated stifle joint. In general, following CrCL transection, ^{99m}Tc-MDP uptake was slightly higher than that of ^{99m}Tc-DPD. However, there were no significant differences between the activity ratios of the ^{99m}Tc-DPD and ^{99m}Tc-MDP groups (data not shown). Scintigraphs were obtained 2 hours and 3 hours after ^{99m}Tc-DPD and ^{99m}Tc-MDP injection, respectively (Figure 1).

Comparison of the LEGP collimator and Pinhole collimator. Stifle joint scintigraphs of ^{99m}Tc-DPD and ^{99m}Tc-MDP, at all weeks after CrCL transaction, disclosed that the Pinhole

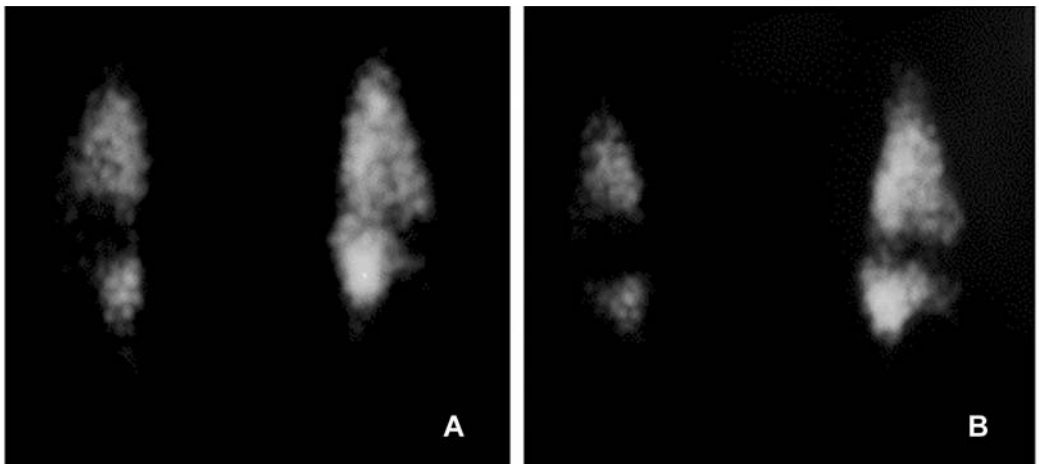

Figure 1. *Scintigraphs of stifle uptake 8 weeks after left cranial cruciate ligament transection. LEGP collimator scintigraphs were obtained 2 h after ^{99m}Tc-DPD (A) and 3 h after ^{99m}Tc-MPD (B) injection. Images A and B show concentrated abnormal increased activity in the left stifle joint.*

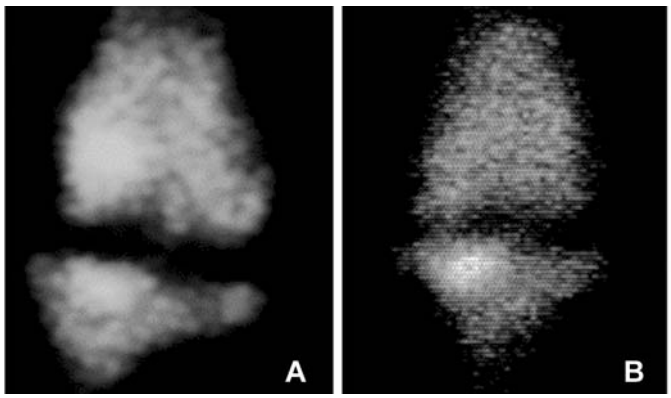

Figure 2. *Scintigraphs at 8 weeks after left cranial cruciate ligament transection. LEGP (A) and Pinhole (B) views of ^{99m}Tc-DPD in the left stifle joints. Pinhole uptake was slightly higher than LEGP uptake.*

uptakes were slightly higher than the LEGP uptakes (Figure 2). However, no significant differences were evident between the data obtained with the two types of collimator (data not shown).

Discussion

The quality of ^{99m}Tc-radiopharmaceutical may be affected by many factors, including: chemical purity and stability of the initial chemicals, the production procedure, radionuclide and radiochemical purity of ^{99m}Tc-eluate (5) and the time interval between labelling and analysis (17, 33). Users claim that new bone-seeking radiopharmaceuticals have higher bone uptake, faster or similar blood clearance and, therefore, better image quality and shorter minimum time between administration and imaging, compared with traditional ^{99m}Tc-MPD agents (7). In the routine skeletal imaging procedure, the patient is usually evaluated 4 to 5 minutes (early phase) and 2.5 to 3.5 hours (late phase) after the intravenous administration of 10-25 mCi of ^{99m}Tc as a diphosphonate or diphosphonate complex (34-36).

Bone scintigraphy is a sensitive and frequently used method in the early detection and follow-up of reactive articular changes in experimental animal models for osteoarthritis (1). CrCL injury is one of the most common orthopedic diseases in the dog hind limb (37). This model shows that progressive structural changes develop in the articular cartilage and subchondral bone of the unstable joint that are typical of osteoarthritis in humans (21). The juxtaarticular bone is the focus of bone-seeking isotopes in osteoarthritis (1). The crystalline structure of the bone salt mineral is hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$. The localization

of intravenously-administered ionic radioactive tracers primarily involves their selective concentration by hydroxyapatite crystals (2, 15). Although the mechanism of ^{99m}Tc-labelled phosphate and diphosphonate localization remains to be clearly defined, several observations strongly suggest that the mineral phase of bone is the primary site of deposition of these materials in the skeleton. The majority of evidence suggests that these materials localize in the bone on the surfaces of hydroxyapatite crystals and amorphous calcium phosphate by chemisorption (2).

Osteoarthritic joints have been identified from animal data (22, 25). Some display increased uptake of bone-seeking agents (21), even in the very early stages of disease (38, 39), and may thus be employed to obtain a better image of the bones, since the lower blood and tissue concentration provides a higher bone to tissue ratio. The average osseous concentration of ^{99m}Tc-DPD is slightly greater than that of ^{99m}Tc-MDP (26). The ratio of bone/muscle is the highest for ^{99m}Tc-DPD and ^{99m}Tc-MDP (17). Computer analysis of ^{99m}Tc-DPD and ^{99m}Tc-MDP kinetics in humans revealed higher bone uptake and lower soft tissue retention by ^{99m}Tc-DPD (27). No significant differences were observed between the means of the bone to soft tissue ratios of the ^{99m}Tc-MDP and ^{99m}Tc-DPD agents at 1 hour. However, at 3 hours, ^{99m}Tc-MDP agents exhibited lower ratios than ^{99m}Tc-DPD ($p<0.01$) (28). Statistical evaluation of quantitative data showed that the lesion to normal bone ratio was significantly higher for ^{99m}Tc-MDP agents. ^{99m}Tc-DPD does not possess clinical advantages over ^{99m}Tc-MDP for the detection of bone metastasis (7, 18). The only significant difference between the two agents was evident in the case of normal bone uptake. However, ^{99m}Tc-MDP was superior with regard to both the time interval and pathologic bone uptake (7). There were no marked differences between the bone-seeking agents with regard to visualization of pathological foci (18, 28).

In this study, ^{99m}Tc-MDP uptake was slightly higher than that of ^{99m}Tc-DPD, consitent with data from Bergqvist *et al.* (7) and Pauwels *et al.* (18). The lesion to normal bone ratio and fractional bone uptake of diphosphonates estimated from the ratio to transfer rates were slightly higher for ^{99m}Tc-MDP than ^{99m}Tc-DPD. Notably, the differences between ^{99m}Tc-DPD and ^{99m}Tc-MDP were not significant. Additionally, increased early uptake was observed for both ^{99m}Tc-MDP and ^{99m}Tc-DPD in osteoarthritis models with no differences between the two agents.

In summary, no significant differences were observed in the activity ratios of ^{99m}Tc-DPD and ^{99m}Tc-MDP in experimentally-transected CrCL dogs. Scintigraphs were obtained 3 hours and 2 hours after the intravenous injection of ^{99m}Tc-DPD and ^{99m}Tc-DPD, respectively. ^{99m}Tc-DPD is more effective than ^{99m}Tc-MDP in reducing the overall procedure time.

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