

# Extravasation of Hydroxymethylene Diphosphonate–Induced Subcutaneous Inflammation, Histologically Demonstrated in BALB/c Mice

Ilhan Lim<sup>1</sup>, Keon Wook Kang<sup>2,3</sup>, Jae Kyung Myung<sup>4</sup>, Kyeong Cheon Jung<sup>4</sup>, Yong Hyun Jeon<sup>2,3</sup>, Ji Yeon Kang<sup>2</sup>, Young Hwa Kim<sup>2,3</sup>, June-Key Chung<sup>2,3</sup>, and Dong Soo Lee<sup>2,5</sup>

<sup>1</sup>Department of Nuclear Medicine, Korea Cancer Center Hospital, Seoul, Korea; <sup>2</sup>Department of Nuclear Medicine, Seoul National University College of Medicine, Seoul, Korea; <sup>3</sup>Cancer Research Institute, Seoul National University College of Medicine, Seoul, Korea; <sup>4</sup>Department of Pathology, Seoul National University College of Medicine, Seoul, Korea; and <sup>5</sup>Department of Molecular Medicine and Biopharmaceutical Sciences, WCU Graduate School of Convergence Science and Technology, Seoul National University, Seoul, Korea

We encountered local reactions at injection sites in 7 patients after an intravenous injection of <sup>99m</sup>Tc-hydroxymethylene diphosphonate (<sup>99m</sup>Tc-HDP). Archived bone scans showed radiopharmaceutical extravasation at injection sites in all patients. To identify the mechanism underlying these local reactions, we challenged BALB/c mice with an <sup>99m</sup>Tc-HDP injection and investigated the mechanism involved. **Methods:** Seven solutions were prepared: 0.1 M acetic acid, 0.1 M NaOH, 2.1 mM <sup>99m</sup>Tc-HDP, 4.7 mM <sup>99m</sup>Tc-methylene diphosphonate (<sup>99m</sup>Tc-MDP), 1.05 mM <sup>99m</sup>Tc-HDP diluted with normal saline, 37 MBq of <sup>99m</sup>Tc-pertechnetate, and normal saline. Six female BALB/c mice were subcutaneously injected in their backs with 0.04 mL of each solution. Backs were observed, and skin and subcutaneous tissues were acquired on days 5, 7, and 19 after injection. Two pathologists interpreted the histologic specimens. **Results:** The pH values of the 0.1 M acetic acid, 0.1 M NaOH, 2.1 mM <sup>99m</sup>Tc-HDP, 4.7 mM <sup>99m</sup>Tc-MDP, 1.05 mM <sup>99m</sup>Tc-HDP diluted with normal saline, 37 MBq <sup>99m</sup>Tc-pertechnetate, and normal saline were 3.28, 14.3, 4.79, 6.45, 6.16, 6.85, and 6.53, respectively. A slight redness was observed at <sup>99m</sup>Tc-HDP injection sites on day 5 after injection, and many inflammatory cells, such as neutrophils, were found to infiltrate subcutaneous tissues by light microscopy, suggesting active inflammation. However, no abnormality was found at <sup>99m</sup>Tc-MDP injection sites. On day 19, <sup>99m</sup>Tc-HDP injection sites appeared to have recovered and showed healed scars and almost-normal microscopic features. **Conclusion:** Our results indicate that the local reaction induced by extravasation of <sup>99m</sup>Tc-HDP is probably caused by high acidity, and <sup>99m</sup>Tc-HDP should be administered carefully to avoid adverse reactions.

**Key Words:** <sup>99m</sup>Tc-hydroxymethylene diphosphonate (<sup>99m</sup>Tc-HDP); <sup>99m</sup>Tc-methylene diphosphonate (<sup>99m</sup>Tc-MDP); extravasation

J Nucl Med 2010; 51:1573–1575

DOI: 10.2967/jnumed.110.078774

Received May 4, 2010; revision accepted Jul. 8, 2010.

For correspondence and reprints contact: Keon Wook Kang, Department of Nuclear Medicine, Seoul National University College of Medicine, 28 Yonggondong, Chongno-gu, Seoul, 110-744, Korea.

E-mail: kangkw@snu.ac.kr

COPYRIGHT © 2010 by the Society of Nuclear Medicine, Inc.

Diphosphonate is a widely used radiopharmaceutical for bone scanning, and no significant toxic effects have been reported to date (1). Regarding adverse reactions, only 0.5 were reported for every 100,000 administrations in 1984 (2).

We started to use <sup>99m</sup>Tc-hydroxymethylene diphosphonate (<sup>99m</sup>Tc-HDP) as a bone scanning agent instead of <sup>99m</sup>Tc-methylene diphosphonate (<sup>99m</sup>Tc-MDP) at the Seoul National University Hospital in January 2008 because <sup>99m</sup>Tc-HDP has better physiochemical and biologic characteristics than <sup>99m</sup>Tc-MDP (3). After this change, 7 of 2,700 patients (0.26%) complained of a sharp pain at the injection site immediately after <sup>99m</sup>Tc-HDP administration and continued to experience pain and inflammation at injection sites 2 wk later. Retained bone scans showed radiopharmaceutical extravasation in all patients. The reported adverse reactions to diphosphonate included typical drug reactions such as rash, headache, dizziness, nausea, myalgia, and fever (4). However, we have not encountered adverse reactions of pain or inflammation over more than 30 y of <sup>99m</sup>Tc-MDP use, and we infer that these <sup>99m</sup>Tc-HDP reactions are not systemic but local because patient responses were immediate and the pain was localized.

No previous study has been conducted on the local adverse reactions induced by <sup>99m</sup>Tc-HDP. Accordingly, we compared histologic changes induced by <sup>99m</sup>Tc-HDP and <sup>99m</sup>Tc-MDP after administering them subcutaneously to BALB/c mice and investigated the mechanism underlying the pain and redness at <sup>99m</sup>Tc-HDP injection sites in an effort to avoid these reactions.

## MATERIALS AND METHODS

### Animals

Six adult female BALB/c mice (18–20 g) were used in this study. The animals were 6–8 wk old at the start of the experiment.

### Radiopharmaceuticals and Control Materials

<sup>99m</sup>Tc-HDP and <sup>99m</sup>Tc-MDP were synthesized in the usual manner, as described in the manufacturer's directions for use,

and the concentration of  $^{99m}\text{Tc}$ -HDP and  $^{99m}\text{Tc}$ -MDP were 2.1 and 4.7 mM, respectively.  $^{99m}\text{Tc}$ -HDP vials (Mallinckrodt Medical BV) contained oxidronate sodium (3.0 mg), stannous chloride (0.24 mg), gentisic acid (0.84 mg; a stabilizer), and sodium chloride (30.0 mg).  $^{99m}\text{Tc}$ -MDP vials (GE Healthcare) contained sodium medronate (6.25 mg), stannous fluoride (0.34 mg), and sodium *p*-aminobenzoate (2 mg) as a freeze-dried mixture.

As a positive control, we used 0.1 M acetic acid as an acid solution and 0.1 M sodium hydroxide as an alkali solution. Normal saline was used as a negative control. For comparison purposes, 37 MBq of  $^{99m}\text{Tc}$ -pertechnetate and 1.05 mM  $^{99m}\text{Tc}$ -HDP diluted with normal saline were prepared.

### Measurement of pH

The pH values of each solution (2-mL aliquots) were measured in triplicate and averaged. To evaluate pH changes caused by radiopharmaceutical decay, we checked the pH values of each solution after 2 d of cold storage.

### Injection and Assay

The 7 solutions (0.04 mL) were injected subcutaneously, more than 1 cm apart, into the backs of each mouse. Injection sites were observed daily. Two mice were sacrificed on days 5, 7, and 19 after injection.

Subcutaneous tissues for histologic examinations were fixed in 10% buffered formalin for 24 h. Paraffin-embedded tissue sections were stained with hematoxylin and eosin.

## RESULTS

### Measurement of pH

The pH values of the 7 solutions—0.1 M acetic acid, 0.1 M NaOH, 2.1 mM  $^{99m}\text{Tc}$ -HDP, 4.7 mM  $^{99m}\text{Tc}$ -MDP, 1.05 mM  $^{99m}\text{Tc}$ -HDP diluted with normal saline, 37 MBq of  $^{99m}\text{Tc}$ -pertechnetate, and normal saline—were 3.28, 14.3, 4.79, 6.45, 6.16, 6.85, and 6.53, respectively. Because the pH of  $^{99m}\text{Tc}$ -HDP (4.79) was substantially lower than that of  $^{99m}\text{Tc}$ -MDP (6.45), its higher acidity could have caused the pain during injection. The pH values of radiopharmaceutical solutions were not found to change significantly during storage.

### Time Course of Inflammation

Red ulceration developed at  $^{99m}\text{Tc}$ -HDP injection sites in BALB/c mice macroscopically, but no abnormality was found at  $^{99m}\text{Tc}$ -MDP injection sites at 5 d after injection. Mononuclear and polymorphonuclear leukocytes were present in subcutaneous tissues with fibrinoid necrosis at  $^{99m}\text{Tc}$ -HDP injection sites at 5 d after injection, suggesting active inflammation, and this inflammation completely resolved on day 19 (Fig. 1). However, no severe inflammation was seen at  $^{99m}\text{Tc}$ -MDP injection sites at 5 or 19 d after injection.

Large ulceration developed at acetic acid and NaOH injection sites macroscopically at 5 d after injection. Neutrophils, eosinophils, and necrotic materials were observed at acetic acid injection sites at 5 d after injection, suggesting active inflammation. Necrotic epidermal ulceration, liquefaction, coagulation, subcutaneous fibrinoid necrosis, and multiple neutrophils were observed at NaOH injection sites at 5 d after injection. Fibrotic healings were observed at acetic acid and NaOH injection sites at 19 d after injection.

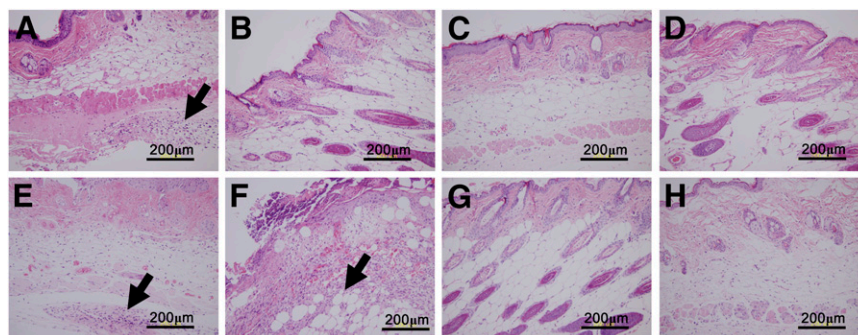
Only minimal inflammatory cells were found at injection sites for  $^{99m}\text{Tc}$ -HDP diluted with normal saline and  $^{99m}\text{Tc}$ -pertechnetate at 5 d after injection, an almost normal finding. No evidence of inflammation was found at normal saline injection sites at this time.

## DISCUSSION

The present study demonstrates that  $^{99m}\text{Tc}$ -HDP injections into subcutaneous tissues induce active inflammation. To explain the adverse reaction at  $^{99m}\text{Tc}$ -HDP, but not at  $^{99m}\text{Tc}$ -MDP, injection sites, we considered the greater acidity of  $^{99m}\text{Tc}$ -HDP to be the potential cause.

The results of the present study concur with those of an earlier study, in which the incidence of infusion thrombophlebitis increased when a solution with a low pH and high tonicity was administered subcutaneously (5).

**FIGURE 1.** Comparison of histologies at  $^{99m}\text{Tc}$ -HDP and  $^{99m}\text{Tc}$ -MDP injection sites.  $^{99m}\text{Tc}$ -HDP injection site at 5 d after injection (A) shows multiple neutrophils in subcutaneous area (black arrow), suggesting active inflammation and fibrinoid necrosis.  $^{99m}\text{Tc}$ -HDP injection site at 19 d after injection (B) revealed only focal inflammatory cells. No severe inflammation was seen at  $^{99m}\text{Tc}$ -MDP injection sites at 5 (C) or 19 d after injection (D). Acetic acid injection site at 5 d after injection (E) has multiple neutrophils in subcutaneous area (black arrow), suggesting active inflammation. NaOH injection site at 5 d after injection (F) shows multiple neutrophils and liquefaction necrosis in subcutaneous area (black arrow). However, no evidence of inflammation was found at injection site for  $^{99m}\text{Tc}$ -HDP diluted with normal saline (G) or injection site for just normal saline (H) at this time. (Hematoxylin and eosin,  $\times 200$ ).



The ideal pH of a radiopharmaceutical is 7.4 (the pH of blood), although pH can vary between 2 and 9 because blood has a substantial buffering effect (6). However, stricter standards for subcutaneous administration are required because the buffering ability of subcutaneous tissues is less than that of blood. An allowable pH range of 5.5–8.0 for radiopharmaceuticals has been suggested by Chung and Lee (7), and caution is necessary when  $^{99m}\text{Tc}$ -HDP is administered because the pH of  $^{99m}\text{Tc}$ -HDP is less than 5.5 in the present study and  $^{99m}\text{Tc}$ -HDP extravasation might cause irritation.

With respect to chemical skin injury, solutions with pH values of less than 2 can produce coagulation necrosis on contact with the skin, and similarly, alkalis with pH values greater than 11.5 can produce severe tissue injury due to liquefaction necrosis (8). Regarding the allowable pH range for subcutaneous infusions, both acidic agents (ondansetron at pH 3.5, morphine sulfate at pH 2.5–6.5) and alkaline agents (sodium phenobarbitone at pH 9.2–10.3) are being administered successfully subcutaneously without inducing skin irritation (9). However, in this previous study, successful administration without skin irritation was assessed physiologically and was not based on patients' complaints.

We experienced 7 adverse reactions out of 2,700  $^{99m}\text{Tc}$ -HDP administrations, and because these patients appeared to have serious complaints, we stopped using  $^{99m}\text{Tc}$ -HDP as a bone scanning agent and resumed  $^{99m}\text{Tc}$ -MDP use.

## CONCLUSION

Patient complaints of a sharp pain after an  $^{99m}\text{Tc}$ -HDP injection could be explained by the low pH of  $^{99m}\text{Tc}$ -HDP. Available information provides direct in vivo evidence that

extravasation of  $^{99m}\text{Tc}$ -HDP may cause active inflammation. Accordingly, we recommend that  $^{99m}\text{Tc}$ -HDP be administered carefully to avoid adverse reactions.

## ACKNOWLEDGMENTS

We thank Dr. Yun-Sang Lee, who kindly provided radiopharmaceutical information, and Dr. Hyo Sang Lee, Jin Uee Kim, and Sung Wook Cho, for their excellent technical and generous support. This work was supported by Priority Research Centers Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009-0093820).

## REFERENCES

1. Weiner R. Bone Imaging radiopharmaceuticals. In: Henkin R, Bova D, Dillehay G, et al., eds. *Nuclear Medicine*. 2nd ed. Philadelphia, PA: Mosby Elsevier; 2006:1116–1119.
2. Atkins H. Reported adverse reactions to radiopharmaceuticals remain low in 1984. *J Nucl Med*. 1986;27(3):327.
3. Francis MD, Ferguson DL, Tofe AJ, Bevan JA, Michaels SE. Comparative evaluation of three diphosphonates: in vitro adsorption (C-14 labeled) and in vivo osteogenic uptake (Tc-99m complexed). *J Nucl Med*. 1980;21:1185–1189.
4. Hoving J, Roosjen HN, Brouwers JR. Adverse reactions to technetium-99m methylene diphosphonate. *J Nucl Med*. 1988;29:1302–1303.
5. Oliver DJ, Sykes NP, Carter RL. Histopathological study of subcutaneous drug infusion sites in patients dying of cancer [letter]. *Lancet*. 1988;1:478.
6. Saha G. *Fundamentals of Nuclear Pharmacy*. 5th ed. New York, NY: Springer; 2004.
7. Chung J-K, Lee MC. *Nuclear Medicine*. 3rd ed. Seoul, Korea: Korea Medicine; 2008:144.
8. Cartotto RC, Peters WJ, Neligan PC, Douglas LG, Beeston J. Chemical burns. *Can J Surg*. 1996;39:205–211.
9. Agar M, Webster R, Lacey J, Donovan B, Walker A. The use of subcutaneous omeprazole in the treatment of dyspepsia in palliative care patients. *J Pain Symptom Manage*. 2004;28:529–531.