Atom Indonesia

Journal homepage: http://aij.batan.go.id

Pharmacokinetics Interaction of Nonsteroidal Anti-Inflammatory Drugs with ^{99m}Tc-MDP Radiopharmaceuticals for Bone Imaging

I. Mahendra[1*], I. Daruwati[1], I. Halimah[1] and S.R. Pajrin[2]

[1]*Center for Applied Nuclear Science and Technology, National Nucler Energy Agency, Jl. Tamansari No.71, Bandung 40132, Indonesia*

[2]*Indonesian College of Pharmacy (STFI), Soekarno Hatta 345, Bandung, Indonesia*

ARTICLE INFO

Article history:
Received 01 November 2017
Received in revised form 17 August 2018
Accepted 27 August 2018

Keywords:
Pharmacokinetics interaction
Biodistribution
^{99m}Tc-MDP
Meloxicam
Sodium diclofenac

ABSTRACT

^{99m}Tc-MDP has been developed as a radiopharmaceutical for bone imaging in nuclear medicine. A drug therapy can alter the pharmacokinetic profiles and biodistribution patterns of radiopharmaceuticals. To achieve an optimum diagnostic outcome, this research focused on pharmacokinetics interaction between two kinds of nonsteroidal anti-inflammatory drugs (NSAID) drugs, meloxicam and sodium diclofenac with ^{99m}Tc-MDP using mice (*Mus musculus*). There were five groups of animal model and each group consists of three mice except for group II and III which consists of six mice. The groups were classified as untreated mice (I), mice treated with meloxicam for 3 days (II), treated with sodium diclofenac for 3 days (III), treated with meloxicam once or at onset (IV), and mice with sodium diclofenac once or at onset (V). Pharmacokinetics interaction and biodistribution test were conducted by injecting 100 µCi/100 µL ^{99m}Tc-MDP intravenously. Blood samples were withdrawn from each mouse which were then weighted and counted using single channel analyzer. The %ID/g of ^{99m}Tc-MDP in blood of untreated mice (I), mice treated with meloxicam (II) and sodium diclofenac (III) 5 minutes post injection were 3.71, 8.96 and 9.15 % respectively, then decrease to 0.12, 0.01, and 0.01 %, respectively, 24 hours post injection. The results of T-test showed there were no significant differences in distribution of ^{99m}Tc-MDP in untreated mice (I) and in treated mice either with meloxicam (II) or sodium diclofenac (III). However, there was significant difference in elimination of ^{99m}Tc-MDP in untreated mice (I) and in treated mice either with meloxicam (II) or sodium diclofenac (III). The bone uptakes of ^{99m}Tc-MDP were 9.03 ± 0.41, 3.52 ± 0.52, 3.62 ± 0.45, 8.44 ± 1.39, and 8.09 ± 0.86 % in group I, II, III, IV, and V, respectively. T-test showed there were significant differences in bone uptake of ^{99m}Tc-MDP in mice with previously treated with meloxicam and sodium diclofenac for 3 days. From these result, it can be concluded that an administration of meloxicam and sodium diclofenac could accelerate elimination half-life that cause low uptake of ^{99m}Tc-MDP radiopharmaceutical on the bone as the primary target. Therefore, it is necessary to follow up using image study to determine the significance of the effects on image quality.

INTRODUCTION

Bone scintigraphy still represents the second greatest volume procedure in nuclear medicine with broad, diverse applications. The clinical utility, sensitivity, specificity, and predictive value of bone imaging have been developed on the basis of planar bone imaging data. Some of the radiopharmaceuticals for detection of early bone metastasis are phosphonate-based compounds such as ^{99m}Tc-MDP (medronate) [1-3].

Metastatic cancer is a common symptom in advancing malignancy and often determines the quality of life in the later stages of disease [4]. Bone metastases disrupt the normal homeostasis between bone formation and resorption by promoting osteoclast maturation, activity, and increased

*Corresponding author.
E-mail address: isamahendra@batan.go.id
DOI: https://doi.org/10.17146/aij.2018.741

bone resorption. The shift toward increased bone resorption may result in bone destruction and skeletal-related events (SREs), such as pathologic fracture, spinal cord compression, severe pain and the need for skeletal radiation or surgery [5].

Pharmacological management of cancer-induced bone pain involves the use of analgesic such as nonsteroidal anti-inflammatory drugs (NSAID) in combination with another treatment such as chemotherapy and radiotherapy. The commonly-used NSAIDs are meloxicam and sodium diclofenac [6].

There is considerable evidence that radiopharmaceutical biodistribution or pharmacokinetics may be altered by a variety of drugs. It can have a significant clinical impact on safety, scan interpretation, and diagnostic imaging accuracy [7]. In a previous study, several drugs such as methotrexate, nifedipine, gentamicin, and vitamin D can influence image quality of ^{99m}Tc-MDP [8]. This research's aims was to determine the effect of NSAID (i.e. meloxicam and sodium diclofenac) on the pharmacokinetic interaction of ^{99m}Tc-MDP radiopharmaceutical in mice (*Mus musculus*) and its biodistribution.

EXPERIMENTAL METHODS

Material

Meloxicam, sodium diclofenac, MDP (Sigma Aldrich), $SnCl_2$.2HO (Sigma Aldrich), NaOH (E. Merck), HCl and acetone (E. Merck), aquadest (IPHA laboratories), radionuclide Technetium-99m from generator ^{99}Mo/^{99m}Tc (Polatom), 3MM Whatman chromatography paper and pH Universal Indicator (E. Merck), and in-house prepared MDP radiopharmaceuticals kits. The equipment used were a single channel analyzer with an NaI(Tl) detector (Ortec), TLC scanner (Bioscan 2000), Deluxe Isotope Calibration (Victoreen), 10-mL vials, micropipette (Eppendorf), analytical scale (Mettler Toledo), oven (Memmert), disposable syringe (Terumo), and other glass tools.

Radiolabelling of ^{99m}Tc-MDP

All steps were performed aseptically. ^{99m}Tc-pertechnetate (3 mCi/3 mL) was added into vial containing MDP kit. The solution was shaken and incubated for 30 minutes at room temperature, resulting in ^{99m}Tc-MDP radiopharmaceutical. The radiochemical purity of ^{99m}Tc-MDP was determined using two paper chromatography systems. The first one used 3MM Whatman chromatography paper as stationary phase and 100 % acetone for separating reduced-^{99m}Tc ($^{99m}TcO_2$) and ^{99m}Tc-MDP from ^{99m}Tc-pertecnetate. The second one used 3MM Whatman chromatography paper as stationary phase and 0.9 % NaCl as a mobile phase for separating $^{99m}TcO_2$ from ^{99m}Tc-MDP and ^{99m}Tc-pertecnetate [3].

Animal preparation

All experiments were performed according to the guidelines and approved protocol by Institutional of Animal Care and Use Committee (KEPPHP-BATAN) with approval number 003/KEPPHP-BATAN/IV/2015.

There were five groups of animal model and each group consists of three mice except in group II and III which consists of six mice. The groups were classified for control (I), mice treated with meloxicam for 3 days (II), mice treated with sodium diclofenac for 3 days (III), mice treated with meloxicam once or at onset (IV), and mice treated with sodium diclofenac once or at onset (V).

Pharmacokinetic and biodistribution study

The Pharmacokinetics test used in this research was adopted from Petriev *et al*. [9]. The pharmacokinetics test was applied to the group I, II, and III. The treated mice were injected with 100 µCi/100 µL of ^{99m}Tc-MDP through their tail veins. At specified times of 5 minutes and 1, 2, 3, 4, 5, 6, 24, 25, and 26 hours post-injection of ^{99m}Tc-MDP, blood samples were withdrawn from the tail of each mouse. Blood samples were then weighted and counted using a single channel analyzer with an NaI(Tl) detector. The results of measurement expressed as percentage of injected dose per gram organ (blood in this case) (%ID/g) [9]. In order to determine the biological half-life of ^{99m}Tc-MDP, the %ID/g of blood samples plotted again time of their withdrawal. Pharmacokinetic parameters were calculated by fitting to a first-order two-compartment model with Multifit pharmacokinetic software [10]. T-test was used to compare the biological half-life of ^{99m}Tc-MDP injected to mice in control group with treated group.

A biodistribution study was performed on normal animal group (I) and animal models group IV and V by injecting each mice with 0.1 mL of ^{99m}Tc-MDP (100 µCi) intravenously through the tail. The mice were then euthanized 3 hours post injection and vital organs such as muscle, bone,

blood, intestine, stomach, liver, spleen, kidneys, heart and lungs were taken. The collected samples were weighed and then counted using single channel analyzer with NaI(Tl) detector. The %ID/g for each sample was then calculated [11]. The significance of differences was determined using two-way ANOVA.

RESULTS AND DISCUSSION

Radiochemical purity is one of quality requirements for a radiopharmaceutical in addition to clarity, sterility, non-pyrogenicity, and pH. In this study, radiochemical purity testing was performed using two ascending paper chromatography systems. Fig. 1 shows a radiochromatogram of ^{99m}Tc-MDP where acetone used as a mobile phase, $^{99m}TcO_2$ and ^{99m}Tc-MDP stayed at origin (Rf = 0) while ^{99m}Tc-pertecnetate moved with the solvent to give an Rf of 1. Figure 2 shows a radiochromatogram of ^{99m}Tc-MDP where saline solution (0.9 % NaCl) used as a mobile phase, $^{99m}TcO_2$ stayed at origin (Rf = 0) while ^{99m}Tc-MDP and ^{99m}Tc-pertecnetate moved with the solvent to give an Rf of 1. The results of this test showed that ^{99m}Tc-MDP radiopharmaceuticals had 96.76 ± 2.27 % radiochemical purity (n=3) with impurity contents of free ^{99m}Tc-pertechnetate and ^{99m}Tc-reduced being 1.1 ± 1.49 % and 3.00 ± 2.28 %, respectively. This ^{99m}Tc-MDP sample conformed to the United States of Pharmacopoeia, which states that the percentage of radiochemical purity has to be greater than 95 %. The solution of ^{99m}Tc-MDP is clear and colorless with pH between 5.8 and 6.0.

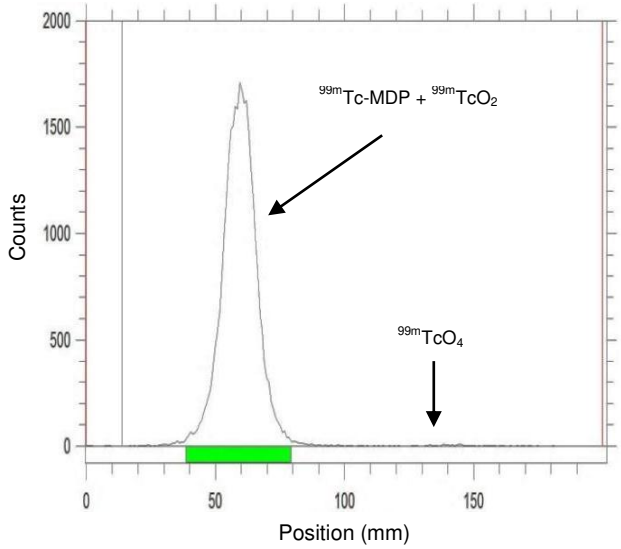

Fig. 1. Radiochromatogram of ^{99m}Tc-MDP using acetone as mobile phase.

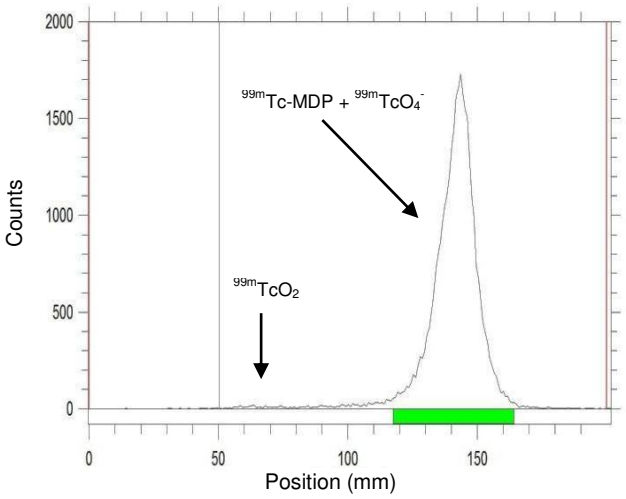

Fig. 2. Radiochromatogram of ^{99m}Tc-MDP using saline solution as mobile phase.

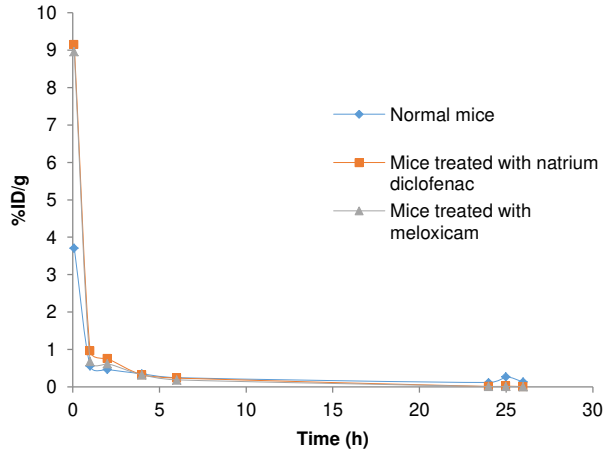

Fig. 3. Pharmacokinetic profile of ^{99m}Tc-MDP in mice treated by sodium diclofenac and meloxicam.

Table 1. Biological half-life of ^{99m}Tc-MDP.

Groups	Biological half-life (h)	
	Distribution	Elimination
I	0.196 ± 0.176	15.367 ± 0.898
II	0.235 ± 0.029	4.224 ± 0.378
III	0.167 ± 0.020	4.252 ± 0.303

I. Control/untreated mice, II. Treated with meloxicam, III. Treated with sodium diclofenac

The blood clearance profile tests were performed in order to determine changes in pharmacokinetic profiles and biological half-life of ^{99m}Tc-MDP in mice which had been previously treated with meloxicam and sodium diclofenac. The results in Fig. 3 show that administration of meloxicam and sodium diclofenac affected the pharmacokinetic profile (%ID/g) of ^{99m}Tc-MDP in blood of normal/untreated (I), treated with meloxicam (II) and sodium diclofenac (III) mice

5 minutes post injection which were 3.71, 8.96 and 9.15 %, respectively, then decrease to 0.12, 0.01, and 0.01 %, respectively, 24 hours post injection.

Based on the calculation of %ID/g of blood, biological half-life (distribution and elimination) can be determined as shown in Table 1. The results of T-test showed there were no significant differences in distribution of ^{99m}Tc-MDP between normal/untreated mice (I) and treated mice either with meloxicam (II) or sodium diclofenac (III). However, there were significant differences in elimination of ^{99m}Tc-MDP between normal/ untreated mice (I) and treated mice either with meloxicam (II) or sodium diclofenac (III). These results showed that treatment with meloxicam and sodium diclofenac can accelerate cleareance of ^{99m}Tc-MDP in the blood.

A biodistribution study was performed 3 hours after intravenous injection of ^{99m}Tc-MDP to the animal model. In nuclear medicine, bone scintigraphy using ^{99m}Tc-MDP is usually performed 2-5 hours post injection for allowing a clearance of administrated radiopharmaceutical from the intravascular compartment and from extracellular nonosseous soft tissues [12]. Biodistribution study (Fig. 4 and Fig. 5) showed that the highest %ID/g organ was found in bone as the primary target of the ^{99m}Tc-MDP radiopharmaceutical.

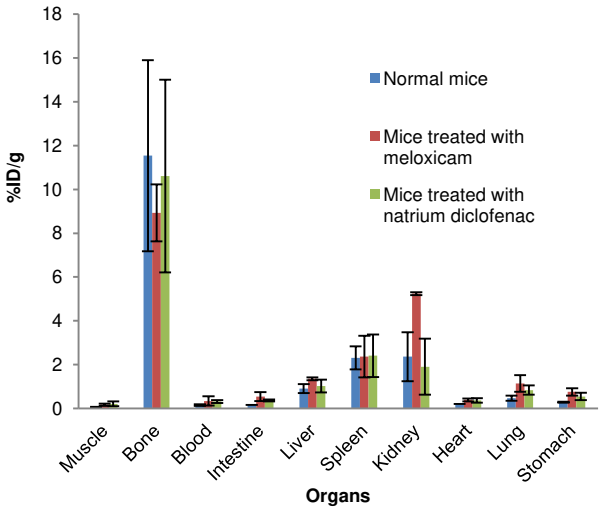

Fig. 4. Biodistribution pattern of ^{99m}Tc-MDP in mice treated by sodium diclofenac and meloxicam for 1 time (onset).

It can be seen from Fig. 4 that the %ID/g organ (bone) of ^{99m}Tc-MDP were 9.03 ± 0.41 %, 3.52 ± 0.52 %, and 3.62 ± 0.45 % in control/ untreated mice (I), mice treated with meloxicam for 3 days (II), mice treated with sodium diclofenac for 3 days (III) respectively. It can be seen from these results (shown in Fig. 6) that the bone uptake of ^{99m}Tc-MDP in mice previously treated with meloxicam and sodium diclofenac for 3 days exhibited significant differences with the control. It can be seen from Fig. 5 that the %ID/g organ (bone) of ^{99m}Tc-MDP were 8.44 ± 1.39 %, and 8.09 ± 0.86 % in mice treated with meloxicam once or at onset (IV), and mice treated with sodium diclofenac once or at onset (V) respectively. We can see in Fig. 6 that there were no significant differences in bone uptake of ^{99m}Tc-MDP between control mice and mice previously treated with meloxicam and sodium diclofenac once (at onset). These results are consistent with pharmacokinetics results that showed that giving meloxicam and sodium diclofenac for 3 days could accelerate the elimination half-life of ^{99m}Tc-MDP radiopharmaceutical.

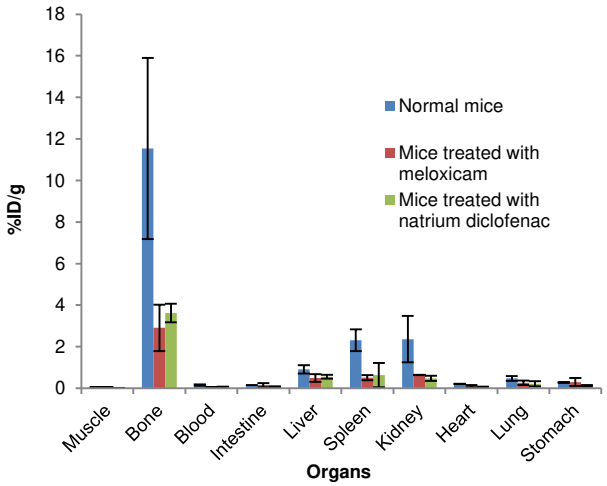

Fig. 5. Biodistribution pattern of ^{99m}Tc-MDP in mice treated by sodium diclofenac and meloxicam for 3 days.

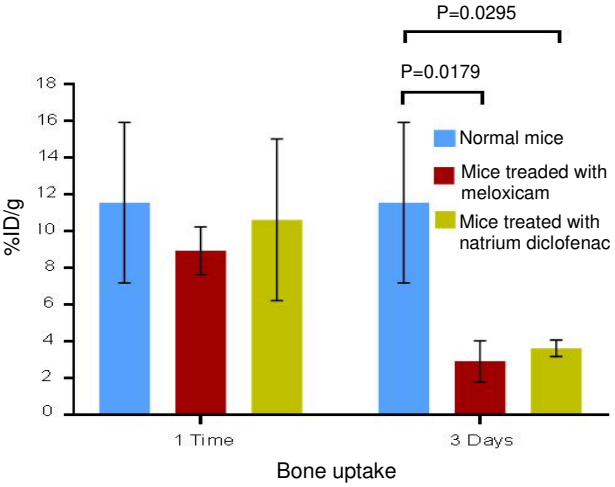

Fig. 6. Bone uptake of ^{99m}Tc-MDP for one-time and 3 days treatments.

The bone uptake of ^{99m}Tc-MDP correlates with both osteoblast differentiation and mineralization. Phosphate is a specific signal that modulates osteoblast differentiation by regulating

protein function and gene expression. Osteoblasts actively gather phosphate through transporter mechanisms, which is important for primary calcification of the bone matrix [13].

NSAID's mechanism inhibits the enzyme cyclooxygenase which catalyzes the conversion of arachidonic acid to prostaglandins (PGs). There are two cyclooxygenase isoforms: COX-1 and COX-2. COX-1 is expressed constitutively in most tissues for homeostatic functions, and COX-2 is induced by an array of stimuli, including inflammation, injury, and mechanical stress. The prostaglandins produced via COX-1 or COX-2 subsequently amplify or sustain the inflammatory response [14,15]. Specifically, PGs have been shown to elicit and participate in inflammatory responses, increase osteoclast activity and subsequent bone resorption, and increase osteoblast activity and new bone formation [16]. As for PG inhibitors, NSAID could decrease osteoblast activity. Decreasing osteoblast activity also effects decreasing ^{99m}Tc-MDP bone uptake.

CONCLUSION

Administration of NSAID such as meloxicam and sodium diclofenac could accelerate cleareance of ^{99m}Tc-MDP in the blood, causing low uptake of ^{99m}Tc-MDP radiopharmaceutical on the bone as the primary target.

ACKNOWLEDGMENT

The authors would like to thank BATAN, whose financial support made this research project possible. They would also like to thank Prof. Aang Hanafiah Wangsaatmadja, Mr. Iswahyudi, Mr. Ahmad Sidik, and Mr. Epy Isabela who helped in conducting this research.

REFERENCES

1. A.I. Brenner, J. Koshy, J. Morey *et al*., Semin. Nucl. Med. **42** (2012) 11.
2. K.K. Wong and M. Piert, J. Nucl. Med. **54** (2013) 590.
3. R.J. Sugiharti, Y. Sumpena and Misyetti, Indones. J. Nucl. Sci. Technol. **X** (2009) 89.
4. E. Bombardieri, L. Setti, M. Kirienko *et al*., Q. J. Nucl. Med. Mol. Imaging **59** (2015) 381.
5. J.J. Body, H.G. Bone, R.H. de Boer *et al*., Eur. J. Cancer **51** (2015) 1812.
6. A. Caraceni, E. Zecca, F. Formaglio *et al*., *Bone Metastases from Prostate Cancer: From Symptom Control to Pain Palliation*, in: Bone Metastases from Prostate Cancer Biology, Diagnosis, and Management, Springer, Switzerland (2017) 251.
7. J.L. Gómez Perales and A.A. Martínez, J. Nucl. Med. Technol. **41** (2013) 212.
8. S. Vallabhajosula, R.P. Killeen and J.R. Osborne, *Altered Biodistribution of Radiopharmaceuticals: Role of Radiochemical/ Pharmaceutical Purity, Physiological, and Pharmacologic Factors*, in: Semin. Nucl. Med., Elsevier Inc. (2010) 220.
9. V.M. Petriev, V.K. Tishchenko, O.V Siruk *et al.*, J. Pharm. Chem. **48** (2014) 357.
10. M.E.M. Dolman, I.J.M. Den Hartog, J.J. Molenaar *et al*., J. Pharm. Biomed. Anal. **88** (2014) 216.
11. H.M. Rashed, F.A. Marzook and Farag. H, Radiol. Med. **121** (2016) 935.
12. L.S. Zuckier and P. Martineau, Semin. Nucl. Med. **45** (2015) 81.
13. Z.A. Zhong, A. Peck, S. Li, *et al*., Bone Res. **3** (2015) 15013.
14. B. Pablos, S.A. Ramalho, B. Ko *et al.,* J. Periodontol **79** (2008) 300.
15. B. Su and J.P. O'Connor, J. Appl. Physiol. **115** (2013) 892.
16. A.T. Harder and Y.H. An, J. Clin. Pharmacol. **43** (2003) 807.