Original Article

The evaluation of omeprazole effect on the sensitivity of ^{99m}Tc-MDP bone-seeking to detect simulated closed fracture in the rat's foot

Alireza Doroudi[1], Mostafa Erfani[2], Seyyed Arash Moradpour[1], Seyyed Mostafa Saadati[3], Farmarz Ahmadi[3], Ali Kiasat[3], Mohammad Javad Khodayar[1], Behrooz Etesami[3]

[1]School of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[2]Radiation Application Research School, Nuclear Science and Technology Research Institute (NSTRI), Tehran, Iran
[3]Nuclear Medicine Department, Golestan General Hospital, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

(Received 4 September 2016, Revised 30 December 2016, Accepted 5 January 2017)

ABSTRACT

Introduction: Omeprazole (OP) is one the proton pump inhibitor (PPI) agents, and available over the counter in generic formulations. Epidemiological investigations have been confirmed a relationship between long term use of PPI agents and bone metabolism. Bone scintigraphy is commonly used for analysing the bone metabolism in clinical practice. This study was conducted to assess the effect of omeprazole on the sensitivity of bone imaging to distinguish simulated closed fracture in the rat's foot.

Methods: A total number of 32 adult, male NMRI were chosen. The animals were divided into two principle groups equally for four and eight weeks experiment. Each group was equally divided into four groups. These subgroups included the standard or control rats (4W S and 8W S) not receiving omeprazole and the others that received 25 (4W OP 25 and 8W OP 25), 50 (4W OP 50 and 8W OP 50), 100 (4W OP 100 and 8W OP 100) mg/kg of the drug. Reproducible closed fracture was created in the rat's foot after 4 and 8 weeks of experiments. Radioisotope studies have been undertaken.

Results: All closed fractures created in the feet of animals were visualized by images. The radiotracer uptake ratio at affected foot (target) versus contralateral healthy foot (non-target) was not influenced by omeprazole in the different animal groups. The quantitative investigation indicated that biodistribution of radiotracer was not affected by omeprazole in the other parts of the skeleton.

Conclusion: Radioisotope imaging by ^{99m}Tc-MDP is sensitive modality to identify the occult fracture and is not affected by omeprazole.

Key words: Bone imaging; Omeprazole; Proton pump inhibitor; ^{99m}Tc-MDP

Iran J Nucl Med 2017;25(2):92-99
Published: July, 2017
http://irjnm.tums.ac.ir

Corresponding author: Dr. Alireza Doroudi, School of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, 6135733184, Ahvaz, Iran. E-mail: doroudi-a@ajums.ac.ir

INTRODUCTION

Bone radioisotope imaging is commonly used in nuclear medicine practice [1-3]. It is a very efficient assay to assess the function of bone. Any significant injury to the bone can increase in bone metabolism that can be easily identified in the bone imaging. This technique is an efficient diagnostic modality to detect bone metastases in many types of tumors as well as fractures, joint or Paget's disease. Different radiopharmaceutical kits have been developed for bone imaging [4-9]. Methylene diphosphonate is one the bisphosphonate derivatives which potentially accumulates in bones. This ligand has the capability to label with ^{99m}Tc radioisotope (^{99m}Tc-MDP) that is widely used in nuclear medicine departments. Its popularity is mainly to the matter that the radioisotope can be readily produced by ^{99}Mo/^{99m}Tc generators. It has the ideal γ-ray energy (140keV) which is suitable for gamma camera detection. Therefore, ^{99m}Tc-MDP cold kit has been developed as a bone-seeking radiotracer to distinguish the bone lesions. The radiotracer uptake in bones is dependent to the blood flow and the osteogenesis activity at the lesion site [10-12]. Therefore, the sensitivity of radioisotope imaging can be influenced by the above mentioned factors. Drug interaction is one the most important factors to alter biodistribution of radiotracers [13]. These interactions between drugs and radiopharmaceutical agents may alter the result of radioisotope imaging [14-19]. This matter may lead to a misdiagnosis or repeat the radioisotope imaging procedure, causing unnecessary exposure to ionization radiation. Therefore, it is highly desirable to develop the reliable experimental tools to investigate these interactions for the intelligent interpretations of scintigraphy images in the clinical practice. Omeprazole (OP) is one the proton pump inhibitor agents (PPI) and is commonly used for the different gastrointestinal complications such as peptic ulcer, duodenal ulcer, gastro esophageal reflux disorder and with the combination antibiotic agents for eradication of Helicobacter Pylori [20-22]. Omeprazole is the prototypical PPI agents, available over the counter and in expensive generic formulations [23, 24]. The PPI agents like omeprazole can suppress gastric acid secretion by the parietal cells of the stomach due to inhibition H^+K^+ ATP_{ase} enzyme. This prevents acid production and raises the pH of the stomach content, which has been demonstrated to be an important component of therapy for the treatment of different gastro intestinal disorders. According to the literature, there is a relationship between prolong consumption of PPI and bone metabolism. Several studies indicated that PPI therapy may be accompanied with an increased risk for osteoporosis and related fractures of the hip, wrist and spine. The potential risk was enhanced in the patients who received high dose of PPI for long term therapy [25-28]. The main aim of this study is to assess the effect of different doses of omeprazole during 4 and 8 weeks drug therapy on the sensitivity of ^{99m}Tc-MDP bone-seeking radiotracer to detect closed fracture created in the rat's tibia.

METHODS

All chemical materials have been purchased from Merck. The chemicals and solvents were the highest purity and analytical grade and used without further purification. Omeprazole granules have been supplied by Daru Paksh Company. The freeze-dried MDP kits and ^{99}Mo/^{99m}Tc generators have been provided by Pars Isotope Company. Technetium 99m as sodium pertechnetate was obtained from an in-house ^{99}Mo/^{99m}Tc generator using 0.9 % saline. The rats with 135±15 g were obtained from research center and experimental animal house of Ahvaz Jundishapur University of Medical Sciences.

MDP labeling by ^{99m}Tc

Technetium 99m as sodium pertechnetate (Na $^{99m}TcO_4$) was obtained from an in-house ^{99}Mo/ ^{99m}Tc generator using 0.9% saline. Commercial MDP kits were used, the labeling and quality control procedures were undertaken on the basis of manufacturer's instructions.

Animal study

This study was approved by the ethics committee of Ahvaz Jundishapur University of Medical Sciences. All the ethics issues were considered based on the Ahvaz University of Medical Sciences Protocols (AUMP) on animal experiments. A total number of 32 adult, male NMRI were acclimated to the conditions for one week before the experiment. The animals were kept in individually wire-bottom stainless steel cages in an air-conditioned room at 24±1ºC with a 12 h light-dark cycle and were fed with standard pellet diet and had free access to water. The rats were randomly divided into two main groups equally. One group was allocated for four weeks and the other group for eight weeks experiment respectively. Each principle group was randomly assigned into four distinct sub groups equally. These subgroups included the standard or control rats (4W S and 8W S) not received omeprazole and the others taken 25 (4W OP 25 and 8W OP 25), 50 (4W OP 50 and 8W OP 50), 100 (4W OP 100 and 8W OP 100) mg/kg drug respectively. Distilled water was used in all experiments. Omeprazole granules were ground up with the aid of mortar and dispersed in a vehicle containing 0.25% hydroxyethyl cellulose 4400 in a 0.1 M solution of sodium bicarbonate (pH≈ 7.4). The suspensions were prepared at concentrations of 25, 50 and 100 mg/kg. The fresh omeprazole suspensions were prepared daily and administered

orally to animals. The control group received only the vehicle without medicine.

Simulated closed fracture in rat's foot

Simulated closed fracture was created in the rat's tibia approximately 1 cm from knee joint after 4 and 8 weeks of experiments. The subjects were anesthetized with diethyl ether and fixed on the board. Simulated closed fractures were created on the right leg of animals in order to avoid any misinterpretation. The small blunt rod stainless steel was placed and fixed on the tibia bone and the metal with weight 500 gr was fallen from the height of 20 cm to hit the fixed metal. The radioisotope imaging with ^{99m}Tc-MDP has been already performed in order to confirm this technique could produce the consistent closed fracture in the rats' feet. The outcome of imaging indicated that reproducible simulated closed fracture could be successfully created in the rat's tibia without displacement and minimal soft tissue trauma. The injured region was irrigated by normal saline. The rats with closed fracture were returned back to their cages. The experiment was continued for 5 more days. The rats were fed with standard pellet diet and had free access to water and were not on omeprazole or placebo solution during this period.

Radioisotope investigation

The rat with closed fracture was placed in the restrainer device and the 37 MBq (1 mCi) ^{99m}Tc-MDP was injected intravenously by contra lateral tail vein in all studies. The rats were returned back to their cages and kept individually. Radioisotope analysis was undertaken 1 h post injection. Therefore, the animal were anesthetised by diethyl ether and placed on the board in the supine position with limbs spread out and fixed on the board with surgical tape. A single-headed gamma camera (E-Cam, Siemens USA) employing a low-energy high resolution collimator was used in all investigations. Acquisition parameters were as follow: matrix size 256×256, Zoom factor ×3, anterior and posterior views for 5 min and energy window 140 Kev, reconstitution method: filter back projection. Anterior and posterior static images was acquired using a large field of view gamma camera peaked at 140 Kev with a 15 % window and a low energy all-purpose collimator for 500 kilo counts per image. The gamma camera was positioned to image the affected area and contralateral healthy side. Two criteria were considered for interpretation of scintigraphic radioisotope imaging. First, the visual inspection of radiotracer uptake at the affected foot to the contralateral healthy side was considered. Second, using available commercial software the activity ratio at the affected foot compared to the contralateral healthy side was quantified. For this reason the region of interest (ROI) was drawn on the affected foot as a target, and then second ROI was created on the contralateral healthy side as a non-target zone, anteriorly. The ratio of target to non-target was calculated by dividing count per pixel in affected foot to count per pixel in unaffected foot. The back ground subtraction was not used. All images were observed and interpreted by three independent nuclear physicians and their final opinion was achieved by consensus. This study was double-blinded and therefore, the observers were unaware about which animal groups participated in radioisotope imaging.

Quantitative assessment

The quantitative analysis has been performed after imaging analysis. The rats were sacrificed by diethyl ether. The organs of interest such as affected foot, contra lateral healthy side foot, kidneys, liver, stomach, spleen, intestine, bladder, heart, and lungs were removed and weighted. The relative activity of each organ to the organ of interest was calculated.

Statistical analysis

The calculation of means and standard deviations were made on Microsoft Excel. The data were shown as the mean±SD. Repeated measure design analysis of variance followed by Tukey test was used to investigate the difference between accumulations of radiotracer at the stressed foot and non-stressed foot. Statistical significance was considered at $p<0.05$.

RESULTS

The radiochemical analysis was performed by Instant Thin Layer Chromatography (ITLC) according manufacturer's instructions. The yields of ^{99m}Tc-MDP, $^{99m}TcO_4$ and $^{99m}TcO_2$ were 96.35± 1.16, 2.46± 0.28 and 1.19± 0.12 %, respectively demonstrating that the cold MDP kits were reconstituted by ^{99m}Tc radionuclide successfully. Omeprazole at different doses from 25 to 100 mg/kg were administrated to the rats for 4 or 8 weeks in this study. It has provided the condition to investigate not only the effect of omeprazole therapy but also the effect of concentrations of drug on the sensitivity of ^{99m}Tc-MDP radioisotope imaging to detect the simulated closed fracture in the rat's foot. Three criteria such as qualitative, semi-quantitative and quantitative analysis have been considered. First, the visual inspection of images was applied (Fig 1). The quality of images was suitable in each case and did not change over the time. Radiotracer accumulation at the affected foot was observed in all images. Therefore, the simulated closed fracture could be easily detected and identified in all images by ^{99m}Tc-MDP radioisotope imaging. The affected rat's foot could be tagged and localized by radiotracer. The target to non-target ratio was the second criterion in this approach.

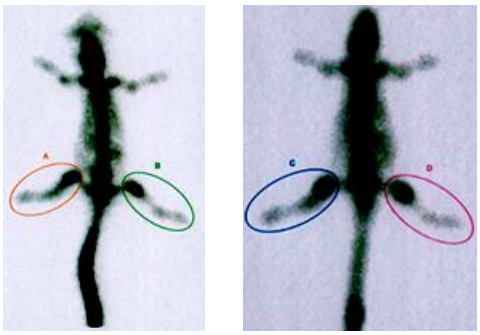

Fig 1. Bone scan has been performed 1h after injection of 37 MBq (1 mCi) ^{99m}Tc-MDP via contralateral tail vein. The anterior view images identified closed fracture lesion in the rats' feet have received omeprazole with 100 mg/kg: (left) 4 weeks and (right) 8 weeks treatment.

These ratios for different groups of rats were as follow: 4W S (n= 4): 1.07±0.04, 4W OP 25(n= 4): 1.1±0.08, 4W OP 50(n=4): 1.15±0.03, 4W OP 100 (n=4): 1.2±0.05, 8W S (n=4): 1.12±0.07, 8W OP 25 (n=4):1.4±0.08, 8W OP 50 (n=4): 1.17±0.02 and 8W OP100 (n=4): 1.21± 0.04, respectively. Therefore, the target to non-target ratios did not demonstrate significant differences in the drug treatment groups versus control or standard groups. There was no difference of the radiotracer uptake at the affected foot relative to contralateral healthy foot in comparison to standard group. It revealed that omeprazole could not influence the accumulation of ^{99m}Tc-MDP radiotracer at the simulated closed fracture in the rat's foot. For this reason all induced lesions could be visualized by imaging. The quantitative analysis has been performed in order to assess the effect of omeprazole on the biodistribution of ^{99m}Tc-MDP radiotracer on other organs of animals. As it is stated in Table 1 drug treatment could not lead to a significant difference in the radiotracer biodistribution. The highest activity was measured in affected foot followed by unaffected foot, intestine, kidneys and liver. Similar pattern of organ distribution of ^{99m}Tc-MDP radiotracer has been observed among the different groups of animals designed for this investigation. It was revealed that omeprazole in comparison of control group did not influence the accumulation ^{99m}Tc-MDP at the affected foot. All created closed fractures have been detected by bone scan imaging. In addition, no considerable change by the administration of omeprazole on biodistribution of ^{99m}Tc-MDP radiotracer have not been observed in any other animal organ.

DISCUSSION

The accuracy of a clinical diagnosis is the most challenging step for every clinical practice. The intelligent treatment is usually the result of a perfect diagnosis with high accuracy. Radioisotope imaging can be considered as a part of the diagnosis procedures. This imaging technique can be very helpful, because the entire body is assessed and providing information on physiologic changes of organs. During this process, a radiotracer agent is administrated to a patient and then the biodistribution of radiotracer is evaluated. The biodistribution of radiotracer includes absorption, distribution, metabolism and excretion. Radiotracer agents are usually used in tiny amounts in order to assess and discriminate disorders. These agents are effective as markers of some biochemical and molecular events. Any factors can influence the normal biodistribution of radiotracers in the body, may lead to misinterpretation of radioisotope images. Drug and radiopharmaceutical interactions can be considered as the most important factors. Therefore, the interpretation of radioisotope imaging can be easily influenced by such drug –radiopharmaceutical interactions. Drug-radiopharmaceutical interactions are divided into two main categories intended and unintended interactions.

The different pharmacological agents are usually used to increase the accuracy of diagnostic procedures. Dipyridamole or adenosine are widely used to stimulate stress blood flow in perfusion myocardial imaging [29, 30], or the use of angiotensin converting enzyme inhibitors in renal scintigraphy investigations [31]. The above mentioned interactions are considered as examples for intended drug-radiopharmaceutical interactions.

The unintended drug-radiopharmaceutical interactions are the result of drug therapy for concomitant disease during the radioisotope scintigraphy imaging. It is necessary to consider this matter that some drug agents may modify the effects of the radiopharmaceutical agents used for imaging and can indirectly influence on the results of the procedure. Captopril renography may be misleading in patients in whom a hypotensive effect is induced by calcium channel antagonist agents [32]. Caffeine or methyl xanthine derivatives such as aminophylline and theophylline may each inhibit dipyridamole or adenosine induced coronary vasodilatation and cause to false negative result in myocardial perfusion imaging procedure [33].

The information concerning drug-radiopharmaceutical has been usually obtained from case report studies in clinical practice or from prospective investigations performed in animal models. There is a lot of information available for drug-drug interactions in the texts and literature. Unfortunately, a little information can be found in the literature for drug-radiopharmaceutical interactions. It is highly desirable to launch a reliable investigations in order to provide the information about drug-radiopharmaceutical interactions for intelligent interpretation of radioisotope imaging.

Table 1: Relative uptake in the biodistribution study of ^{99m}Tc-MDP.

		Affected foot	Unaffected foot	Intestine	Kidney	Liver	Bladder	Spleen	Lungs	Stomach	Heart
4 weeks	Control	33.03±1.34	26.86±0.89	12.83±1.37	11.94±0.90	6.70±0.75	5.34±0.55	0.72±0.15	0.80±0.12	1.50±0.50	0.28±0.02
	OP* 25	35.90±1.06	30.38±1.16	12.01±1.54	09.17±0.17	5.01±0.53	2.30±0.23	1.30±0.54	1.45±0.56	1.22±0.23	0.31±0.03
	OP 50	34.52±2.13	29.42±1.23	13.50±0.70	10.62±1.67	5.86±0.26	2.02±0.20	1.10±0.22	1.67±0.20	1.08±0.20	0.21±0.50
	OP 100	35.98±1.47	29.51±1.19	12.30±1.94	10.30±0.43	5.80±0.22	2.20±0.08	1.29±0.14	1.30±0.18	1.10±0.15	0.22±0.05
8 weeks	Control	33.79±1.34	27.22±0.48	12.55±0.10	11.44±1.02	6.44±0.68	5.61±0.53	0.65±0.01	0.90±0.15	1.17±0.15	0.23±0.03
	OP 25	32.84±1.20	28.11±1.07	11.83±1.03	11.10±1.05	6.51±1.20	4.17±1.50	1.48±0.36	1.61±0.75	1.37±0.25	0.98±0.38
	OP 50	34.69±2.87	25.11±2.15	13.79±0.82	11.12±1.90	6.18±0.98	4.24±2.20	0.82±0.51	2.03±0.44	1.07±0.10	0.68±0.40
	OP 100	36.06±1.10	27.78±1.95	10.88±1.25	10.70±1.02	8.03±1.18	2.20±0.29	1.20±0.15	1.7±0.20	0.9±0.20	0.55±0.03

OP 25, OP 50, OP 100: Different omeprazole doses from 25 to 100 mg/kg were orally administrated to the rats for 4 or 8 weeks.

Bone radioisotope imaging with ^{99m}Tc-MDP radiopharmaceutical is commonly used in nuclear medicine in order to identify a variety bone lesions such as metastatic foci, occult or stress fractures, infection, Paget disease or other miscellaneous conditions. The exact mechanism of accumulation of ^{99m}Tc-MDP radiotracer on bone has not been elucidated completely .It has been suggested for the presence of phosphate group in the ligand structure, phosphonates derivatives (like MDP) have a very high affinity for bone mineral because they bind to surface of hydroxyapatite crystals . Therefore, ^{99m}Tc radionuclide can be readily transferred to bone when the labeled ^{99m}Tc-MDP radiocomplex is formed and administrated to patient .The radiotracer uptake depends on the blood flow and the rate of osteogenesis activity. Osteogenesis activity is increased after bone injury and during repair period. Any modalities can influence the above mentioned factors, may have effect on the sensitivity of bone radioisotope imaging. Awareness of these factors may clarify some unexpected bone scanning findings. Mc Devitt et al. reported that extra skeletal ^{99m}Tc-MDP uptake has observed in the abdominal region of a patient while undergoing continuous ambulatory peritoneal dialysis who had no symptoms or finding referable to the abdomen. They suggested that radiotracer crossed the peritoneal membrane across a concentration gradient. An in-vitro simulation model confirmed that ^{99m}Tc-MDP could cross a semi-permeable membrane [34]. Kuno et al. reported that a 29 year old with several years of back pain was referred for bone radioisotope imaging. The images of head demonstrated increased ^{99m}Tc-MDP radiotracer uptake in several areas of the skull. The patient had received acupuncture treatment for his back pain several times in the same areas as the enhanced ^{99m}Tc-MDP uptake. They concluded that acupuncture could cause increased bone metabolism and increased uptake observed on the images [35]. According to the literature, different number of case reports could be found that etidronate is principally used for treatment of post-menopausal osteoporosis and Paget disease. This drug demonstrated to influence the normal biodistribution of ^{99m}Tc-MDP radiotracer in bone scan imaging [36]. Park et al. reported that two patients with hypochromic microcystic anemia received intravenous iron (Blutal) therapy. Blutal is an iron colloid chondroitin sulphate complex and is used to treat iron deficiency anemia in Korea. These patients demonstrated diffuse hepatic ^{99m}Tc-MDP uptake radiotracer on bone imaging. Scanning has undertaken for evaluation of lower back pain in order to rule out skeletal metastasis from gastric cancer. They concluded that ^{99m}Tc radionuclide could bind to colloid chondroitin sulphate due to transmetal chelating process. Then the labeled colloid was phagocytised into the reticuloendothelial system and visualized the patient's liver. The consumption of glucocorticoid agents at the pharmacologic doses for a long time period, may induced osteoporosis with loss of bone density and potential risk for fracture. The ^{99m}Tc-MDP uptake could be reduced by skeleton for the presence of sever osteoporosis induced by glucocorticoids.

A case report documented failure of ^{99m}Tc-MDP radioisotope imaging to identify an occult interochanteric hip fracture in a young woman on long term steroid therapy [37].

Scott et al. investigated the effect of hydrocortisone on the sensitivity of ^{99m}Tc-MDP bone imaging to detect the surgically created simulate bone fracture in rabbits receiving different doses of glucocorticoid agent.

They reported that the high dose of hydrocortisone could influence on the sensitivity of bone scan [38]. Several documents have been reported that PPI agents are accompanied with an increased risk for osteoporosis related fractures of the wrist; hip and spine .The potential risk of fracture was enhanced in patients who received high doses for long term PPI therapy. Omeprazole is the prototype of PPI agents and widely used in clinical practice. However, the relationship between PPI agents and bone demineralization associated with prolong use of omeprazole is unknown. Two main assumptions have been recommended for induced osteoporosis for long term PPI agents. First, the effect on calcium absorption has been received much attention. Omeprazole can effectively raise pH of stomach content by inhibition H^+K^+ ATP $_{ase}$ enzyme. The release of ionized calcium from insoluble calcium salts depends to the acidic condition in the stomach. Therefore, the calcium solubilizing is not occurred in basic pH and calcium absorption could be interrupted [39-41]. Second assumption is that prolong use of PPI agents lead to reduced absorption calcium from upper small intestine resulting in negative calcium balance, and cause the development of secondary hyperparathyroidism. For this reason, the potential risk of osteoporosis and bone loss could be increased [42, 43].

The data obtained from our investigation indicated that omeprazole does not any effect on the biodistribution of the ^{99m}Tc-MDP radiotracer in the rats. Therefore, all simulated closed fracture in animals detected and visualized by radioisotope images. ^{99m}Tc-MDP bone-seeking radiopharmaceutical adsorbs on the hydroxyapatite crystal surfaces during bone formation. The radiotracer uptake depends on the blood flow in bone and the rate of osteoblast cells activity. Regional blood flow and osteogenesis are increased during repair period and are responsible for increased radiotracer uptake at the site of injury site. In addition to the above mentioned factors, the ligand dose in the cold freeze-dried kit versus to the pharmacologic dose is too small. The tiny dose of ligand is intentionally used to transfer ^{99m}Tc radioisotope at the desired location in order to detect and tag the bone lesions. Therefore, omeprazole at different doses could not show significant effect not only on the radiotracer uptake at the induced lesion in bone but also on the biodistribution of bone-seeking in the other part of rats in this study. The outcome of this study demonstrated that radiotracer reaches at the site of injury in bone due to blood flow and when minimum functional mineral bone density is available at the lesion foci. Therefore, the bone-seeking radiotracer can effectively bond to the hydroxyapatite crystal surfaces and simulated closed fracture can be readily detected.

CONCLUSION

The result of this assessment demonstrated that bone imaging with ^{99m}Tc-MDP radiopharmaceutical is highly sensitive to discriminate the occult fracture. Omeprazole has not shown to affect the sensitivity of bone scan to identify simulated closed fracture in the rat's tibia. However, in clinical practice should be taken into consideration for the intelligent interpretation of radioisotope imaging. Radioisotope imaging technique using radiotracer agents like ^{99m}Tc-MDP bone-seeking is highly sensitive to subtle changes in bone metabolism and physiological processes. Therefore, it is necessary the nuclear medicine specialists to consider all of the variables including drug-radiopharmaceutical interactions that might potentially affect distribution of the bone agents.

Acknowledgement

This study is part of Pharm-D thesis of Arash Moradpour. The authors have no relevant financial interests related to the material in this manuscript. They also have no conflict of interests to declare. This work has been carried out with financial support from Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

REFERENCES

1. Love C, Din AS, Tomas MB, Kalapparambath TP, Palestro CJ.. Radionuclide bone imaging: an illustrative review. Radiographics. 2003 Mar-Apr;23(2):341-58.
2. Zuckier LS, Freeman LM.. Nonosseous, nonurologic uptake on bone scintigraphy: atlas and analysis. Semin Nucl Med. 2010 Jul;40(4):242-56.
3. Wong KK, Piert M. Dynamic bone imaging with 99mTc-labeled diphosphonates and 18F-NaF: mechanisms and applications. J Nucl Med. 2013 Apr;54(4):590-9.
4. Marì C, Catafau A, Carriò I.. Bone scintigraphy and metabolic disorders. Q J Nucl Med. 1999 Sep;43(3):259-67.
5. Even-Sapir E, Martin RH, Barnes DC, Pringle CR, Iles SE, Mitchell MJ. Role of SPECT in differentiating malignant from benign lesions in the lower thoracic and lumbar vertebrae. Radiology. 1993 Apr;187(1):193-8.
6. Okamoto YM. Mechanism of accumulation of 99mTc-MDP to bone: correlation of in vivo data with in vitro data. Radiat Med. 1997 Jul-Aug;15(4):209-15.

7. Savelli G, Maffioli L, Maccauro M, De Deckere E, Bombardieri E. Bone scintigraphy and the added value of SPECT (single photon emission tomography) in detecting skeletal lesions. Q J Nucl Med. 2001 Mar;45(1):27-37.

8. Wellman HN, Schauwecker D, Robb JA, Khairi MR, Johnston CC. Skeletal scintimaging and radiography in the diagnosis and management of Paget's disease. Clin Orthop Relat Res. 1977;(127):55-62.

9. Pennington WT, Mott MP, Thometz JG, Sty JR, Metz D. Photopenic bone scan osteomyelitis: a clinical perspective. J Pediatr Orthop. 1999 Nov-Dec;19(6):695-8.

10. Genant HK, Bautovich GJ, Singh M, Lathrop KA, Harper PV. Bone-seeking radionuclides: an in vivo study of factors affecting skeletal uptake. Radiology. 1974 Nov;113(2):373-82.

11. Galasko CS. The pathological basis for skeletal scintigraphy. J Bone Joint Surg Br. 1975 Aug;57(3):353-9.

12. Kanishi D. 99mTc-MDP accumulation mechanisms in bone. Oral Surg Oral Med Oral Pathol. 1993 Feb;75(2):239-46.

13. Bernardo-Filho M, Santos-Filho SD, de Moura EG, Maiworm AI, de Camões Orlando MM, Penas ME, Cardoso VN, Bernardo LC, de Carvalho Brito L. Drug interaction with radiopharmaceuticals: a review. Braz Arch Biol Technol. 2005;48:13-27.

14. Yu MD, Shaw SM. Potential interference of agents on radioiodide thyroid uptake in the euthyroid rat. J Nucl Med. 2003 May;44(5):832-8.

15. Solanki KK, Bomanji J, Moyes J, Mather SJ, Trainer PJ, Britton KE. A pharmacological guide to medicines which interfere with the biodistribution of radiolabelled meta-iodobenzylguanidine (MIBG). Nucl Med Commun. 1992 Jul;13(7):513-21.

16. Schneider JA, Divgi CR, Scott AM, Macapinlac HA, Seidman AD, Goldsmith SJ, Larson SM. Flare on bone scintigraphy following Taxol chemotherapy for metastatic breast cancer. J Nucl Med. 1994 Nov;35(11):1748-52.

17. Hesslewood S, Leung E. Drug interactions with radiopharmaceuticals. Eur J Nucl Med. 1994 Apr;21(4):348-56.

18. Abu-Judeh HH, Naddaf SY, el-Zeftawy HA, Abdel-Dayem HM. G-CSF induced bone marrow hyperplasia: characteristic appearance on total body blood pool and delayed Tc-99m MDP bone scan. Clin Nucl Med. 1998 Jan;23(1):39-41.

19. Peylan-Ramu N, Haddy TB, Jones E, Horvath K, Adde MA, Magrath IT. High frequency of benign mediastinal uptake of gallium-67 after completion of chemotherapy in children with high-grade non-Hodgkin's lymphoma. J Clin Oncol. 1989 Dec;7(12):1800-6.

20. Kromer W, Horbach S, Lühmann R. Relative efficacies of gastric proton pump inhibitors: their clinical and pharmacological basis. Pharmacology. 1999 Aug;59(2):57-77.

21. Dean BB, Gano AD Jr, Knight K, Ofman JJ, Fass R. Effectiveness of proton pump inhibitors in nonerosive reflux disease. Clin Gastroenterol Hepatol. 2004 Aug;2(8):656-64.

22. Moayyedi P, Talley NJ. Gastro-oesophageal reflux disease. Lancet. 2006 Jun 24;367(9528):2086-100.

23. Karam SM, Forte JG. Inhibiting gastric H(+)-K(+)-ATPase activity by omeprazole promotes degeneration and production of parietal cells. Am J Physiol. 1994 Apr;266(4 Pt 1):G745-58.

24. Fendrick AM, Shaw M, Schachtel B, Allgood L, Allgood G, Grender J, Peura D. Self-selection and use patterns of over-the-counter omeprazole for frequent heartburn. Clin Gastroenterol Hepatol. 2004 Jan;2(1):17-21.

25. Yang YX, Lewis JD, Epstein S, Metz DC. Long-term proton pump inhibitor therapy and risk of hip fracture. JAMA. 2006 Dec 27;296(24):2947-53.

26. Kirkpantur A, Altun B, Arici M, Turgan C. Proton pump inhibitor omeprazole use is associated with low bone mineral density in maintenance haemodialysis patients. Int J Clin Pract. 2009 Feb;63(2):261-8.

27. Gray SL, LaCroix AZ, Larson J, Robbins J, Cauley JA, Manson JE, Chen Z. Proton pump inhibitor use, hip fracture, and change in bone mineral density in postmenopausal women: results from the Women's Health Initiative. Arch Intern Med. 2010 May 10;170(9):765-71.

28. Yang YX. Chronic proton pump inihibitor therapy and calcium metabolism. Curr Gastroenterol Rep. 2012 Dec;14(6):473-9.

29. Merhige ME, Breen WJ, Shelton V, Houston T, D'Arcy BJ, Perna AF. Impact of myocardial perfusion imaging with PET and (82)Rb on downstream invasive procedure utilization, costs, and outcomes in coronary disease management. J Nucl Med. 2007 Jul;48(7):1069-76.

30. Doroudi A, Erfani M, Norouzi B, Saadati SM, Kiasat A, Ahmadi F, Etesami B, Baghersad MH. Clinical application of ultrasound for preparation of (99m)Tc-sestamibi complex. Ann Nucl Med. 2015 Apr;29(3):295-301.

31. Davidson RA, Wilcox CS. Newer tests for the diagnosis of renovascular disease. JAMA. 1992 Dec 16;268(23):3353-8.

32. Ludwig V, Martin WH, Delbeke D. Calcium channel blockers: a potential cause of false-positive captopril renography. Clin Nucl Med. 2003 Feb;28(2):108-12.

33. Böttcher M, Czernin J, Sun KT, Phelps ME, Schelbert HR. Effect of caffeine on myocardial blood flow at rest and during pharmacological vasodilation. J Nucl Med. 1995 Nov;36(11):2016-21.

34. McDevitt GR Jr, Heironimus JD, Toney MO, Billingsley JL. Diffuse abdominal uptake of technetium-99m-methylene diphosphonate in a patient on continuous ambulatory dialysis during bone scintigraphy. J Nucl Med. 1992 Nov;33(11):2052-4.

35. Kuno RC, Cerqueira MD. Enhanced bone metabolism induced by acupuncture. J Nucl Med. 1995 Dec;36(12):2246-7.

36. Park CH, Kim HS, Shin HY, Kim HC. Hepatic uptake of Tc-99m MDP on bone scintigraphy from intravenous iron therapy (Blutal). Clin Nucl Med. 1997 Nov;22(11):762-4.

37. Scott S, Alazraki N, Manaster B. Failure of bone scanning to detect fractures in a woman on chronic steroid therapy. Skeletal Radiol. 1984;12(3):204-7.

38. Scott SM, Manaster BJ, Alazraki N, Wooten WW, Murphy K. Technetium-99m imaging of bone trauma: reduced sensitivity caused by hydrocortisone in rabbits. AJR Am J Roentgenol. 1987 Jun;148(6):1175-8.

39. Wright MJ, Proctor DD, Insogna KL, Kerstetter JE. Proton pump-inhibiting drugs, calcium homeostasis, and bone health. Nutr Rev. 2008 Feb;66(2):103-8.

40. Fournier MR, Targownik LE, Leslie WD. Proton pump inhibitors, osteoporosis, and osteoporosis-related fractures. Maturitas. 2009 Sep 20;64(1):9-13.

41. Yanagihara GR, de Paiva AG, Neto MP, Torres LH, Shimano AC, Louzada MJ, Annoni R, de Oliveira Penoni ÁC. Effects of long-term administration of omeprazole on bone mineral density and the mechanical properties of the bone. Rev Bras Ortop. 2015 Mar 14;50(2):232-8.

42. Mizunashi K, Furukawa Y, Katano K, Abe K. Effect of omeprazole, an inhibitor of H+,K(+)-ATPase, on bone resorption in humans. Calcif Tissue Int. 1993 Jul;53(1):21-5.

43. Yang YX. Proton pump inhibitor therapy and osteoporosis. Curr Drug Saf. 2008 Sep;3(3):204-9.