

## The effect of prednisolone on the sensitivity of Technetium 99m-methylene diphosphonate ( $^{99m}\text{Tc-MDP}$ ) scintigraphy to detect simulated closed fracture in the rat tibia

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(Received 8 September 2012, Revised 8 October 2012, Accepted 10 October 2012)

### ABSTRACT

**Introduction:** The purpose of the present study was to evaluate the effect of prednisolone on the sensitivity of bone scanning to detect the simulated closed fracture in the rat tibia.

**Methods:** A total number of forty eight adult, male NMRI rats randomly assigned into two parts, one part for 4 and other for 8 weeks experiments. Each part has been divided into four groups, one group not receiving prednisolone (control group) and the other groups receiving 5, 10 and 20 mg/kg prednisolone respectively. After four and eight weeks of experiments a simulated closed fracture was created in the tibia approximately 1 cm near knee joint. The scintigraphy imaging has been performed on second and fifth day after tibial bone fracture and the ratio of the activity of tibia bone fracture to the contra lateral healthy side was calculated (R factor).

**Results:** All scintigraphy images showed the rat tibia bone fracture. Statistically significant difference in the R factors have been observed, when scintigraphy images were performed in fifth day after injury.

**Conclusion:** Bone radionuclide scanning by  $^{99m}\text{Tc-MDP}$  is very sensitive for detection of occult fracture. In order to enhance bone scan sensitivity, it may be reasonable to postpone  $^{99m}\text{Tc-MDP}$  scintigraphy imaging at least 2 to 3 days after suspected trauma in patients receiving glucocorticoid medication.

**Key words:** Bone Scintigraphy, Fracture, Prednisolone,  $^{99m}\text{Tc-MDP}$

Iran J Nucl Med 2012;20(1):32-38

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## INTRODUCTION

The structure of methylene diphosphonate (MDP) is similar to the phosphonates and preferentially accumulates in bones and with its capability to label by technetium-99m,  $^{99m}\text{Tc}$ -Methylene diphosphonate ( $^{99m}\text{Tc}$ -MDP) has been developed as a radiopharmaceutical product for scintigraphic imaging in order to visualize bone lesions. Bone scintigraphy imaging is widely used for diagnosing bone lesions like occult or stress fractures, infection, metastatic disease and miscellaneous condition such as Paget disease, etc [1, 2]. The uptake of  $^{99m}\text{Tc}$ -MDP in bones depends to the blood flow and the rate of osteogenesis [3, 4]. Any factor influencing these two processes may affect the sensitivity of bone radioisotope scanning. Drug interaction is one of the most important factors that can influence the distribution of radiopharmaceuticals in the body [5, 6, 7]. This may alter the result of scintigraphy imaging in nuclear medicine in a patient under treatment due to a concomitant disease. This matter may lead to a misdiagnosis or repeat of the procedure causing unnecessary exposure to ionizing radiation. Thus the developments of real models to assess these interactions are highly useful and desirable.

Glucocorticoids are common prescribed drugs in clinical practice for treatment of several conditions [8]. Administration of glucocorticoids especially for long term induces bone loss [9, 10]. A case report documented failure of  $^{99m}\text{Tc}$ -MDP bone scintigraphy to detect an occult interchanteric hip fracture in a young woman on long term steroid therapy [11].

To examine this effect of glucocorticoids on the sensitivity of  $^{99m}\text{Tc}$ -MDP scintigraphy imaging one study has been performed to investigate the effect of hydrocortisone in detection of surgically created simulate bone fractures in rabbits receiving different doses of hydrocortisone [12]. The result from this study indicated the high dose of hydrocortisone could influence the sensitivity of the procedure.

Glucocorticoid agents can induce bone loss in rat [13, 14, 15, and 16]. Therefore this suitable model has been suggested to investigate the side effects of glucocorticoids on bony structures [17, 18, 19, and 20].

The purpose of this study was to evaluate the effect of different doses of prednisolone during 4 and 8 weeks following treatment on the  $^{99m}\text{Tc}$ -MDP scintigraphy of closed created simulate fracture in the rat tibia.

## METHODS

All chemical materials have been purchased from Merck or Fluka. The chemicals and solvents were of

the highest purity and analytical grade and used without further purification. The freeze-dried kit of MDP and  $^{99}\text{Mo}/^{99m}\text{Tc}$  generator have been provided by Radioisotope Division of Atomic Energy Organization of Iran. The pure prednisolone has been supplied by Iran Hormone Company. The rats with average weight of

$150 \pm 10$  g were obtained from research center and experimental animal house of Ahvaz Jundishapur University of Medical Sciences.

This study was approved by the ethics committee of Ahvaz Jundishapur University of Medical Sciences. A total number of forty eight adult, male NMRI rats were acclimated to conditions for one week before the experiment. These rats were kept in individually wire-bottom stainless steel cages in an air-conditioned room at  $24 \pm 1$  °C with a 12-h light-dark cycle and were fed a regular diet.

They were randomly assigned into two experiments, one for 4 and other for 8 weeks experiments and each one included into four groups, one group not receiving prednisolone (standard or control group) and the other groups receiving 5, 10 and 20 mg/kg prednisolone respectively. Body weight was measured once a week. The powder of prednisolone was dissolved with minimum amount of tween 80 in distilled water. The freshly prednisolone solutions were prepared daily and administered to rats orally.

After four and eight weeks of experiments a simulated closed fracture was created in the tibia approximately 1 cm near knee joint. The rat was anesthetized with diethyl ether and fixed on the board. The left leg of rats were chosen to avoid misinterpretation. The small blunt rod stainless steel were put and fixed on the tibia bone near about 1 cm to the knee joint and the metal with weight of 500 gr was dropped from the height of 20 cm to hit the stainless steel metal.

The preliminary tests by  $^{99m}\text{Tc}$ -MDP scintigraphy images have already been performed to confirm this method can create reproducible closed fracture in the rat tibia without displacement and minimal soft tissue trauma. The injured area was irrigated by normal saline and then the rats with closed fracture were returned to their cages and experiment was considered for another five days.

### *Labeling of MDP by $^{99m}\text{Tc}$*

Technetium-99m as sodium pertechnetate ( $\text{Na}^{99m}\text{TcO}_4$ ) was obtained from an in-house  $^{99}\text{Mo}/^{99m}\text{Tc}$  generator using 0.9% saline. A commercial MDP kit (AEOL, Tehran, Iran) was used and the labeling and quality control procedures were performed according to the manufacturer's instructions.

### Imaging protocol

The rat was placed in the restrainer apparatus and the (37 MBq)  $^{99m}\text{Tc}$ -MDP was administered intravenously by contra lateral tail vein. Two hours after injection of

$^{99m}\text{Tc}$ -MDP, the rat was anesthetized with diethyl ether and fixed on the board for scintigraphy imaging. For all studies a single-headed camera (PICKER PRISM 1000) employing a low energy high resolution collimator was used. The spot view of interested region with collection of 500 kilo count was acquired and then the same time was used for acquisition of the contra lateral healthy side view. For all rats the ratio of the activity of tibia bone fracture to the contra lateral healthy side were obtained (R factor). In order to measure the R factor, the site of scintigraphic rat tibia fracture and contra lateral healthy side were identified. Available software was used to quantify the counts within the fracture and healthy tibia bones. By dividing the counts in the tibia fracture to contra lateral healthy side, the R factor was calculated. All the scintigraphy images were interpreted by two nuclear medicine physicians independently and their final opinion was achieved by consensus.

### Statistical analysis

Repeated measures analysis of variance was used to test the effect of day of fracture on the ratio of measured radioactivity in fractured tibia bone to the healthy contra lateral side (R factor). To check the influence of duration of treatment with prednisolone and its dose on both R factor and weight gain in the treated rats two-factor analysis of variance test was used. Statistical significance was considered at p-value of less than 0.05.

## RESULTS

Fig. 1 shows the weight gain in the rats treated with different doses of prednisolone for 4 and 8 weeks. Duration of treatment with prednisolone showed a statistically significant effect on the amount of weight gain ( $p < 0.0001$ ). Increasing the dose of prednisolone led to a decreased weight gain for both periods of treatment. However, it seems that the effect of dose is more considerable after eight weeks of treatment with prednisolone. Amount of decrease in weight gain for all doses of prednisolone was significantly greater after 4 weeks as compared to those after eight weeks ( $p < 0.0001$ ).

When scintigraphy imaging has performed 5 days after the closed simulate fracture in the rat tibia in comparison to those obtained in second day, significant difference in R factor was observed ( $p < 0.0001$ ) (Fig 2).

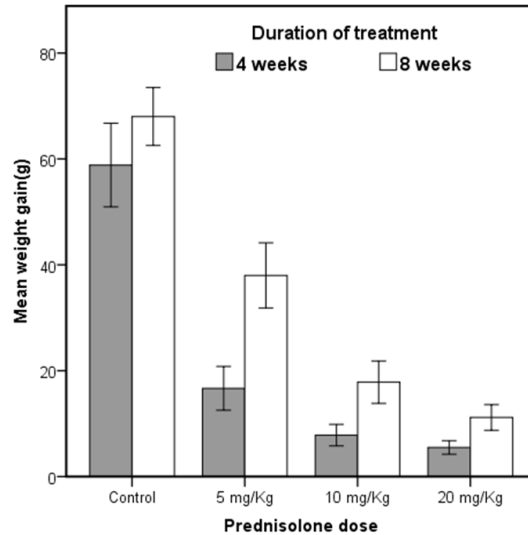


Fig 1. Effect of prednisolone dose and duration of treatment on weight gain in rats. Vertical bars denote 95% confidence interval around mean values (N=6 in each group of animals). Duration of treatment showed a significant effect on mean weight gain ( $p < 0.0001$ ). Effect of prednisolone dose is significant ( $p < 0.0001$ ) except for the difference of mean weight gain between 10 and 20 mg/Kg of prednisolone dose.

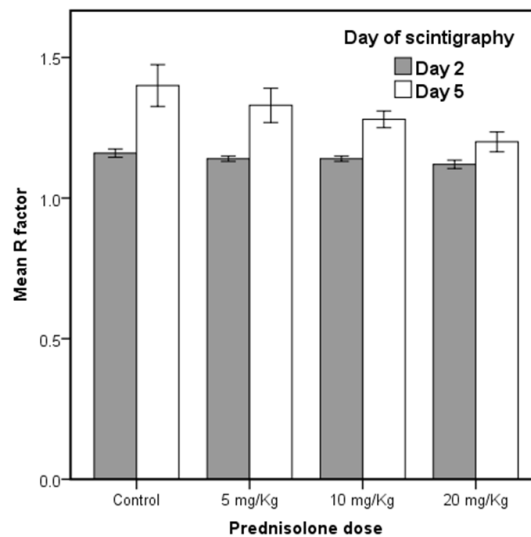
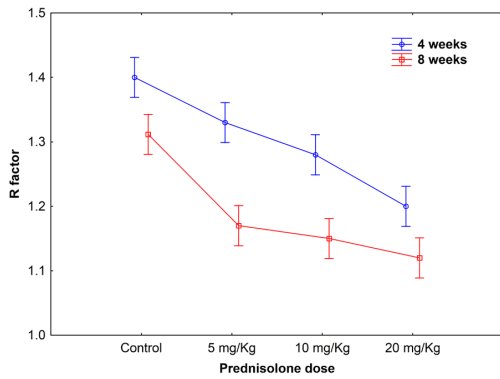


Fig 2. Effect of prednisolone dose and day of scintigraphy on the ratio of measured activity in fractured tibia bone to contra lateral healthy one (R factor). Vertical bars denote 95% confidence interval around mean values (N=6 in each group of animals). Day of scintigraphy showed significant effect on mean R factor ( $p < 0.0001$ ). Effect of prednisolone dose is significant ( $p < 0.024$ ) except for the difference of mean R factor between 5 and 10 mg/Kg of prednisolone dose.

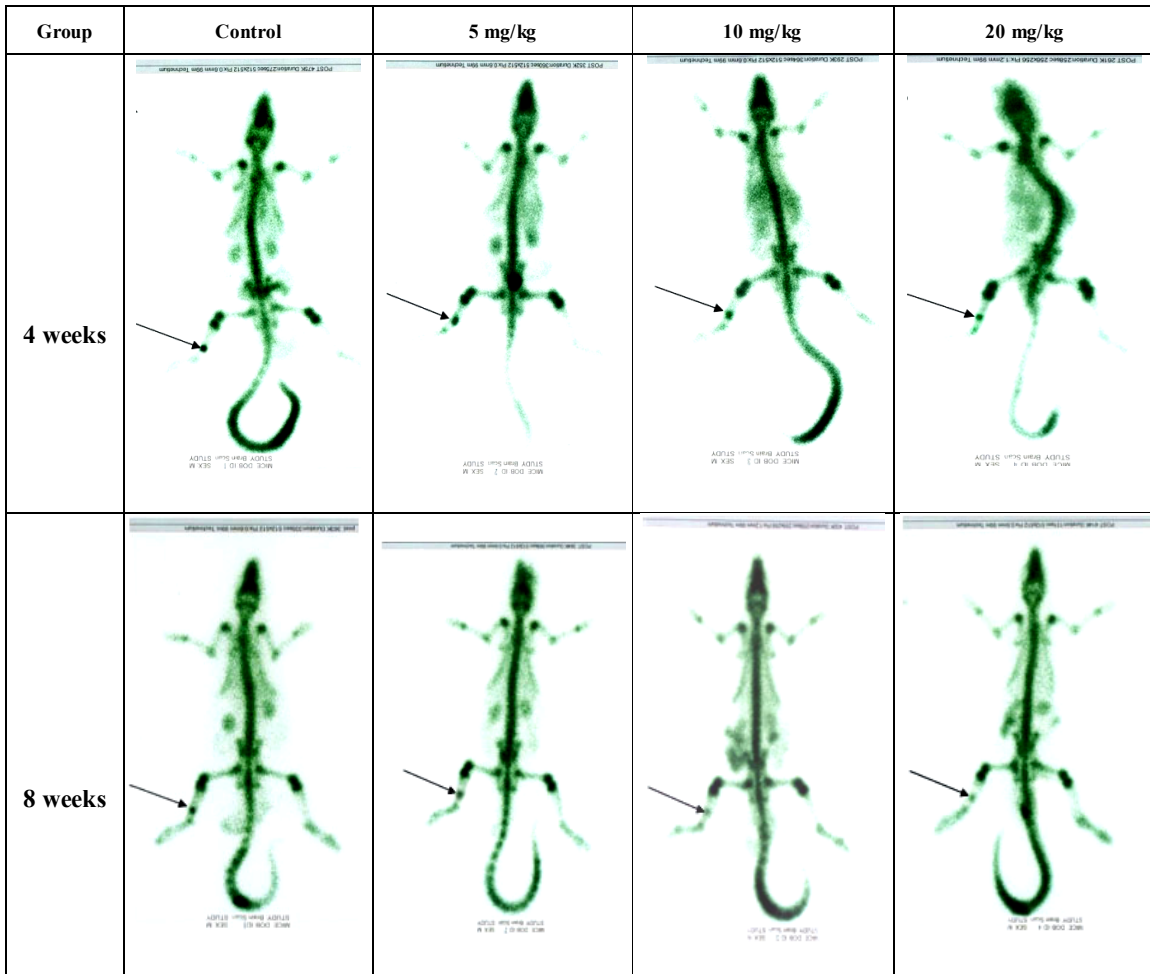


Both duration of treatment and its dose had statistically significant effect on the R factor. The values of measured R factor were statistically lower following 8 weeks of treatment at all levels of prednisolone doses ( $p < 0.0001$ ). The effect of dose seems to be greater after 8 weeks of treatment with this drug (Fig 3).

The scintigraphy images have been performed at 5 days after tibia bone fracture showing the distribution of bone -seeking radiopharmaceutical in the interested area to contra lateral healthy side (Fig 4).

**Fig 3.** Effect of prednisolone dose and duration of treatment on the ratio of measured activity in fractured tibia bone to contra lateral healthy one (R factor). Vertical bars denote 95% confidence interval around mean values (N=6 in each group of animals). Duration of treatment had a significant effect with  $p < 0.0001$ . Difference of mean R factor between different doses of prednisolone is significant ( $p < 0.024$ ) except for 5 and 10 mg/Kg of prednisolone doses.

**Fig 4.** The Scintigraphy images in different groups of rats have been obtained after  $(37\text{MBq})^{99\text{m}}\text{Tc-MDP}$  administered intravenously by contra lateral tail vein, two hours after injection at 5th day after injury and shown the tibia fracture.



These scanning have been performed at 2 and 5 days after injury and determined the R factor. According to Fig 2, prednisolone influenced the R factor in comparison to control but it was not significant at 2 days after injury. Although all images revealed the lesion but the images at 2 days after injury were less clear. During this study the images of treated rats with prednisolone obtained at 2 days after injury were not significantly different from control group. The scintigraphy images performed in fifth day after injury, they were more distinct and clear. The R factors have shown statistically significant difference not only between control and prednisolone treatment groups, but also among drug treatment groups. At this time period, primary bone formation is the dominating process and causes to enhance the uptake of bone -seeking radiopharmaceutical at the site of injury.

### DISCUSSION

Glucocorticoids are commonly used in clinical practice to control or treat several inflammatory diseases. One of the most serious and important side effects of glucocorticoid agents especially for long-term prescription is bone loss. This side effect can be induced by glucocorticoids due to different mechanisms such as the direct inhibition of osteoblast [22,23,24,25], termination of differentiation of osteoblast precursors into mature osteoblast [26], inhibition of the synthesis and the activity of insulin-like growth factor 1 a main stimulator of osteogenesis [27], a reduction in the amount of calcium absorbed from the intestine [28,29], increased secretion of parathyroid hormone [30,31] and a decreased in renal tubular calcium reabsorption with a consequent increase in urinary calcium excretion [32]. Glucocorticoids particularly for long-term administration decrease quality and quantity of mineral bone density and rate of osteogenesis and do not influence significantly on blood flow in bone. The long-term glucocorticoids prescription can lead to decrease bone mineral density and induce osteoporosis and patients have potentially the risk of occult fracture after minimal trauma [33,34,35].

Preferential diagnosis this kind of fracture is of utmost importance in medicine. Although most fractures are diagnosed by plain radiography but bone scintigraphy imaging is the most useful for detecting occult fractures in patients with a history of trauma and equivocal or frankly negative plain radiographies. Most fractures are detectable by means of scintigraphy images during 24 hours after their occurrence. It is recommended in elderly patients particularly with osteopenia, to postpone the time of

radioisotope imaging until 72 hours following their occurrence in order to increase sensitivity [36].

The exact mechanism which  $^{99m}\text{Tc}$ -MDP binds to bone is not well determined. It has been suggested for the presence of the phosphonate group in the MDP structure, this

bone-seeking radiopharmaceutical adsorb on the hydroxyapatite crystal surfaces during bone formation [21, 37]. The accumulation of  $^{99m}\text{Tc}$ -MDP depends on the blood flow in bone and the rate of osteoblast activity. Enhanced regional blood flow and osteogenesis after bone injury and during repair period are responsible for increased bone-seeking radiopharmaceutical uptake at the site of injury. The rats have been participated in this study were young, healthy and without any co-existence disease. All scintigraphy images with  $^{99m}\text{Tc}$ -MDP have been shown the closed fracture in rat tibia. This finding is contrary to Scott et, al study [12]. He reported the sensitivity of bone scan 48 hours after bone injury was reduced to 41 % in rabbits which received high dose of hydrocortisone. The data have been obtained from our research indicated that  $^{99m}\text{Tc}$ -MDP bone-seeking radiotracer has the ability to be incorporated into the bone fracture. The scintigraphy images alone could not determine significant difference between the control and drug treatment groups. For this reason the R factor has been chosen as criteria. The R factors have measured in second day after injury could not show any statistically significant among different groups. The increased blood flow was the dominant process at the site of injury. Prednisolone could not influence greatly on blood supply and focal blood flow to fractured bone, therefore the bone-seeking radiotracer accumulated efficiently at the site of injury to provide positive scintigraphic images. Significant difference in the R factors have been observed, when scintigraphy images performed in fifth day after injury. At this time period, osteogenesis and bone formation was the predominating process at the site of injury. This finding may be considered that  $^{99m}\text{Tc}$ -MDP bone scintigraphy can provide a method to evaluate net bone formation during healing. Prednisolone could influence the osteogenesis due to the different mechanisms such as the inhibition of osteoblasts, decreased absorption of calcium from intestine, increased urinary excretion of calcium and enhanced parathyroid hormone secretion. The effect of prednisolone on mentioned process depends on the dose and the duration of administration. The results of this study indicated the effect of prednisolone on the R factor was very paramount when the dose of prednisolone administration has been grown from 5 to 20 mg/kg and the period time of drug treatment increased from 4 to 8 weeks. Prednisolone with the



dose of 20 mg/kg during 8 weeks administration has shown the great effect on the sensitivity of  $^{99m}\text{Tc}$ -MDP scintigraphy imaging in our study.

The high uptake of  $^{99m}\text{Tc}$ -MDP radiotracer in primary bone formation is completely consistent with the result of study by Schwartz et al reported significant increased  $^{99m}\text{Tc}$ -MDP radiopharmaceutical uptake in the sixth day after injury in rat tibial bone [21].

Therefore, if  $^{99m}\text{Tc}$ -MDP bone-seeking radiotracer reaches to the site of injury by blood flow in bone and when minimum functional mineral bone density requirements are available, the bone-seeking radiotracer can bind to the hydroxyapatite crystal surfaces, so that it is possible to detect the occult fracture by radioisotope scanning.

### CONCLUSION

This investigation indicates that  $^{99m}\text{Tc}$ -MDP scintigraphy imaging is very sensitive for detection of occult fracture. In order to maximize the sensitivity of bone scanning, it is logical to postpone scintigraphy imaging later than 2 to 3 days after suspected trauma in patients receiving high doses of glucocorticoids for long-term.

### Acknowledgments

This study is part of Pharm-D thesis for Hossein Heidari Kaydan and special thanks to Ahvaz Jundishapur University of Medical Sciences for the financial support and also from Radioisotope Division of Atomic Energy Organization of Iran for providing the freeze-dried MDP kits and  $^{99}\text{Mo}/^{99m}\text{Tc}$  generator.

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