

# The Effect of Calcium Carbonate and Cholecalciferol on Pharmacokinetic Interaction of $^{99m}\text{Tc}$ -CTMP Radiopharmaceuticals for Bone Scanning in Rats (*Rattus norvegicus*)

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## ABSTRACT

Hypocalcemia is one of manifestation of bone metastases which could be treated using calcium carbonate and cholecalciferol. Tc- $^{99m}$  radiolabeled 1,4,8,11-tetraazacyclo tetradecyl-1,4,8,11-tetramethylene phosphonic acid (against  $^{99m}\text{Tc}$ -CTMP) on the other hand is a radioactive complex compound which has an affinity toward bone. Therefore, it could be used as bone tracer (radiopharmaceutical) in bone imaging. However, there has been a concern regarding the use  $^{99m}\text{Tc}$ -CTMP on patients who have been treated with calcium carbonate and cholecalciferol. In this circumstance  $^{99m}\text{Tc}$ -CTMP could interact with calcium carbonate and cholecalciferol and it might then interfere with the imaging results. Therefore, the aim of this study was to determine the pharmacokinetic behavior of  $^{99m}\text{Tc}$ -CTMP in rats (*Rattus norvegicus*) that had been previously treated with calcium carbonate and cholecalciferol. The pharmacokinetic studies were performed using four groups of animal model and each groups consists of three rats. The groups were classified as control (I), rats treated with calcium carbonate (II), rats treated with cholecalciferol (III), and rats treated with both calcium carbonate and cholecalciferol (IV). After the rats were treated for 3 days,  $^{99m}\text{Tc}$ -CTMP radiopharmaceutical was injected through a tail vein of each rat. At specific time intervals after  $^{99m}\text{Tc}$ -CTMP administration, blood was then extracted from the tail, weighed and counted using a single channel analyzer. The percentage of radioactivity in blood at a certain interval was then calculated to determine the distribution half-time and the elimination half-time. The distribution half-time of group I, II, III, and IV were  $0.43\pm 0.13$ ,  $0.25\pm 0.18$ ,  $0.32\pm 0.10$ , and  $0.47\pm 0.07$  hours, respectively, while the elimination half-time were  $2.56\pm 0.18$ ,  $4.48\pm 0.56$ ,  $4.47\pm 1.13$ ,  $6.19\pm 1.97$  respectively. The results of T-test showed that there was no significant difference of distribution half-time between the three treated groups and the control group. However, there was significant difference of elimination half-time between the three treatment groups and control. This research showed that giving calcium carbonate or, cholecalciferol, and both for 3 days prior administration of  $^{99m}\text{Tc}$ -CTMP would maintain elimination half-time which resulted in longer excretion/ elimination time.

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## INTRODUCTION

In radiopharmaceuticals, the radioactive tracers are the main components for examining the function of body systems. Many radiopharmaceuticals are available for imaging

purposes; they differ in terms of their physical characteristics, biodistribution, and radiation exposure. Medical images provide very helpful information to medical specialists for taking the important and right decision for diagnosis and therapeutic actions [1].

Bone scintigraphy still represents the second greatest volume procedure in nuclear medicine with broad diverse applications. The clinical utility,

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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  $^{99m}\text{Tc}$ -MDP (medronate), and  $^{99m}\text{Tc}$ -CTMP [2-4].

Metastatic cancer is a common symptom in advancing malignancy and often determines quality of life in the later stages of disease [5]. Bone metastases disrupt the normal homeostasis between bone formation and resorption by promoting osteoclast maturation, activity, and increased 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. Antiresorptive agents such as denosumab are used to treat bone metastases. They inhibit osteoclastic bone resorption but reduce skeletal calcium release into circulation which promote hypocalcaemia [6]. One of the treatments of hypocalcemia is giving oral calcium and cholecalciferol (D3 vitamin). Calcium carbonate has the greatest proportion of elemental calcium (40 %) and is easily absorbed [7,8].

Cyclam is a macrocyclic compound that is widely used in the medical field. One of cyclam derivatives is 1,4,8,11-tetraazacyclotetradecyl-1,4,8,11-tetramethylene phosphonic acid (CTMP) which has four phosphonate groups and four amine groups. The amine groups are a side to be bound with technetium atom that make tetraphosphonate affinity to bone higher because there are phosphonate groups in free form that are not bound to the technetium atom. It was reported that labeling of CTMP with  $^{99m}\text{Tc}$  radionuclide will form a complex compound which has a stronger affinity for bone than other phosphate compounds such as pyrophosphate compounds and diphosphonates [4,9,10].

Research on the effect of calcium carbonate on the radiopharmaceutical  $^{99m}\text{Tc}$ -CTMP has been conducted and published by our group in 2015 [11]. However, a repeating experiment in order to validate the results of research has yet to be done.

Considerable evidence showed that radiopharmaceutical biodistribution or pharmacokinetics may become altered by a variety of drugs. These phenomena have a significant clinical impact on safety, scan interpretation, and diagnostic imaging accuracy [12]. Therefore, the aim of this research was to determine the effect of calcium carbonate and cholecalciferol on the pharmacokinetic interaction of  $^{99m}\text{Tc}$ -CTMP radiopharmaceutical in rats (*Rattus norvegicus*) of

Sprague Dawley stock. This research will determine drug-radiopharmaceutical interaction possibility to pharmacokinetic profile of  $^{99m}\text{Tc}$ -CTMP.

## EXPERIMENTAL METHODS

The materials used were calcium carbonate (E. Merck), cholecalciferol, CTMP radiopharmaceutical kit,  $^{99}\text{Mo}/^{99m}\text{Tc}$  generator (Polatom), Whatman 3MM chromatography paper, NaCl 0.9 % (IPHA), acetone (E. Merck), and aquabidest (IPHA). The tools used were analytical scale (Mettler Toledo), oven (Memmert), rat strainer, single channel analyzer (Ortec), and dose calibrator (Victoreen).

This research used 12 rats (*Rattus norvegicus*) of Sprague Dawley strain. All experiments were performed according to the guidelines and approved protocol by Ethical Committee for Care and Use of Laboratory Animals with approval number 003/KEPPHP-BATAN/IV/2015.

All phases were performed aseptically. A total of 3 mL of  $^{99m}\text{Tc}$ -pertechnetate with 3 mCi radioactivity was added into a vial containing CTMP kit that had been kept in a Pb container. The solution was shaken and incubated, resulting in the  $^{99m}\text{Tc}$ -CTMP radiopharmaceutical. The  $^{99m}\text{Tc}$ -CTMP radiochemical purity was then determined using two paper chromatography systems. The first system, with Whatmann 3MM as stationary phase and acetone as mobile phase, was used to separate impurities in the form of free  $^{99m}\text{Tc}$ -pertechnetate. The second system, with Whatmann 3MM as stationary phase and NaCl as mobile phase, was used to separate impurities in the form of free  $^{99m}\text{Tc}$ -reduced [4].

The pharmacokinetics tests used in this research were adopted from the method given in Petriev *et al.* [13]. The pharmacokinetics tests were performed using four groups of animal models and each groups consists of three rats. The groups were classified as control (I), rats treated with calcium carbonate (II), rats treated with cholecalciferol (III), and rats treated with both calcium carbonate and cholecalciferol (IV). Rats that had been treated for 3 days were injected through a tail vein with  $^{99m}\text{Tc}$ -CTMP radiopharmaceutical with a dose of 200  $\mu\text{Ci}/200 \mu\text{L}$ . At designated-time intervals of 5 minutes, 15 minutes, and 1, 2, 3, 24, 25, and 26 hours post injection of  $^{99m}\text{Tc}$ -CTMP, blood was extracted from the tail of each rat, weighed, and then counted using a single channel analyzer. Measurement results are expressed as a percentage of radioactivity per gram of blood (%ID/g) which was calculated based on eq. (1) below [13]:

$$\frac{\text{blood per gram count}}{\text{injected dose count}} \times 100\% \quad (1)$$

The percentage of radioactivity in the blood at various times was plotted in a graph and the biological half-time of  $^{99m}\text{Tc}$ -CTMP was then determined. Pharmacokinetic parameters were calculated by fitting the data to a first-order two-compartment model with Multifit pharmacokinetic software package [14]. The resulting biological half-times of  $^{99m}\text{Tc}$ -CTMP were compared using T-test.

## RESULTS AND DISCUSSION

Radiolabeling of CTMP with  $^{99m}\text{Tc}$  resulted in  $^{99m}\text{Tc}$ -CTMP radiopharmaceutical with pH and radiochemical purity of 5-6 and  $96.42 \pm 2.20\%$  ( $n = 6$ ), respectively. The radiochemical impurities in form of free  $^{99m}\text{Tc}$ -pertechnetate and  $^{99m}\text{Tc}$ -reduced in this  $^{99m}\text{Tc}$ -CTMP radiopharmaceutical were  $0.19 \pm 2.20\%$  and  $3.39 \pm 2.13\%$ , respectively. Based on these results (radiochemical purity > 95%), the  $^{99m}\text{Tc}$ -CTMP radiopharmaceutical

could be used for pharmacokinetics test [4].

The blood pharmacokinetic profile was tested to determine any change in pharmacokinetic profiles and biological half-time when  $^{99m}\text{Tc}$ -CTMP radiopharmaceutical was injected to rats which previously had been treated with specified drugs. The results (Figs. 1 and 2) showed that administration of calcium carbonate, cholecalciferol, or both of them in experimental animals affected the pharmacokinetic profile of  $^{99m}\text{Tc}$ -CTMP radiopharmaceutical. The pharmacokinetic profile of groups I, II, III, and IV in the blood 5 minutes post injection were 35.19, 22.88, 36.53, and 22.35 %, respectively. These then decreased to 0.003, 0.07, 0.02, and 0.04 %, respectively, 24 hours post injection.

From the calculation of radioactivity per gram of blood, biological half-time data which are divided into distribution and elimination half-times were obtained. The distribution and elimination half-times are shown in Table.1.

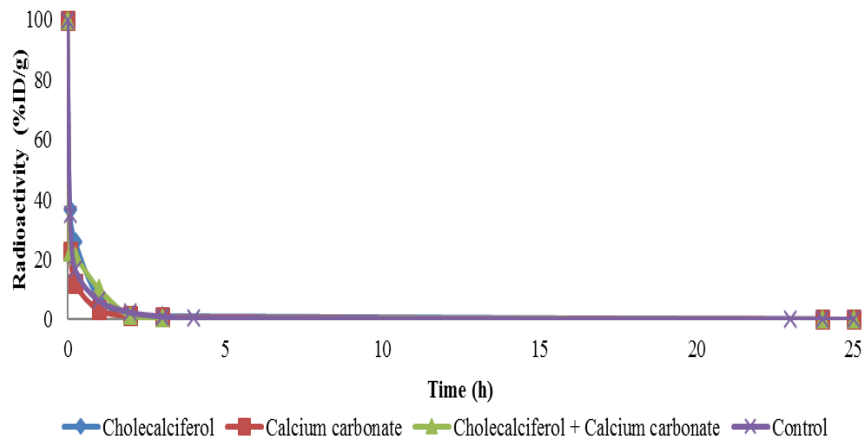


Fig. 1. Pharmacokinetic profile of  $^{99m}\text{Tc}$ -CTMP.

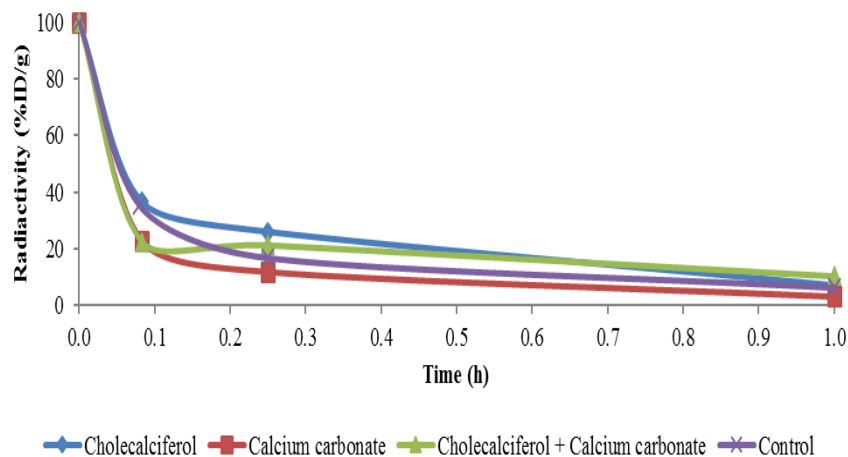


Fig. 2. Pharmacokinetic profile of  $^{99m}\text{Tc}$ -CTMP for 1 hour.

**Table 1.** Biological half-time of  $^{99m}\text{Tc}$ -CTMP

Groups	Biological half-time (h)	
	Distribution	Elimination
I	0.43±0.13	2.56±0.18
II	0.25±0.18	4.48±0.56
III	0.32±0.10	4.47±1.13
IV	0.47±0.07	6.19±1.97

It can be seen from the above results that the standard deviation for each group is quite wide. This might be caused by the limited number of rats used and each the different metabolism condition of each rat. The results of T-test showed no significant differences of distribution half-time between the three experimental groups compared and the control group. However, there were significant differences of elimination half-time between the three treatment animal groups and the control group.

The aforementioned results showed that giving calcium carbonate and cholecalciferol prior to administration of  $^{99m}\text{Tc}$ -CTMP might cause interactions that affect the pharmacokinetic profile of  $^{99m}\text{Tc}$ -CTMP. Figures 1 and 2 show that the percentage of injected dose per gram organ (%ID/g) of  $^{99m}\text{Tc}$ -CTMP in rats that were previously treated with combination of calcium carbonate and cholecalciferol. These figures show that administration of the aforementioned drugs prior to administration of  $^{99m}\text{Tc}$ -CTMP are able to change the pharmacokinetic profile pattern of  $^{99m}\text{Tc}$ -CTMP. The radioactivity in blood was found to be more maintained in rats treated (calcium carbonate and cholecalciferol) than in the untreated rats. As a consequence, the excretion time of  $^{99m}\text{Tc}$ -CTMP of the latter became longer.

The selective accumulation of radionuclides in bony tissues is explained by the following mechanisms of adsorption of phosphonates labeled with radioactive isotopes by hydroxyapatites [15]:

(1) Adsorption of alkali-substituted by exchange for calcium ions in bone tissues, (2) Interaction of charged tri- and tetravalent cations with hydroxyapatites to form insoluble phosphates or hydroxides, which attach tightly to the mineral components of bone tissues and (3) Chemosorption of phosphates and diphosphonates onto the hydroxyapatite surface.

The results showed that radioactivity of  $^{99m}\text{Tc}$ -CTMP in the blood that is maintained relatively longer in the group treated with the combination of calcium carbonate and cholecalciferol (Group IV) is possibly caused by the mechanism of action of cholecalciferol that would be synergistic with the homeostasis of calcium [15,16]. Increased calcium is associated with the concentration of  $^{99m}\text{Tc}$ -labeled phosphonate.

Phosphonates act as ligands which absorb into calcium in tissues, fixing Tc-99m into the mineral phase with little interaction of organic substrates [3,17]. Vitamin D3 or cholecalciferol is hydroxylated in the liver into 25-hydroxyvitamin D3 (25(OH)D) and subsequently in the kidney into 1,25-dihydroxyvitamin D3 (1,25(OH)2D). This is an active metabolite, which stimulates the calcium absorption from the gut. The active metabolite 1,25(OH)2D enters the cell and binds to the vitamin D receptor. The classic effect of 1,25(OH)2D on active calcium transport occurs in the intestinal cell. Calcium enters the cell through membrane proteins. In the intestinal cell, 1,25(OH)2D binds to the vitamin D receptor and the calcium binding protein is synthesized and this regulates the active transport through the cell. The 1,25(OH)2D has its effect on the classic target organs bone, intestine and kidney and stimulates calcium transport from these organs to the blood. The production of 1,25(OH)2D is stimulated by parathyroid hormone (PTH) which functions as a calcium sensor. PTH then stimulates the kidney to produce calcitriol, the hormonal form of vitamin D, and activates bone resorption, which will increase extracellular calcium levels. Calcitriol acts in an endocrinal manner on the intestine, bone, and kidney to raise serum calcium levels. It also acts on the intestine and, to some extent, the kidneys to raise serum phosphorus levels. As the serum calcium level rises, the feedback mechanism causes the calcium sensing receptor to be turned off and PTH secretion to drop. If there is an overshoot in serum calcium levels, the "C" cells (parafollicular) cells of the thyroid gland secrete calcitonin, which can block bone calcium resorption, helping to keep serum calcium levels in the normal range. Calcitriol, through its receptor, also provides feedback relative to suppressing the production and release of PTH, commonly referred to as PTH suppression [18-20].

Presently, there is no publication that explains the implications on image result when the elimination half-time of radiopharmaceuticals becomes longer than usual. However, never-before-seen untoward effects had been reported following administration of radiopharmaceuticals, as unanticipated reactions occurred from unknown pharmacological actions of a nonradioactive pharmaceutical. The time delay of phosphonates radiopharmaceutical consistently reported could cause allergic reaction and another symptoms such as dermatographism, nausea, malaise, vertigo, and pruritus. Those adverse reactions related to radiopharmaceuticals do occur and can be very severe [21].

Research on the effect of calcium carbonate on the radiopharmaceutical  $^{99m}\text{Tc}$ -CTMP has been conducted and published by our group in 2015 [11]. Then, a repeating experiment to validate the results

of previous research was performed. The validating results was found to give a different of elimination half-time only on control group. In the previous experiment, the elimination half-time on the control group was  $7.294 \pm 0.319$  hours, but in the repeating repetition experiment was  $2.563 \pm 0.177$  hours. In the previous eperiment the radiochemical purity was  $93.54 \pm 0.68$  %. This different result can be caused by the higher radiochemical purity of the radiopharmaceutical  $^{99m}\text{Tc}$ -CTMP in the repeating experiment than the radiochemical purity of  $^{99m}\text{Tc}$ -CTMP in the previous experiment, and can also be caused by individual factors of the rats [11].

## CONCLUSION

Research to assess the effect of calcium carbonate and cholecalciferol against  $^{99m}\text{Tc}$ -CTMP pharmacokinetic interaction in rats (*Rattus norvegicus*) had been conducted. The finding showed that giving calcium carbonate, cholecalciferol, or combination of both for 3 days prior to admistration of  $^{99m}\text{Tc}$ -CTMP would maintain the elimination half-time of  $^{99m}\text{Tc}$ -CTMP which resulted in a longer elimination half-time. Based on the results of this research, another research is needed about the toxicity and biodistribution profile of interaction between calcium carbonate and calciferol to  $^{99m}\text{Tc}$ -CTMP radiopharmaceutical.

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