Dependence of Technetium-99m Radioactivity on the Stability of Tc-99m Tetrofosmin as Injectable Radiopharmaceuticals

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ABSTRACT

Technetium-99m (Tc-99m) labeled tetrofosmin kit has been widely used in hospitals including in Indonesia. Usually, tetrofosmin kits and Tc-99m pertechnetate are supplied separately, but recently there has been a new trend where Tc-99m tetrofosmin is supplied in the form of the ready-to-inject product. To prepare such a product it has to be proven that tetrofosmin can be labeled with high activity of Tc-99m and the resulted Tc-99m tetrofosmin, remains stable within shipping time until being used in hospitals. In this investigation, locally produced tetrofosmin kits were labeled with various radioactivity of Tc-99m ranging from 150 mCi to 400 mCi, their radiolabeling yields or radiochemical purity was analyzed using SepPak C18 column, and the radiochemical stability was assessed within 30 hours. The worst storage condition was also studied by analyzing its radiochemical purity after being stored at extreme temperatures for several hours. The results showed that tetrofosmin kit can be highly labeled with up to 415 mCi of Tc-99m, and stable up to 30 hours in room temperature, and there is a tendency that higher radioactivity leads to more decreasing in radiochemical purity. Stability study in extreme temperatures showed that the product can withstand its stability within 6 hours in 40°C, but it decreased rapidly to less than 70% within 1 hour when stored in 50°C. It concludes that the quality of Tc-99m tetrofosmin as an injectable radiopharmaceutical can be maintained during transportation by storing it at cool temperature.

Key word: Tc-99m, tetrofosmin, injectable radiopharmaceuticals, radiochemical purity, stability.

INTRODUCTION

To date, heart diseases and cancers are two of the highest death cause diseases in Indonesia, and their prevalence is increasing day by day. Nuclear medicine has a role to contribute the management of these diseases, using Positron Emission Tomography (PET) and Single Emission Computed Tomography (SPECT) modalities. SPECT using technetium-based radiopharmaceuticals has been widely used in several hospitals in Indonesia for a few decades, one of which is Tc-99m tetrofosmin. Tc-99m tetrofosmin is commonly used for myocardial perfusion imaging agent, mainly at Harapan Kita Cardiac Hospital, Jakarta and Hasan Sadikin General Hospital, Bandung [1]. However, in recent year the use of Tc-99m tetrofosmin has been expanding not only for myocardial imaging but also for detection of several tumors and cancers such as brain cancer (glioma, glioblastoma, and meningioma), breast cancer and parathyroid adenoma [2-5]. The chemical structure of Tc-99m tetrofosmin has been discussed elsewhere [6].

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Another SPECT radiopharmaceutical used in hospitals which has the same function and comparable performance as Tc-99m tetrofosmin is Tc-99m sestamibi. However, its radiolabeling preparation in hospitals is more time consuming since it requires heating for several minutes. The use of Tc-99m sestamibi on one has been doubting some radiopharmacists for a possibility of inconsistency in radiolabeling results since it requires skilled and trained personnel. On the other hand, apart from the ease in preparation, Tc-99m tetrofosmin has a favorable pharmacokinetic behavior, i.e. faster clearance from the liver, and lower dose needed for the patient both at rest and stress condition. Therefore, for practical reason, some hospitals prefer to use Tc-99m tetrofosmin [7-9].

Some hospitals in Indonesia has been using Tc-99m tetrofosmin for years, and the products are imported in the forms of Mo-99/Tc-99m generator and lyophilized tetrofosmin kit, for which tetrofosmin kit is to be mixed with Tc-99m (pertechnetate solution) to prepare Tc-99m tetrofosmin. Center for Radioisotope and Radiopharmaceutical Technology (PTRR), as one of the centers in BATAN, has been developing Tc-99m tetrofosmin as an injectable radiopharmaceutical, in which the resulted product showed an excellent and consistent quality that complied with radiopharmaceuticals requirement as written in the US Pharmacopoeia [10-11]. Tetrofosmin kit is recommended to be labeled with Tc-99m with radioactivity up to 240 mCi and concentration up to 30 mCi/mL [12]. Tetrofosmin kit which was developed in PTRR-BATAN has undergone patient trial in Hasan Sadikin General Hospital which showed comparable imaging performance from that of Myoview, and the test was applied to 28 volunteers [13].

Some countries are now developing centralized radiopharmacy, i.e a center which produces injectable radiopharmaceuticals mainly based on Tc-99m and distributing to hospitals nearby that can be reached within a few hours. These centers are Syncor (USA), Gipharma (Italy) and Nihon Medi-Physics (Japan). The availability of injectable Tc-99m labeled radiopharmaceuticals helps most hospitals which have limited human resources since its use is less time consuming, but has the disadvantage as the half-life of Tc-99m is short, of only 6 hours, making it impossible to send to far distances [14].

Gamma radiation has been considered to have radiolysis effect on molecules, especially that of high radioactivity. The higher the specific activity and the more energetic the radiation, the greater the radiolysis effect. When the radiation breaks down the chemical bond of its own molecule, it is called autoradiolysis. The mechanism of decomposition could be direct or indirect decomposition. The indirect one can be the effects of gamma irradiation on the water that lead to the formation of various radiolysis products of water, i.e. $\text{H}_2\text{O}$, $\text{H}_2\text{O}_2$, and $\cdot\text{H}_2$. Peroxide and free radical species are known as strong oxidants which can also destroy DNA of living cells and lead to cells death [15-20].

Previously, it has been reported that mostly in-house prepared tetrofosmin kits were labeled with low activity of Tc-99m i.e up to 75 mCi [13]. However, these kits have to be proven if they could be labeled with higher activity of Tc-99m ( up to 240 mCi or higher) since the labeled products are supposed to be prepared as injectable radiopharmaceutical which will decay during transportation to the end users in hospitals. Moreover, as a pre-prepared injectable radiopharmaceutical, it needs to be proven that the stability will remain constant during shipment. This work was aimed to study radiolabeling of tetrofosmin kits with high activity of Tc-99m (higher than 240 mCi) and to study the stability of the labeled product (Tc-99m tetrofosmin) in various temperatures and storage condition until the radiochemical purity decrease to less than 90% (a minimal requirement of radiochemical purity stated in the US Pharmacopoeia) [21].
The radiochemical purity of Tc-99m tetrofosmin is the main quality parameter which is commonly determined using thin layer chromatography where instance thin layer chromatography-silica gel (ITLC-SG) is used as stationary phase and the mixture of acetone-dichloromethane (35:65) is used as mobile phase. However, sometimes this system does not give a consistent result, therefore alternatively, the analysis method using SepPak C18 column with comparable results which are relatively simpler and faster can be used [22-24].

This research is expected to provide sufficient information regarding the stability of high radioactivity Tc-99m tetrofosmin in relation with radiolysis effect of gamma radiation on the intact of Tc-99m tetrofosmin molecules in order to find the way of minimizing this impact.

EXPERIMENT
Chemicals and instrumentation

Material used for radiolabeling and analyzing Tc-99m tetrofosmin were in-house-prepared tetrofosmin kits (PTRR-BATAN), sodium pertechnetate Tc-99m from Mo-99/Tc-99m generator (Polatom), water for injection (IPHA), physiological NaCl solution or saline (IPHA), ethanol (Merck), SepPak C18 column (Waters), various size of syringes and glasswares.

The equipment used was vortex mixer (VWR) and dose calibrator (AtomLab 300).

Procedure

Radiolabeling of tetrofosmin kit to form the complex compound of Tc-99m tetrofosmin was done by adding various radioactivities of pertechnetate Tc-99m solution (150 up to 400 mCi) into a vial containing liquid tetrofosmin kit. The concentration of Tc-99m solution used in this procedure must not exceed 30 mCi/mL. The mixture was vortexed and allowed to react at room temperature for 15 min.

The quality of labeled compound which was represented as the radiochemical purity of Tc-99m tetrofosmin was analyzed by separation method using SepPak C18 column. The small volume of sample (0.1 to 1 mL) was injected into a SepPak column which prior to its use had been activated using 5 mL of ethanol 95% followed by 5 mL of water and 5 mL of air consecutively. The polar impurity in Tc-99m tetrofosmin solution in the form of free pertechnetate Tc-99m was removed from the column by eluting with 10 mL of saline and collected in an empty vial. The purified Tc-99m tetrofosmin was then eluted with 10 mL of ethanol-saline mixture (4:1) and collected into an empty vial. Radioactivity of both fractions was measured, as well as the remained radioactivity in the column, and altogether was calculated as total radioactivity. The percentage of radioactivity in the first fraction represented polar impurity or free pertechnetate (TcO₄²⁻) and percentage of the remained radioactivity in the column represented colloidal impurity (TcO₂). The percentage of radioactivity in the second fraction represented the radiolabeling yield or radiochemical purity of Tc-99m tetrofosmin.

Radiochemical stability of Tc-99m tetrofosmin was assessed by measuring the radiochemical purity after storing the labeled compound at room temperature for several hours. Tc-99m tetrofosmin of various radioactivity was stored at RT, every 3 hours a small amount of sample was taken out and measured for radiochemical purity using the same SepPak C18 method. Radiochemical purity measurement was ended when radioactivity of the sample was no longer detectable or its radiochemical purity has become less than 90%. The dependence of radiochemical stability on high temperatures was tested by analyzing the
radiochemical purity of Tc-99m tetrofosmin which has been stored at various temperatures, i.e. room temperature (~25°C), 40 and 50°C for 1 to 6 hours.

RESULT AND DISCUSSION

Liquid tetrofosmin kits labeled with various radioactivity of Tc-99m resulted in a high radiochemical purity of Tc-99m-tetrofosmin which conformed to the requirement stated in Pharmacopoeia (> 90%). The radiochemical purity of Tc-99m tetrofosmin which was prepared with various radioactivity of Tc-99m is shown in Table 1. It can be seen that there is no significant difference in radiochemical purity of Tc-99m tetrofosmin, i.e. 98.3% ± 0.4% in average, which impurities in the form of polar impurity (TeO$_4^-$ or free Tc-99m pertechnetate) and colloid impurity (TeO$_2$) were 0.8% ± 0.3% and 0.9 ± 0.2% respectively. However, the various radioactivity of Tc-99m used in this experiment was very much lower in molar, so the mole ratio of ligand to Tc-99m was almost the same to form Tc-99m tetrofosmin complex compound. Therefore, in radiolabeling of tetrofosmin kit, although using much higher radioactivity of Tc-99m the mole of tetrofosmin can still be assumed excessive.

| Radioactivity (mCi) | Free Tc-99m pertechnetate (%) | Tc-99m Colloids (%) | Tc-99m tetrofosmin (%) |
|---------------------|-------------------------------|---------------------|------------------------|
| 150                 | 1.2                           | 0.8                 | 98                     |
| 282                 | 0.7                           | 1                   | 98.3                   |
| 366                 | 1.1                           | 1.1                 | 97.8                   |
| 392                 | 0.5                           | 0.7                 | 98.8                   |
| 415                 | 0.6                           | 0.7                 | 98.7                   |
| Average             | 0.8% ± 0.3%                   | 0.9 ± 0.2%          | 98.3% ± 0.4%           |

The stability test results of Tc-99m tetrofosmin of various radioactivity at room temperature which was observed up to 30 hours is shown in Figure 1. In general, it can be seen that radiochemical purity of Tc-99m tetrofosmin which was stored in the above-mentioned condition was still higher than 90% indicating that this product has a good stability. However, radiochemical purity of high radioactivity Tc-99m tetrofosmin (i.e. 415 mCi) seemed to decrease quite significantly from 98.7 to 90.3% within 30 hours whereas the one with radioactivity of 150 mCi decreased from 98.3 to 95.4% within the same time of storage. The decrease of radiochemical stability seems to correlate with radioactivity of Tc-99m tetrofosmin. Although the radioactivity of this Tc-99m tetrofosmin was still acceptable (> 90%) when this pattern compared with those of lower radioactivity, the former seemed to experience auto-radiolysis. Auto-radiolysis, in this case, is degradation of radioactive molecules caused by gamma radiation emitted by those molecules themselves. Gamma radiation is considered to have the capability of breaking down chemical bonds within the molecules or with other molecules which are in close proximity with those radiation emitter which then leads to the instability of the products [15-20].
The results as mentioned above conformed with a publication reported by Jia et al. that gamma radiation can induce degradation of cyclohexanebutyric acid in water caused by a major role of OH radical [20]. Gamma radiation may have radiolysis effect to material which can break down the chemical bonds of the molecules directly or through the decomposition of water molecules producing hydrogen peroxide or perhydroxyl free radical which then acts as strong oxidants that will oxidize labeled compound.

Stability of Tc-99m tetrofosmin (150 mCi) upon storage at various temperature was observed within 6 hours, and the result of stability test is shown in (Figure 2). It can be seen that the product which was stored at 25°C and 40°C was relatively stable within observation time (15 minutes, 1 up to 6 hrs post labeling), whereas the one that was stored at 50°C decreased rapidly in radiochemical purity from 92.4 to 68.8% after 1 hour. Based on these results, it could be suggested that radiolysis effect is greater in higher temperatures of more than 40°C (Figure 2). Therefore, in order to minimize radiolysis effect during storage or transportation, it is recommended to pack the labeled product, in this case, Tc-99m tetrofosmin radiopharmaceutical below 40°C, for example in a cool box during shipment, or storing the products under room temperature before its use.
Understanding the characteristic of Tc-99m tetrofosmin and ensuring its quality does not change during transportation toward the users, is very important in term of providing a ready-to-inject radiopharmaceutical. The shortcoming of providing Tc-99m radiopharmaceuticals as the labeled compound is the short half-life of Tc-99m which is only 6 hours that makes it inefficient to be used at the next day after preparation or after 24 hours.

CONCLUSION

Tetrofosmin kit can be labeled with high radioactivity of Tc-99m which is much higher than stated in commercial product monograph, but high radioactivity of Tc-99m influences the stability of Tc-99m tetrofosmin due to auto-radiolysis caused by gamma energy emitted by highly radioactive Tc-99m on the intact molecules, and this effect increases by elevating the storage temperature.

Technetium Tc-99m tetrofosmin can be supplied as injectable radiopharmaceutical dosage form, it will help end users in providing better service to their patients, and also to hospitals with improper facility for nuclear medicine services, but limited to hospitals located nearby which can be reached in only a few hours from the manufacturer location.

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