Pharmacokinetics interaction study of $^{99m}$Tc-glutathione radiopharmaceutical with doxorubicin in mice (mus musculus)

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Abstract. The use of radiopharmaceuticals for cancer diagnosis and therapy is increasing. The radiopharmaceutical is also used to monitor the progress of a disease and determine the appropriate treatment. However, the use of radiopharmaceuticals needs to pay attention to the alteration in pharmacokinetics, pharmacology, and toxicity that are influenced by the drugs consumed by patients. Interaction between drugs with radiopharmaceuticals will cause alteration in pharmacokinetic effects. Therefore, this study aimed to determine the pharmacokinetic interaction of $^{99m}$Tc-Glutathione radiopharmaceuticals with doxorubicin cancer drug in mice (Mus musculus). The pharmacokinetic studies were performed using four groups of animal model and each group consists of three rats. The groups were classified to normal mice without treatment of doxorubicin as normal mice control (I), normal mice treated with doxorubicin (II), cancer model mice without treatment of doxorubicin as cancer model mice control (III), and cancer model mice treated with doxorubicin (IV). The radioactivity in blood at a certain interval was then calculated to determine the distribution and elimination half-time. The distribution half-time of group I, II, III, and IV were 0.004±0.001, 0.0037±0.0001, 0.003±0.0001, and 0.0037±0.0001 hours, respectively, while the elimination half-time were 5.310±1.050, 10.7344±0.4692, 72.712±2.427, and 26.9320±7.8152 hours, respectively. The results of the T-test showed that there was a significant difference in elimination half-life of $^{99m}$Tc-Glutathione between the treated group and the control group. These results indicate that administration of the doxorubicin before administration of $^{99m}$Tc-Glutathione needs to be avoided because it can alter the elimination half-life of $^{99m}$Tc-Glutathione. The results of this study are expected to provide benefits for clinicians in nuclear medicine to avoid the interpretation of incorrect diagnosis results, to achieve high-quality health service and will have a positive impact on the appropriate treatment for patients.

1. Introduction
Cancer is one of the causes of death in the world, wherein 2018 there were 18.1 million new cases and 9.6 million cases of cancer-related deaths. Also, as many as 48.4% or 8.75 million new cases occurred in Asia [1]. In Indonesia, the prevalence of cancer in all ages in 2013 was 1.4% or estimated to be around 347,792 people. Province D.I. Yogyakarta has the highest prevalence of cancer, which is 4.1%. Based on the estimated number of cancer sufferers, Central Java, and East Java Provinces are provinces with the highest estimated cancer sufferers, namely around 68,638 and 61,230 people [2]. Cancer is caused by two factors, including internal factors (such as genetic, hormonal and body conditions) and environmental factors (such as cigarette smoke, food, radiation, and infectious organisms) [3].

Doxorubicin is an anthracycline that has antineoplastic activity with a wide spectrum and has a lot used to treat cancer [4], such as breast cancer [5], leukemia [6], non-Hodgkin lymphomas [7], and soft tissue sarcomas [8]. Doxorubicin is isolated from the species Streptomyces peucetius [9].

Deaths due to cancer can be reduced through early detection, to increase the chances of healing cancer patients. One way to detect cancer early is to use radiopharmaceutical. This is the reason for the
use of radiopharmaceuticals for cancer diagnosis and therapy is increasing [10], [11]. According to Mukiza, more than 10,000 medical facilities worldwide use radiopharmaceuticals for both diagnosis and medical therapy [12].

One of the radiopharmaceuticals that have been developed at PSTNT-BATAN is the \(^{99m}\)Tc-Glutathione radiopharmaceutical. \(^{99m}\)Tc-Glutathione is a radiopharmaceutical developed for the diagnosis of cancer. The glutathione plays a role in the reaction of detoxification and cell protection against chemical damage is one reason it is used as a ligand [13].

In addition to initial diagnosis and therapy, the radiopharmaceutical is also used to monitor the progress of a disease and determine the appropriate treatment measures. However, the use of radiopharmaceuticals in hospitals needs to pay attention to the occurrence of pharmacological changes, pharmacokinetics, and toxicity that is influenced by the drug consumed by the patient, the patient's illness, and in some cases can be affected by the surgical process. This can allow errors or misdiagnosis by clinicians in nuclear medicine. Interactions that occur between a cancer drug such as doxorubicin with radiopharmaceuticals can cause alteration in the pharmacokinetics profile of radiopharmaceuticals and competition occurs at the same binding site. Therefore, pharmacokinetic studies need to be done on the interaction of doxorubicin cancer drugs with \(^{99m}\)Tc-Glutathione radiopharmaceutical.

2. Methodology

2.1. Material and Instrument

Glutathione and SnCl\(_2\) (Sigma Aldrich), doxorubicin (Kalbe Farma), Na\(^{99m}\)TcO\(_4\) (Polatom), acetone (E.Merck), NaCl (IPHA), aquabidest (IPHA), TLC-SG (Merck), and universal pH indicator (Merck). Instruments used include radio-thin layer chromatography (TLC) scanners (Bioscan), a single-channel analyzer (Ortec), isotope calibrators (Biodex), oven (Memmert), freeze dryer (Labconco), and other glassware.

2.2. Method

2.2.1. Glutathione dry kit preparation. The Glutathione kit composition was 20 mg glutathione, 0.3 mg SnCl\(_2\)2H\(_2\)O. The pH was adjusted to pH 7. The mixture was dried using a Freeze Dryer for 22 hours and stored at 4°C. Glutathione was labeled by adding a solution of 2.5 mL Na\(^{99m}\)TcO\(_4\), then the mixture was shaken until homogeneous, then stored at room temperature for 5 minutes.

2.2.2. \(^{99m}\)Tc-Glutathione radiochemical purity test. The purity of the \(^{99m}\)Tc-Glutathione radiochemical was determined by the thin layer chromatography method. As the stationary phase TLC-SG (1x10 cm) was used which was marked -1 to 8, while for the mobile phase used dry acetone and physiological NaCl solution (0.9%). The \(^{99m}\)Tc-Glutathione radiopharmaceutical sample was spotted at the zero points of TLC-SG, then eluted with both types of the mobile phase. After elution was complete, the TLC-SG plate was dried, then counted using a radio-TLC scanner.

2.2.3. Pharmacokinetics study. This pharmacokinetic study was conducted according to the method used by Petriev et al [14]. Animals test were divided into four groups and each group consisted of three mice. Each group was classified as (I) normal mice that were only given \(^{99m}\)Tc-Glutathione, (II) normal mice given the drug doxorubicin and \(^{99m}\)Tc-Glutathione, (III) cancer model mice that were only given \(^{99m}\)Tc-Glutathione, and (IV) cancer mice that were given the drug doxorubicin and \(^{99m}\)Tc-Glutathione. Test animals were given 0.1 mL of doxorubicin via intravenous to the tail. After 5 minutes, 0.1 mL of \(^{99m}\)Tc-Glutathione radiopharmaceutical with 100 µCi radioactivity was given. Then the blood was collected at intervals of 15 minutes, 30 minutes, 1, 2, 3, 4, 5, 6, 24, 25, and 26 hours. Blood was drawn from the tail, weighed using an analytical balance, and counted using a single channel analyzer. Blood count results
can be used to calculate the total injection dose (% ID). Pharmacokinetic profile, biodistribution and elimination were calculated using PK Solver 2.0 [15].

2.2.4. Data analysis. The data obtained from the pharmacokinetic interaction study of ~Tc-Glutathione with doxorubicin cancer drugs were processed using a T-test.

3. Result and Discussion
Radiopharmaceutical ~Tc-Glutathione which will be used for pharmacokinetic interaction studies must comply with the specified requirements, such as clarity, pH, and radiochemical purity. Radiopharmaceutical purity of ~Tc-Glutathione radiopharmaceutical was carried out using the radio-thin layer chromatography method using TLC-SG as the stationary phase. The mobile phase used was dry acetone to separate radiochemical impurities in the form (~TcO₄⁻) with an Rf value of 0.9 - 1.0 (Rf ~Tc-Glutathione = Rf ~TcO₄⁻ = 0.0), which is shown in Figure 1.

![Figure 1. ~Tc-Glutathione Radiochromatogram using TLC-SG as stationary phase and dry acetone as mobile phase](image)

While physiological NaCl solvents (0.9%) were used to separate radiochemical impurities in the form of ~Tc-reduced (~TcO₃⁻) with an Rf value of 0.0 - 0.1 (Rf ~Tc-Glutathione = Rf (~TcO₃⁻) = 0.9 - 1) shown in Figure 2.
The percentage of $\sim$Tc-Glutathione radiochemical purity was calculated using equation (1).

\[
\text{Radiochemical purity of } \sim\text{Tc-Glutathione} = 100\% - (\% \sim\text{TcO}_2 + (\% \sim\text{TcO}_4)) \ldots (1)
\]

The result has shown that the percentage of radiochemical purity that complies with the pharmacopeia requirements, which is 99.52±0.007%, pH 7, and a clear solution so that it can be used for pharmacokinetic interaction studies.

Several factors can affect the pharmacokinetics of a radiopharmaceutical, such as recent surgery, chemotherapy, radiotherapy, dialysis, etc [16]. Also, there are unexpected changes in radiopharmaceutical patterns, either biodistribution, poor visualization of organs or even misdiagnosis [17], [18]. Therefore it is necessary to study the pharmacokinetic interaction of $\sim$Tc-Glutathione radiopharmaceutical with a doxorubicin cancer drug.

Interaction pharmacokinetic studies of cancer drugs with $\sim$Tc-Glutathione were carried out to determine alteration in pharmacokinetic profiles in Balb/C mice test animals that were previously given doxorubicin cancer drug followed by $\sim$Tc-Glutathione administration. The doxorubicin given in mice was 0.1 mL. When administering cancer drugs and $\sim$Tc-Glutathione, mice were not anesthetized and were treated calmly and gently to reduce pain or stress in mice. The non-anesthetic treatment aims to ensure that $\sim$Tc-Glutathione radiopharmaceutical was normally distributed in the body of mice because it was adapted to its application later when it will be given to patients without anesthesia first. Results (Figures 3-6) show pharmacokinetic profiles of $\sim$Tc-Glutathione in normal mice and cancer model mice. Figures 3-6 shown predictive data which is the optimum picture determined by the software based on observational data. In the graph above there is a difference in Glutathione concentration (% ID/g) shortly after injection where the predicted graph determines the optimum point at 7% ID/g but does not affect the pharmacokinetic parameters. Pharmacokinetic profiles in groups I, II, III, and IV at 5 minutes after successive injections were 7.08, 11.25, 7.17, and 29.48%. After 24 hours after injection the pharmacokinetic profile decreased to 0.01, 0.05, 0.09, and 0.11%, respectively.
Figure 3. Pharmacokinetic profile in normal mice (\textsuperscript{99m}Tc-Glutathione)

Figure 4. Pharmacokinetic profile in normal mice (Doxorubicin + \textsuperscript{99m}Tc-Glutathione)
The results of pharmacokinetic studies in Tables 1 and 2 show that the administration of doxorubicin affects several $^{99m}$Tc-Glutathione pharmacokinetic profiles in normal mice and cancer model mice.
Table 1. 99mTc-Glutathione pharmacokinetic profile in normal mice

| Pharmacokinetic profiles | Normal Mice | Doxorubicin + 99mTc-Glutathione | Sig. |
|--------------------------|-------------|-------------------------------|------|
| K12                      | 0.001±0.001 | 0.0003±0.0003                 | NS   |
| K21                      | 0.134±0.275 | 0.1341±0.0275                 | NS   |
| t1/2 α                   | 0.004±0.001 | 0.0037±0.0001                 | NS   |
| t1/2 β                   | 5.310±1.050 | 10.7344±0.4692                | S    |
| Cmax                     | 6.132±5.161 | 6.1319±5.1613                 | NS   |
| AUC0-inf                 | 12.264±10.322 | 7.4520±2.5616              | NS   |

Table 2. 99mTc-Glutathione pharmacokinetic profile in cancer model mice

| Pharmacokinetic profiles | Cancer Model Mice | Doxorubicin + 99mTc-Glutathione | Sig. |
|--------------------------|------------------|-------------------------------|------|
| K12                      | 0.003±0.003     | 0.0007±0.0003                 | NS   |
| K21                      | 0.067±0.099     | 0.0281±0.0082                 | NS   |
| t1/2 α                   | 0.003±0.000     | 0.0037±0.0001                 | NS   |
| t1/2 β                   | 72.712±2.427    | 26.9320±7.8152                | S    |
| Cmax                     | 2.436±0.569     | 5.4331±5.1505                 | NS   |
| AUC0-inf                 | 4.343±1.159     | 11.1996±10,0057                | NS   |

Pharmacokinetic profiles t-test results in Table 1, administration of doxorubicin before 99mTc-Glutathione in normal mice affects the elimination half-life of 99mTc-Glutathione significantly with a p-value <0.05, being longer than 5.3 hours to 10.7 hours, while for the distribution half-life was relatively similar. The administration of doxorubicin before 99mTc-Glutathione in the cancer model mice shown in Table 2 also significantly affected the elimination half-life by p-value <0.05, but the changes that occurred were different from those of normal mice, the elimination half-time decreased from 72.7 hours to 26.9 hours, 45.8 hours faster than control (without doxorubicin). Distribution and elimination half-life that was thought to occur in radiopharmaceuticals in the blood. Distribution half-life occurred when radiopharmaceutical was spread through various organs and tissues, while elimination half-life occurred when radiopharmaceutical was eliminated by biochemical processes and radioactive decay.

The administration of doxorubicin before 99mTc-Glutathione by venous injection also reduced AUC0-inf value (area under the curve) from normal mice compared to control mice that were only given 99mTc-Glutathione, AUC0-inf value decreased from 12.3 hours to 7.4 hours, which means absorption of 99mTc-Glutathione in normal mice by administration doxorubicin before 99mTc-Glutathione was lower than normal mice that were only given 99mTc-Glutathione, but based on the results of statistical analysis the difference was not significant (p>0.005). The same in cancer model mice, administration of doxorubicin before administration of 99mTc-Glutathione affects the Cmax (peak concentration) and AUC0-inf values. The Cmax value was longer than 3 hours from 2.4 hours to 5.4 hours, while the AUC0-inf value was 6.9 hours longer from 4.3 hours to 11.2 hours, but based on the results of statistical analysis the difference was not significant (p>0.005).

The mechanism of localization of 99mTc-Glutathione was generally through permeability and an increase in blood flow. Blood clearance of 99mTc-Glutathione requires a relatively long time because it was caused by the binding of proteins in the localization to extend 99mTc-Glutathione retention in the target tissue. This was proven by the elimination half-life in cancer model mice (t1/2 elimination 72.7 hours) 67 hours longer than the elimination half-life in normal mice (t1/2 elimination 5.3 hours). The administration of doxorubicin cancer drug before giving 99mTc-Glutathione in cancer model mice accelerates the elimination half-life of 99mTc-Glutathione. These results suggest that the interaction between doxorubicin and 99mTc-Glutathione can alter the pharmacokinetic profile of 99mTc-Glutathione and
may complicate the interpretation of scintigraphic imaging. According to Razaq et al., changes in the distribution, absorption, retention, and elimination of radiopharmaceuticals were caused by changes in blood flow, metabolism and binding of the radiopharmaceutical to blood plasma [4]. This hypothesis was confirmed by the fact that doxorubicin is also a metal chelator [19], and undergoes changes in biodistribution when forming complex compounds [20]. As a result, ~Tc-Glutathione is chelated by doxorubicin and does not accumulate in the target organ [21], in addition to that there is competition between doxorubicin and ~Tc-Glutathione in the target tissue so that the elimination half-life of ~Tc-Glutathione in the target tissue decreases significantly.

4. Conclusion
The results of pharmacokinetic interaction studies show that administration of the cancer drug Doxorubicin before administration of ~Tc-Glutathione needs to be avoided because it can affect the elimination half-life of ~Tc-Glutathione. The alteration in elimination half-life can result in misdiagnosis results. In addition, further studies are needed regarding the biodistribution and toxicity profile of the doxorubicin interaction with the ~Tc-Glutathione radiopharmaceutical.

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