Labelling of Red Blood Cells with Technetium-99m for Nuclear Medicine Studies

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ABSTRACT

Autologous red blood cells can be labelled with the gamma emitting radionuclide, technetium-99m (Tc-99m) and used for nuclear medicine imaging procedures. Over the last 15 years, the use of Tc-99m red blood cells has ranged from placental and spleen imaging studies to gastrointestinal bleeding and ventricular radiography studies. In vitro, in vivo *and* modified in vivo *methods have been described by several authors to maximize the efficiency of the Tc-99m binding to red cells. The mechanism of radiolabelling likely involves the binding of a reduced form of Tc-99m to intracellular components in the red cell. The amount of stannous ion used as a reducing agent is important in providing maximal labelling. Interactions with various drugs including heparin, doxorubicin, iodinated contrast media, methyldopa, quinidine and digoxin have been reported to interfere with the labelling efficiency.*

Key Words: *red blood cells, Tc-99m, drug interactions, nuclear medicine*

RÉSUMÉ

Le gamma radionuclide émis par le technétium 99M (^{99}Tc) peut être étiquetté avec les globules rouges autologues pour l'utilisation de la visualisation de la médecine nucléaire. Durant les quinze dernières années, l'utilisation des globules rouges fixés au ^{99}Tc a démontré une bonne visualisation de la rate du placenta ainsi que des saignements gastro-intestinaux et ventriculaires. Certains auteurs ont décrit les méthodes in vitro, in vivo *et* in vitro modifiées *maximisant l'efficacité de la fixation du ^{99}Tc réduit aux éléments intracellulaires des globules rouges. Il est important de fournir l'étiquetage au maximum à cause du montant d'ions stanneux utilisés comme agent de réduction. Quelques interactions médicamenteuses ont été rapporté avec certains agents dont l'héparine, la doxorubicine, les produits de contrastes iodés, la méthyldolpa, la quinidine et la digoxine qui ont interféré avec l'efficacité de l'étiquetage.*

Mots clés: *globules rouges, interactions médicamenteuses, médecine nucléaire, ^{99}Tc*

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INTRODUCTION

Tc-99m labelled red blood cells have been used in nuclear medicine for more than 20 years. In the latter half of the 1960s, Tc-99m red blood cells (RBCs) were utilized to image the spleen and the placenta.[1] In the early 1970s, the volume of red blood cells in humans was determined using this radiopharmaceutical.[1] Its use was expanded to radionuclide ventriculography studies in the latter half of the 70s and more recently to occult gastrointestinal bleeding studies. In 1989, over 1417 studies were completed at the Toronto General Hospital involving Tc-99m RBCs.

Since Tc-99m must be labelled to the red blood cells, the technique used should be simple and reliable. Smith and Richards in 1975 introduced an *in vitro* method.[2] Although this method produced a high quality product, further improvements were made by the introduction of *in vivo* labelling of red blood cells with Tc-99m by Pavel *et. al.* in 1977.[3] However, only 65-70% of Tc-99m was tagged to the red blood cells. The remaining 30-35% of free Tc-99m caused imaging problems in ventriculography and GI bleeding studies, when the unbound Tc-99m was seen in the kidneys, gastric region and bladder. This led to the development of a "modified *in vivo*" technique by Callahan *et. al.* in 1982.[4]

This technique involves the IV administration of stannous pyrophosphate or any other stannous compound (as in the original *in vivo* method) but later removing a certain volume of the patient's blood, incubating with Tc-99m pertechnetate and re-injecting the labelled blood into the patient. This technique produced a high labelling yield and has gained wide acceptance.[5]

Clinical Utility

Tc-99m RBCs have been used mainly for cardiovascular imaging

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studies. By using a gamma camera linked to a computer, ejection of the radiolabelled erythrocytes by the heart can be visualized and quantified. Measurements of heart wall function and ventricular ejection fraction can be made. These measurements can be utilized to diagnose conditions such as coronary artery disease, cardiomyopathies, doxorubicin cardiotoxicity, valvular heart disease, cardiac shunts, ventricular aneurysms, and myocardial contusions.[6] In 1989, at the Toronto General Hospital, there were 1325 cardiovascular imaging studies carried out using radiolabelled erythrocytes.

Since Tc-99m RBCs are constantly circulating in the body, intermittent lower GI bleeding or colonic hemorrhage can be diagnosed over 12-24 hours. In contrast, another radiopharmaceutical, Tc-99m sulfur colloid can only be used to detect GI bleeding which is active at the time of radiopharmaceutical administration since its clearance from the blood is very rapid. Bleeding sites of the upper GI tract (esophagus and stomach) are best diagnosed by endoscopy rather than radionuclide studies.[6] In 1989, 44 GI bleed studies were done at Toronto General Hospital using Tc-99m red blood cells.

The labelled red cells can also be heat damaged by heating at 50°C for about 20 minutes before injection.[7] These damaged cells once injected are taken up by the spleen to diagnose conditions such as splenomegaly, splenic infarction, tumours of the spleen, hematomas secondary to trauma and accessory spleens.[6]

Other more recent clinical uses of Tc-99m RBCs include equilibrium venography[8] and hepatic hemangioma detection.[9]

Methods of Preparation

Several methods of preparation are available to label red blood cells. They are *in vitro, in vivo* or both (*"modified in vivo"* or *in vivtro*).

***In Vivo* Method:** The *in vivo* method (Figure 1-A) involves the intravenous administration of stannous compounds in order to "pretin" the patient's red blood cells. Stannous ion is usually injected as part of a solution of stannous chloride and sodium pyrophosphate. Stannous pyrophosphate should be injected within 10-15 minutes after reconstitution with saline. This is necessary to avoid oxidation of stannous ion to stannic ion which would then result in a decrease in labelling yield. Tc-99m pertechnetate is then injected 30 minutes later. The pre-tinning of red blood cells allows the Tc-99m pertechnetate to be reduced once it enters the cells. The reduced radionuclide then becomes bound to intracellular components.[10] Labelling efficiency ranges from 60-90%.

Modifications have been proposed for the *in vivo* method. Esquerre *et. al.*[11] was the first to demonstrate that the administration of potassium perchlorate with cold pyrophosphate improves imaging. Bekdik *et. al.*[12] also recom-

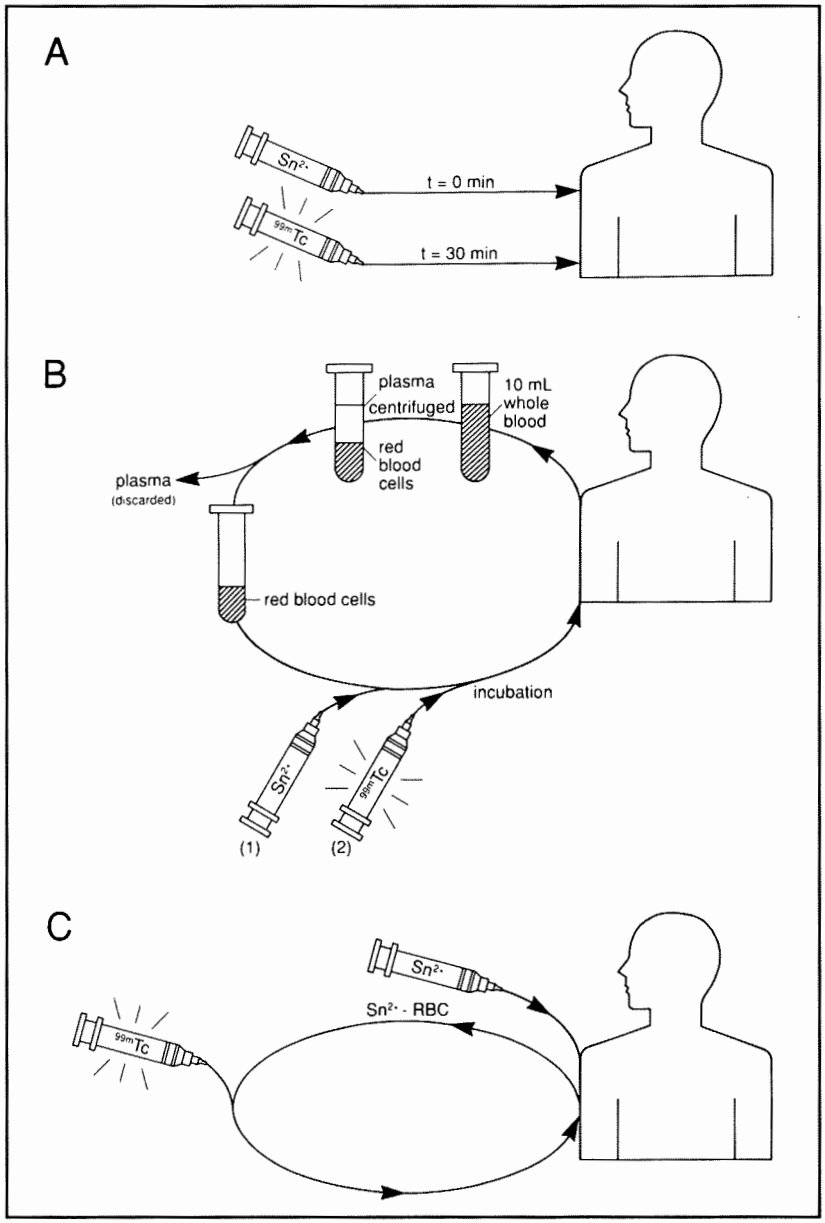

Figure 1: Methods for radiolabelling red blood cells with Tc-99m, A:*in vivo* method, B:*in vitro* method, C:*in vivtro* or modified *in vivo* method.

mended the oral administration of potassium perchlorate before the IV injection of pertechnetate to block the uptake of Tc-99m by the thyroid, salivary glands and gastric mucosa. With these modifications, improved labelling efficiency and quality of gated cardiac imaging was observed. However, despite the usefulness of potassium perchlorate to improve imaging, potassium perchlorate is usually not needed if the timing of injections is followed strictly.

***In Vitro* Method:** *In vitro* radiolabelling (Figure 1-B) can be used when higher labelling efficiencies are needed. In this case, a sample of blood is withdrawn from a patient. The red blood cells are separated and then pre-tinned with a stannous compound. The pretinned red cells are then labelled with Tc-99m and reinjected into the patient. Labelling efficiencies can be as high as 98% or more.[10]

***"In Vivtro"* or Modified *In Vivo* Method:** An alternate method, the *"in vivtro"* technique (Figure 1-C) involves a combination of the *in vivo* and *in vitro* methods. Callahan *et. al.* developed a technique involving the injection of a stannous compound into the patient, removal of a sample of the patient's blood, followed by incubation of the blood sample with Tc-99m.[4,10] This method prevents excess pertechnetate from distributing to the extravascular compartments.[4] The labelled blood is then re-injected into the patient. The combined technique gives reproducible, good labelling yields and improved image quality. ACD (Acid-citrate-dextrose) is the recommended anticoagulant to use for this method.[13,10] If sufficient time is allowed for incubation (ie. 10 minutes), labelling yields are around 90% at the time of injection.[4]

Mechanism of radiolabelling

Presently, the mechanism of radiolabelling of Tc-99m red blood cells is not entirely understood. However, it is known that the quantity of Sn^{2+} agent is important for binding pertechnetate to the red blood cells *in vitro*. It has been postulated that the stannous ion, Sn^{2+}, diffuses into the cell and cannot escape due to intracellular binding. The Tc-99m pertechnetate crosses the cell membrane, becomes reduced by the stannous ion and then binds to proteins such as hemoglobin.[7,14]

Several studies have evaluated the mechanisms by which Tc-99m binds to stannous-primed cells. Selding *et. al.*[14] found a linear relationship between the amount of red cell surface charge and the labelling efficiency. However, a reduction of charge could only decrease labelling efficiency by 80%. It was concluded that the reduced charge increased membrane permeability for intracellular technetium to diffuse out of the cell, resulting in a decreased labelling efficiency. The residual 20% of intracellular Tc-99m may be bound to hemoglobin which is unaffected by the change in membrane permeability.

Billinghurst and Waddell[15] analyzed the mechanisms by which pre-tinned red cells bind with Tc-99m. After the cells were mixed with stannous pyrophosphate, the uptake of Tc-99m was dependent on low stannous ion concentrations. However, at high stannous ion concentrations, the labelling yield decreased possibly due to conditions such as the binding of Tc-99m to plasma proteins or the displacement of the radionuclide by the pyrophosphate within the erythrocyte.

Biodistribution

There are two components to the blood disappearance curves; an initial phase with a half-life of 20 minutes and a terminal phase with a half-life of 29 hours. Five percent of the activity is seen in the spleen probably due to a proportion of the red blood cells that are damaged or due to an equilibrium with the cardiac blood pool. The cardiac chambers are well visualized, (Figure 2) particularly the left ventricle with its activity to background ratio of 2.5-2.7. The cardiac pool occupies less than half the diameter of the chest. Diminished activity is observed between the left ventricular chamber and the lung blood pool.[16]

Drug Interactions

Heparin: Heparin has been shown to decrease the imaging quality of *in vivo* labelled erythrocytes. When Tc-99m is injected through a heparinized catheter, a Tc-99m heparin complex may be formed, resulting in a reduced labelling efficiency. Thus, the injection of a stannous compound and Tc-99m pertechnetate through a heparin lock should be avoided.[17] Tc-99m red blood cells prepared with acid-citrate-dextrose (ACD) yielded superior binding efficiencies than with heparin. ACD is therefore the recommended anticoagulant for preparing *in vivo/in vitro* Tc-99m labelled red blood cells.[13]

Doxorubicin: Doxorubicin also may decrease the labelling efficiency. This decrease is dependent or varies directly with the drug's concentration in the blood at the time of the scan. Thus, attempts should be made for patients to avoid taking the drug on the same day of the GI bleed or ventriculography evaluation. However, it is unlikely that the poor labelling with the *in vivo* method observed one month after a dose of doxorubicin is due to the drug itself. More likely, it results from the lower hematocrit and/or hemoglobin concentration in these patients. It has been shown that *in vitro* and *"in vivtro"* methods result in a reasonably good labelling efficiency.[19] It is somewhat paradoxical that Tc-99m red blood cell ventriculography studies are used to monitor for cardiotoxicity of

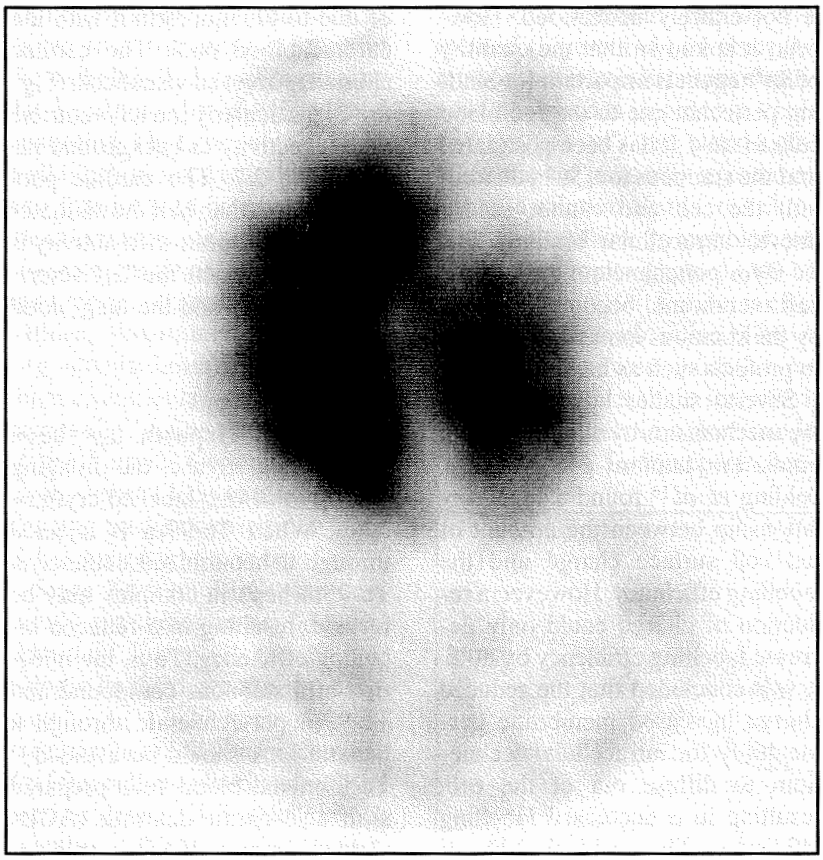

Figure 2: Left anterior oblique image of the heart following *in vivo* labelling of red blood cells with Tc-99m.

doxorubicin in patients taking this drug.

Contrast Agents: Several reports have mentioned the interaction of iodinated contrast media with red blood cell labelling. Tatum *et. al.*[5] have shown a suboptimal labelling efficiency in patients who have received iodinated contrast media prior to a red blood cell labelling study. The mechanism of such an interaction might be a change in redox potential of the stannous ion or technetium, an alteration in intracellular stannous ion concentration, the competition of Tc-99m and iodide to bind to the red blood cells or a direct alteration of binding sites of the red blood cells by iodide. Another mechanism that was postulated by Dawson *et. al.*[20] was a change in red blood cell morphology in the presence of the contrast media. However, Finkel *et. al.*[21] reported consistent and unaltered high labelling yields using different concentrations of iodinated contrast media. Generally, the interference of these contrast agents upon the labelling quality of Tc-99m to the red blood cells requires further study.

Other Drugs: Methyldopa and hydralazine also reduce the labelling efficiency by oxidizing the stannous ion, decreasing its ability to reduce the Tc-99m. Methyldopa and quinidine have also been associated with red blood cell antibody formation which may interfere with the labelling and imaging quality. To avoid any problems, methyldopa, hydralazine and quinidine should be discontinued if possible before any study is carried out.[17] Another potential interaction concerns patients using prazosin or digoxin (but not captopril) since a significant decrease in red blood cell labelling has been reported with these drugs.[18]

Cannulae: Millar and associates[22] have studied the influence of labelling efficiency using different types of cannulae. High labelling yields occurred after the administration of stannous pyrophosphate or a mixture of stannous pyrophosphate and heparin by means of the metal needles of intravenous cannulae. But there was a significant reduction in labelling efficiency and a more rapid clearance of Tc-99m from the blood when the tannous compound was given via teflon cannulae. This phenomenon was not because of the adsorption of stannous ion or pyrophosphate onto the teflon cannulae or the retention of stannous pyrophosphate in the cannulae. Generally, stannous compounds should not be injected by means of a teflon cannula.

Stannous Ion: The quantity of Sn^{2+} agent is an important determinant in blood pool imaging quality. If the amount of Sn^{2+} is too large (ie. 12.5 μg/kg body weight) the pertechnetate will be bound to other species found in the plasma other than the red blood cells. This can be shown *in vitro* as the labelling efficiency and the percentage of free Tc-99m found in the plasma decreases when the amount of stannous ion increases. *In vivo,* a more rapid blood clearance with increasing quantities of Sn^{2+} indicates that most of the radioactivity is not associated with the erythrocytes.[23]

If the amount is too small (ie. 2.5 μg/kg body weight), the pertechnetate will not become associated with the cells. The resulting free pertechnetate will show significant activity in the stomach, thyroid and salivary glands.

Some studies have established appropriate quantities of various stannous compounds (stannous pyrophosphate, stannous diphosphonate and stannous tartrate) to be injected. Maximal *in vivo* labelling efficiency was achieved with an IV dose of 10 μg Sn^{2+}/kg followed 5-30 minutes later by an injection

of sodium pertechnetate.[24] Billinghurst *et. al.*[23] attempted to determine optimal concentrations of stannous pyrophosphate for *in vivo* red cell labelling by incubating a blood sample containing heparin, with Tc-99m and a known volume of dilute stannous ion solution. A plot of the percentage of free Tc-99m in the plasma versus the volume of diluted tin solution used was obtained. It was concluded from this study that the volume of diluted stannous pyrophosphate to be used for maximal labelling corresponded to a level of 50% free Tc-99m in the plasma. From this, the volume of undiluted stannous pyrophosphate which should be injected could be calculated and is approximately 7.5 to 10 μg/kg. However, the optimum amount of stannous ion required for red blood cell labelling is highly dependent on the proportion of stannous and stannic ions in the pre-tinning agent. Each formulation may be different and the quantity of stannous ion reagent required should be individualized.

Salchi and associates[25] also tried to use optimal and minimal stannous quantities by using a novel technique involving an infusion catheter that has stannous ion adsorbed on its surface.

PROBLEMS AND LIMITATIONS

Though Tc-99m red blood cells have many uses, several problems and limitations are apparent. These include: (i) Organs like the salivary and thyroid glands and gastric mucosa compete with the red blood cells for the uptake of the Tc-99m pertechnetate. This competition may diminish *in vivo* labelling yields producing imaging problems. Also, possible visualization of the stomach may result in an inaccurate calculation of the left ventricular ejection fraction;[12] (ii) The *in vitro* procedure is tedious, time consuming and has an increased risk of microbial contamination due to the large number of steps involved;[10] (iii) The *in vivo* technique results in a labelling yield which is lower relative to the other methods. (iv) Heparin as an anticoagulant within the syringe is reported to decrease the labelling efficiency. Acid-citrate dextrose is a preferred anticoagulant;[13] (v) During the manufacturing of stannous reagents, the oxidation of stannous ion to stannic ion will result in a decreased capacity to reduce the Tc-99m *in vivo*; (vi) Another problem concerns the use of a Tc-99m generator with a long "in-growth" time because the accumulation of stable Tc-99 reduces the ability of radioactive Tc-99m to bind to the erythrocytes; (vii) Agglutination of labelled red cells is possible by the presence of aluminum in the Tc-99m eluate. Thus, tests to check for aluminum should be carried out;[7] (viii) Anatomical abnormalities of the red cells like sickle cell hemoglobin may decrease labelling efficiency;[26] (ix) Red blood cell antibody formation[26] in the presence of methyldopa and quinidine decreases labelling efficiency; (x) Drug and non-drug interactions may decrease labelling efficiency and these should be considered; (xi) The use of teflon cannulae instead of intravenous cannulae is not recommended.

SUMMARY

As well as labelling various reagents and compounds, Tc-99m can also be bound to biological entities such as red blood cells. The labelling of red cells requires the pretinning of red blood cells prior to the addition of Tc-99m sodium pertechnetate. Despite the possible interference in labelling efficiency by some drugs and other biological and chemical factors, Tc-99m labelled red blood cells are an important tool for diagnostic imaging studies in nuclear medicine.

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