The University of New Mexico ◆ Health Sciences Center
COLLEGE OF PHARMACY

UNM RADIOPHARMACY 1973 2003 30 YEARS OF EXCELLENCE

.::VOLUME 12, LESSON 1::.

Radiolabeled Red Blood Cells: Method and Mechanisms

Continuing Education for Nuclear Pharmacists and Nuclear Medicine Professionals

By

Ronald J. Callahan, Ph.D.
Associate Professor of Radiology
Harvard Medical School
Director of Nuclear Pharmacy
Massachusetts General Hospital

The University of New Mexico Health Sciences Center College of Pharmacy is accredited by the Accreditation Council for Pharmacy Education as a provider of continuing pharmaceutical education. Program No. 039-000-06-121-H04. 2.5 Contact Hours or .25 CEUs. Expires 05/24/2009.

Radiolabeled Red Blood Cells: Method and Mechanisms

By

Ronald J. Callahan, Ph.D.

RADIOLABELED RED BLOOD CELLS: METHOD AND MECHANISMS

STATEMENT OF OBJECTIVES

Upon completion of this course you will be able to discuss the methods and mechanisms by which human red blood cells are radiolabeled with Tc-99m.

Specifically, the recipient should be able to:

1. List currently available methods by which human red blood cells are labeled with Tc-99m for clinical use.

2. Define the three general steps involved in any method of radiolabeling red blood cells with Tc-99m.

3. Compare and contrast how each of these general steps is accomplished using currently available method.

4. State the relative advantages and disadvantages of currently available methods.

5. Describe the role of each component found in products used to radiolabel human red blood cells with Tc-99m.

6. Describe the pharmacokinetics of radiolabeled red blood cells.

7. Present the currently accepted mechanisms involved in the labeling process.

8. List several drug-drug interactions that interfere in the labeling process.

9. Discuss operator and patient safety issues associated with radiolabeled autologous blood products.

COURSE OUTLINE

RADIOLABELED RED BLOOD CELLS: METHODS AND MECHANISMS

By

Ronald J. Callahan, Ph.D.
Associate Professor of Radiology
Harvard Medical School
Director of Nuclear Pharmacy
Massachusetts General Hospital

INTRODUCTION

Radiolabeled red blood cells have played an important role as diagnostic radiopharmaceuticals for many decades. Their current role as the drug of choice for cardiac blood pool imaging has resulted in an evolution in the methods of labeling and a better understanding of labeling mechanisms. In this article the use of radiolabeled red blood cells as a diagnostic radiopharmaceutical will be reviewed, current labeling methods will be presented and the understanding of the mechanisms by which these cells are labeled will be discussed. Emphasis will be placed on technetium-99m red blood cells due to their importance in contemporary nuclear medicine practice.

Since the first version of this lesson was published in 1992 drug product selection for blood pool imaging has remained essentially unchanged. Autologous blood products remain in our formularies even with the risks and technical difficulties associated with the handling and use of these products. While novel macromolecules like radiolabeled synthetic graft co-polymers and derivatized human serum albumin products have been reported in recent years, including limited use in humans, a commercially available blood pool imaging product has not emerged. It is likely that high development costs and limited market potential for this class of drugs has prevented this from occurring.

While our understanding of basic methods and mechanisms remains essentially unchanged, widespread use of Tc-99m red blood cells has resulted in increased awareness of potential pitfalls and identification of interfering factors that can adversely affect the in vivo behavior, and thus, clinical utility of Tc-99m red blood cell products. This lesson will update our current understanding of these topics.

CLINICAL INDICATIONS

The use of radiolabeled red blood cells includes five major areas:

1. Measurement of total red blood cell volume
2. Measurement of red blood cell survival time
3. Identification of sites of red blood cell destruction
4. Blood pool imaging studies including gated cardiac imaging and gastrointestinal bleeding
5. Selective spleen imaging with damaged red blood cells

The ideal properties of labeled red blood cells used for each of these indications are different. The physical properties of the radionuclide, in vivo stability, and ease of labeling all have different importance depending on the study to be performed. For example, determination of red blood cell

survival time requires a radionuclide with a relatively long physical half-life and good in vivo stability whereas cardiac blood pool imaging studies are usually complete within 1 hour and require a short half-life radionuclide. However, since these studies may be a high volume study in many nuclear medicine departments, the speed and ease of labeling becomes an important consideration.

REVIEW OF LABELING METHODS

The use of radioactive nuclides in the labeling of erythrocytes dates back to the work of Nobel laureate, George de Hevesy, when he introduced in 1942 the use of P-32 labeled erythrocytes for the determination of blood volume in patients. In this method, in vitro incubation of P-32 with red blood cells allows the erythrocyte hexoses and trioses to bind the P-32.

Sterling and Gray who observed that hexavalent Cr-51 in the form of sodium chromate provides a suitable label for red blood cells described an improved labeling technique in 1950. Cr-51 was incubated in a manner similar to that of P-32. The Cr-51 method replaced the P-32 technique and is still a commonly used labeling method. However, the relatively low abundance (9%) of the 320 keV gamma ray of Cr-51 makes it unsuited for external imaging procedures. Nonetheless, Cr-51 labeled red blood cells continue to be used, albeit with decreasing frequency, for certain lab tests involving counting of blood samples, namely measurement of total red cell volume and measurement of red blood cell survival time.

Radioisotopes of iron have been used extensively to study red blood cells. When radioactive iron is injected intravenously, it is cleared rapidly from the circulation (half-time 60 to 120 min) and about 80% of it is incorporated into the newly formed cells over the next 7 to 10 days. Unfortunately, the iron liberated from destroyed red cells is reutilized and rapidly reappears in newly formed cells. Radioactive iron, therefore, cannot be used clinically for autologous red cell survival studies, except in special circumstances, e.g., aplastic anemia when reutilization would be minimal. The principal isotopes of iron used in these studies are Fe-55 and Fe-59. Neither of these iron isotopes is currently marketed as radiopharmaceuticals in the US.

Several other methods for labeling erythrocytes have been reported over the years. Because glycine is incorporated into protoporphyrin during heme synthesis, C-14 labeled glycine has been used as a label for red blood cells. C-14 glycine is not marketed as a radiopharmaceutical in the US.

When Hg-197 or Hg-203 labeled bromomercuryhydroxypropane (BMHP) is incubated at room temperature with whole blood, 90-98% of the label is rapidly bound to red blood cells. When a sufficient concentration of non-radioactive mercuryhydroxypropane (MHP) is added to the cells, they are altered in such a manner that they are selectively removed from the circulation by the spleen. This damage can also be induced by heat and other chemical methods. Labeled red blood cells damaged in this fashion are useful for selective spleen scanning. Although radio-mercury radiopharmaceuticals are no longer marketed in the US, Tc-99m labeled red blood cells (see below) damaged by heat are currently used, albeit infrequently, for selective spleen imaging.

In 1968, Rb-81 was described as a suitable red blood cell label. The main advantage of the Rb-81 is its short physical half-life (4.7 hrs.) and suitable gamma-ray energy. It has also been reported useful for quantitative estimation of red cell uptake in the spleen. Rb-81 is not marketed as a radiopharmaceutical in the US.

A method for the measurement of red cell mass in the spleen by radionuclide scanning after the injection of C-11 labeled carbon monoxide has been described. Because of its short physical half-life (20 min), large amounts of the radionuclide can be administered and the spleen visualized without damage to red blood cells. However, the major disadvantage of this method is the necessity of having a cyclotron nearby for the production of this short half-life position-emitting radionuclide. The recent increase in the number of positron emission tomography (PET) facilities may increase the interest in this novel technique.

The introduction of lipid-soluble complexes of In-111 led to the use of this radionuclide to label platelets and white blood cells. The physical half-life of 2.8 days and suitable gamma emissions of 174 and 247 keV make it ideal for monitoring physiologic processes which are several days in duration. In-111 labeled red blood cells have been proposed for detection of gastrointestinal bleeding and red blood cell sequestration and survival studies. In-111 oxine is a suitable product for labeling red blood cells as an off-label use.

Lipid-soluble complexes of Ga-67 and Ga-68 have also been reported as alternatives to more common methods for special applications such as the use of Ga-68 red blood cells in PET. Such radiogallium products are not marketed as radiopharmaceuticals in the US.

Tc-99m LABELED RED BLOOD CELLS (RBCs)

Many of the radionuclides previously mentioned above lack physical properties that allow for their use in imaging procedures. These limitations restricted the use of red blood cells labeled with these nuclides to in vitro determinations or external probe counting techniques. The availability of a radiotracer with physical properties suited to imaging techniques and with chemical properties which would permit efficient labeling to red blood cells has greatly expanded the usefulness of labeled red blood cells as a diagnostic agent. The introduction of Tc-99m has singularly had the greatest impact on radionuclide procedures, including those with labeled red blood cells.

The use of Tc-99m labeled red blood cells as a blood pool imaging agent in nuclear cardiology is well established. Clinical effectiveness of this agent is based on its ability to distribute primarily within the intravascular pool of the body and to leave this compartment at a slow rate. Such behavior allows for the accumulation of high resolution images which can be obtained with the aid of a physiological gating device. Combined with the gamma scintillation camera, this procedure can yield diagnostic information about dynamic processes such as regional myocardial wall motion and left ventricular ejection fraction.

Tc-99m as the pertechnetate ion is not firmly bound to red blood cells and will diffuse into the extravascular fluid compartment, with accumulation in organs such as the stomach, gut and thyroid gland. Such a distribution pattern results in lower blood-to-background activity ratios, poor detection of myocardial borders, interference with GI blood pool imaging and images which are difficult or impossible to interpret. It is, therefore, important that the Tc-99m be firmly and quantitatively bound to the cells and that this labeling persist in vivo during the observation period. In nuclear cardiology this time period may be 1 hour, while in the evaluation of gastrointestinal bleeding, the observation period may be as long as 24 hours.

Labeling of red blood cells with Tc-99m for spleen scanning was reported in 1967. However, efforts at that time to reproduce Tc-99m labeling of red blood cells had been unsuccessful. In these studies, Tc-99m was added as pertechnetate ion without the addition of any reducing agent. It is now well

known that pertechnetate ion (with Tc-99m in the +7 oxidation state) is nonreactive, and binding to cellular components would not be expected under the reaction conditions employed by these authors. In 1971, a labeling method employing stannous chloride as a reducing agent for technetium was introduced with labeling efficiencies of 50 to 60% reported. The method involved the incubation of washed cells with pertechnetate followed by the addition of stannous chloride solution. It was observed that the presence of plasma greatly reduced the labeling efficiency by this method but that the labeled cells exhibited good in vivo and in vitro stability. All of the early methods involved erythrocyte separation from anticoagulated whole blood by centrifugation with subsequent incubation with stannous chloride followed by pertechnetate, or with pertechnetate followed by stannous chloride.

GENERAL STEPS IN LABELING RBCs WITH Tc-99m

Before presenting details of current labeling methods, it is worthwhile discussing the general steps involved in labeling red blood cells with technetium since they are common to all methods. There are three general steps involved:

1. Treatment of RBCs with stannous ion
2. Removal of excess extracellular stannous ion
3. Addition of pertechnetate

Treatment of Red Blood Cells with Stannous Ion

Although it is technetium in the +7 (pertechnetate) oxidation state that crosses the intact erythrocyte membrane, only technetium that has been reduced to a lower oxidation state will firmly bind to hemoglobin. Stannous ions are most commonly employed for reduction of technetium and stannous chloride (as a stannous pyrophosphate complex) is preferred. At physiologic pH, stannous ions are subject to hydrolysis and precipitation that causes their rapid clearance from blood by the reticuloendothelial system. When complexed with pyrophosphate (or other soluble chelates), however, stannous ions are sufficiently soluble to be resistant to these effects, yet are not so strongly bound to pyrophosphate as to prevent their dissociation and passage into red blood cells. In the in vivo and modified in vivo methods, treatment with stannous ion is accomplished by the direct intravenous administration of stannous pyrophosphate. Other chelates of stannous ions can also be used (such as pentetate, medronate, etc.) and would yield radiolabeled red blood cells with varying degrees of efficiencies. Pyrophosophate seems nearly ideal, however, because (a) it maintains the solubility of stannous ions in serum until they come into contact with the red blood cells and (b) most kits contain an optimal amount of stannous ion.

Reports on the quantity of stannous ion required for RBC labeling have been confusing because the quantity of tin to be given is stated in terms of stannous ions, stannous chloride, or stannous pyrophosphate. For Tc-99m red blood labeling using the in-vivo or the modified in vivo technique, most clinicians utilize 10-20 micrograms Sn^{+2}/kg body weight. Depending upon the commercial formulation chosen, it may be necessary to inject one-third, one-half, or the entire contents of a vial of stannous pyrophosphate to provide required mass of stannous ions. When the in vitro method of radiolabeling is employed, a much smaller number of stannous ions are employed. Currently available in vitro kits contain a stated minimum of approximately 25 micrograms of stannous ion.

Removal of Extracellular Stannous Ions

The presence of stannous ion in the serum can result in the undesirable reduction of Tc-99m pertechnetate prior to its entry into the red blood cell. Only the oxidized form of Tc-99m can be transported by the erythrocyte membrane.

In either the in vivo or the modified in vivo method, biological clearance of excess stannous pyrophosphate is the method by which the concentration of extracellular stannous ions is reduced. The optimal time between the injection of stannous pyrophosphate and the administration of Tc-99m pertechnetate (in vivo method) or the incubation of the stannous ion pretreated cells with Tc-99m pertechnetate (modified in vivo method) is 20-30 minutes.

With the original in vitro labeling method, extracellular stannous ions were removed by centrifugation, a step that physically separates stannous-treated cells from the non-cellular associated stannous ion in serum. A modification of this vitro labeling is now commercially available (Ultra-Tag®, Mallinckrodt Medical, Inc.) and widely used. This product uses the non-penetrating oxidizing agent sodium hypochlorite to oxidize extracellular stannous ions, thus preventing the undesirable extracellular reduction of Tc-99m pertechnetate.

Addition of Tc-99m Pertechnetate

Actual red blood cell labeling with Tc-99m occurs whenever Tc-99m pertechnetate is brought into contact with RBCs that have been previously treated with stannous ions. This can be accomplished by either the in vivo or in vitro addition of Tc-99m pertechnetate to RBCs that have been pretreated with stannous ions.

CURRENT Tc-99m RBC LABELING METHODS

Nuclear medicine and nuclear pharmacy practitioners today have a choice of labeling methods from which to choose. With the approval of the commercially produced in vitro kit (Ultra-Tag®), there are now three methods available, each of which has distinct advantages and disadvantages. These methods use different combinations of physical, chemical and biological means to accomplish the three general steps listed above. The following section will compare and contrast available methods.

In Vitro Kits

Although the stannous chloride method of labeling autologous red blood cells resulted in a clinically useful radiopharmaceutical, the procedure was long and required multiple washing steps as well as the extemporaneous compounding of a stannous chloride solution suitable for intravenous injection. These disadvantages were partially eliminated with the introduction of simple kits for the preparation of Tc-99m red blood cells using stannous citrate and stannous glucoheptonate (gluceptate).

The introduction of these kits, although not widely available, greatly simplified the labeling procedure. One major advantage was that reagents could be prepared in advance and stored while quality control testing was undertaken.

The most widely used kit was that of Smith and Richards and is referred to as the Brookhaven National Laboratory (BNL) kit. A modification of the in vitro kit has been introduced and is commercially available (Ultra-Tag®, Mallinckrodt Medical, St. Louis, MO). With this latter product, a small amount

of sodium hypochlorite is added to whole blood that has been previously treated with stannous ion. Extracellular stannous ions are oxidized to the stannic form, and interference with labeling is minimized. Intracellular stannous ions are not affected by the addition of sodium hypochlorite because this agent does not penetrate the red cell membrane. Unlike the centrifugation method, the chemical oxidation method does not require separation of red cells and can be performed in whole blood. Elimination of centrifugation lessens the degree of cellular damage that may occur during radiolabeling as well as saving time and effort.

As a result of experiments performed in the development of the BNL kit, important observations of problems with some Tc-99m solutions were made. The consequences of the chemical effects of the total mass of technetium present in an eluate may not be routinely considered in the preparation of Tc-99m radiopharmaceuticals. However, in these experiments the Tc-99 in some generator eluates apparently exceeded the reductive capacity of the added stannous ion causing depressed labeling yields. It was pointed out that this problem may exist with other radiopharmaceuticals that use stannous ion, particularly when the quantity of Sn^{+2} used is very small (e.g. Ceretec®) or when poor formulation methods make the stannous ion unstable.

Consequently, in many radiopharmaceutical kits today the manufacturer's suggested methods for preparation include a consideration or restriction on the amount of time between generator elutions and the age of the eluate, factors that determine the total mass of technetium present in a generator eluate.

The chemical form of the stannous ion seems not to affect the labeling reaction since stannous ion has been combined with various anions including chloride, fluoride or citrate, and in conjunction with other ligand molecules such as glucoheptonate, methylene diphosphonate, or pyrophosphate. It is clearly the quantity of stannous ion that is most important, not the chemical form that most effects labeling efficiency.

In Vivo Methods

In 1975, several groups reported altered distribution of Tc-99m pertechnetate in brain scans of patients who had undergone previous Tc-99m pyrophosphate bone scans. In these patients, Tc-99m pertechnetate, which normally distributes throughout the extracellular fluid volume, was distributed primarily in the intravascular compartment. Further investigation showed that the majority of this intravascular radioactivity was associated with red blood cells. The occurrence of this phenomenon is affected by:

1. The amount of stannous ion administered in the bone scan dose
2. The interval between administration of pertechnetate and the brain scan
3. The interval of time between the bone scan and brain scan (no effect was observed when this interval exceeded 6 days)

While this observation was first reported as a drug interaction to be avoided, it was soon realized that this phenomenon could serve as a basis for the development of a new method for the in vivo labeling of red blood cells with Tc-99m using stannous pyrophosphate as the source of stannous ion.

In this method, labeling is accomplished with two consecutive intravenous injections. The first injection of non-radioactive stannous pyrophosphate is followed in 20-30 minutes by a second injection containing Tc-99m pertechnetate. Reported results for average labeling efficiency using the in vivo method vary widely from 71-96%. The interval between pyrophosphate and pertechnetate

injection also affects the composition of the plasma Tc-99m activity. With a short interval, the plasma activity is primarily Tc-99m pyrophosphate while as the interval increases to 30 minutes the technetium is equally divided between pertechnetate and pyrophosphate.

Note: Part of the explanation for the wide range of labeling efficiencies stated above may be related to differing definitions of "labeling efficiency." Some investigators simply centrifuged a blood sample and counted the radioactivity in plasma and in red blood cells; hence, labeling efficiency was simply the fraction of blood pool activity bound to red blood cells, yielding values as high as 96%. This definition, however, ignores Tc-99m that diffused into extravascular spaces or was localized in organs such as thyroid or stomach. Other investigators, in contrast, took into account non-blood pool activity and determined labeling efficiency as the fraction of injected Tc-99m activity bound to red blood cells. Values using these latter definitions of labeling efficiency are accordingly somewhat lower.

Modified in Vivo Methods

Currently, red blood cells can be labeled with Tc-99m by in vivo and in vitro techniques. Clinical comparisons have shown that the in vitro method results in a superior product. The need to remove a blood sample from the patient and the lack of a commercially available kit had prevented the method from gaining widespread acceptance. In vivo methods use readily available components and do not require blood samples to be removed from the patient. However, the quality of images obtained with the standard in vivo method was often of poor quality.

In an attempt to optimize the biological behavior of Tc-99m red blood cells, modifications of existing in vivo labeling techniques have been developed. One such method reported by our laboratory is called the modified in vivo labeling method. This method evolved from observations that the rate of incorporation of Tc-99m pertechnetate into human red blood cells in vivo proceeds at a measurable rate. During the time interval between i.v. injection of Tc-99m pertechnetate and firm binding to red blood cells, the Tc-99m is free to distribute to extracellular compartments and localize in organs such as thyroid and stomach. A standard in vivo technique was, therefore, modified so as to isolate pre-tinned red blood cells and Tc-99m pertechnetate from other body compartments during labeling. If sufficient time is allowed for the reaction to proceed to completion, approximately 90% of the total Tc-99m present will be firmly bound to the red blood cells at the time of injection. This results in increased intravascular retention and improved image quality.

Although any source of stannous ion may be suitable for this procedure, products containing the equivalent of 1 mg of stannous chloride dihydrate per vial are the most efficient and convenient. Products containing enough stannous ions for multiple patient doses may seem to be more economical. However, the possibility of oxidation of stannous ion in the unused portion of the vial may result in poor labeling of subsequent patients. Therefore, single dose preparations of stannous ion should be used in this method. It is important that sufficient time be allowed for distribution and clearance of extracellular stannous ion and pyrophosphate within the intravascular pool. For the modified in vivo method 15 to 20 minutes seems to be optimum.

Anticoagulation of the reaction mixture is provided by the residual heparin solution in the infusion set. It is, therefore, important that the line be first flushed with the heparin-containing solution before red blood cells are withdrawn into the syringe containing pertechnetate. The source of pertechnetate should be a generator which has been previously eluted within 24 hours. This limits the amount of Tc-99 that may be present in the eluate, which has been shown to exert an adverse effect on labeling efficiency. A standard incubation time for this method is 10 minutes at room temperature. However,

since factors such as temperature and hematocrit affect the rate of labeling, in certain instances it may be necessary to increase this incubation time.

Although this method may require a slightly longer labeling time than the standard in vivo method, the increased retention of intravascular Tc-99m results in shortened imaging time and, therefore, the total time necessary for the procedure is not lengthened over the standard in vivo labeling method.

PHARMACOKINETICS OF Tc-99m RBCs

The pharmacokinetics of technetium-99m red blood cells has been studied in patients and normal volunteers. After intravenous injection of pertechnetate during in vivo labeling, maximum whole blood activity was not reached until at least 30 minutes after injection. This suggests that pertechnetate freely diffuses into the extracellular fluid space, then re-enters the intravascular pool as blood levels of pertechnetate fall.

Whole-body clearance was found to be bi-exponential for both in vivo and in vitro methods. The in vivo method of labeling resulted in a short $T_{1/2}$ component of 2.5 ± 0.7 hr (10.9 ± 6.1%) and a long $T_{1/2}$ component of 176.6 ± 163.3 hr (90.5 ± 5.0%), whereas the in vitro method resulted in whole body retention components of 2.7 ± 1.5 hr (25.4 ± 10.4%) and 75.6 ± 25.3 hr (82.2 ± 7.7%).

COMPARISON OF RADIOPHARMACEUTICAL FOR BLOOD POOL IMAGING

Several studies have compared two or more radiopharmaceuticals used for blood pool imaging, including Tc-99m human serum albumin (HSA) and Tc-99m red blood cells (prepared by either the in vitro, modified in vivo, or the in vivo radiolabeling method). Based upon these studies, the following conclusions can be drawn:

1. Whenever Tc-99m HSA was compared to Tc-99m labeled red blood cells prepared by any method, labeled red blood cells were determined to be superior.

2. When in vivo and modified in vivo methods of labeling red blood cells were compared, the modified in vivo method was judged to be superior.

3. When in vitro labeled red blood cells were compared to in vivo and/or modified in vivo methods and judged on labeling efficiency and image quality, in vitro labeled cells were judged superior.

4. When availability and ease of labeling were considered in comparisons among all red blood cell labeling methods, the in vitro kit was found to be inferior because of the increased manipulation required and the potential for administration of cells to the wrong patient.

5. A comparison of all methods of red blood cell labeling showed that the modified in vivo method resulted in image quality approaching that of in vitro methods but is far more easily performed with readily available components.

6. When cardiac gated equilibrium blood-pool imaging studies are performed with Ultra-Tag® or standard in vivo methods, the heart-to-background ratios were significantly higher for the in vitro method.

For any given clinical situation, therefore, the selection of a blood pool agent will depend on the acceptable level of image quality, requirements for patient throughput, and the level of expertise of the technical staff. Patient acceptance may also influence product selection. For example, some patients may refuse in vitro labeled red blood cells based on religious beliefs regarding transfusions.

DRUG INTERFERENCE

Drug interference with Tc-99m red blood cells for equilibrium blood pool imaging can be classified into two general categories: (1) agents that alter, by a direct pharmacological effect, cardiac function and have the potential to interfere with the interpretation of equilibrium blood pool images, or (2) agents that inhibit or diminish the radiolabeling or red blood cells by Tc-99m.

Agents that induce an alteration in cardiac function include (a) the beta adrenergic blockers, such as propranolol (b) calcium channel blockers, including verapamil and (c) the nitrates, notably, nitroglycerin. Studies performed in patients receiving these pharmaceuticals may not detect the presence of coronary artery disease or accurately reflect its severity.

It has been proposed that these interfering drugs be withdrawn from patients prior to exercise ventriculography. For beta blocking medications a 48-hour interval between withdrawal of the drug and the nuclear medicine study has been suggested, while for the calcium channel blockers the proposed interval is 48-72 hours, and 12 hours has been suggested for the nitrates.

Doxorubicin causes a dose-related cardiomyopathy that may interfere with the diagnosis of abnormal cardiac function. However, the radionuclide ventriculogram is often performed to monitor doxorubicin-induced cardiotoxicity.

Poor radiolabeling of red blood cells with Tc-99m or early dissociation of Tc-99m from the labeled red blood cell brought about by concomitant drug therapy can adversely affect image quality. Table 1 lists several of the drugs and conditions reported to interfere with Tc-99m red blood cell labeling or that may be responsible for deterioration of the labeled cell.

The anticoagulant used in red blood cell labeling techniques has been shown to affect the results. Decreased labeling and increased urinary excretion of Tc-99m has been reported when stannous pyrophosphate and Tc-99m pertechnetate were injected through a venous catheter containing heparin. The fact that heparin is used successfully as an anticoagulant in the in vitro and modified in vivo method suggest that the effect of heparin may be dose related or temporally related to the addition of the various components of the reaction. It is known that in the presence of stannous ion, Tc-99m will form a complex with heparin and it may be this complex that contributes to a decrease in image quality.

Both heparin and acid-citrate-dextrose (ACD) solution have been used as the anticoagulant in the modified in vivo method in patients and normal volunteers. Some groups report higher labeling efficiency and improved image quality with minimal renal urinary bladder activity with ACD. Others have shown labeling efficiency to be independent of the selection ACD or heparin as the anticoagulant.

It has been reported that recent transfusions of whole blood have a negative impact on labeling red blood cells with Tc-99m. Blood transfusions, especially frequent in nature, result in an increase in the levels of circulating free hemoglobin. It is known that in the presence of stannous ion, Tc-99m will form a high affinity stable complex with hemoglobin. Biodistribution studies in animals indicate that

this complex is rapidly cleared from the circulation and excreted in the urine. This mechanism may be a factor in the relationship of transfusions to altered biodistribution. Also, some investigators report an increase in anti red blood cell antibodies which could also be involved.

In addition to drugs, devices used to inject various components involved in radiolabeling red blood cells with Tc-99m can adversely affect labeling. Intravenous catheters and Butterfly™ infusion sets can result in binding of stannous ions thus reducing the administered amount of tin to sub-optimal levels, in addition, if Tc-99m is administered through the same device, Tc-99m will bind to the tubing.

With more widespread use of commercially available kits for in vitro labeling of red blood cells reports suggest that interference with cell labeling is decreased. Perhaps the optimum conditions present in the Ultra-Tag Kit® has increased the robustness of the labeling reaction and minimized physiologic and pathophysiologic factors.

Table 1

Drugs Suspected of Interfering with Labeling of Red Blood Cells with Tc-99m	
Drug / Device	**Possible Mechanism**
Heparin	Formation of Tc-99m labeled heparin
Methyldopa, hydralazine	Oxidation of Sn^{+2}
Chemotherapeutic Agents	Unknown
Whole Blood Transfusions	Formation of free Tc-99m hemoglobin; presence of anti-RBC antibodies
Intravenous Catheters	Binding of stannous ions to tubing
Digoxon, prazocin, propranolol	Unknown
Iodinated contrast media	Competition between iodide and pertechnetate for transport by the band-3 anion transport system

RADIATION DOSIMETRY

To calculate accurate radiation dosimetry estimates, detailed knowledge of the pharmacokinetics of the agent must be known and applied. For example, it is known that the initial distribution and rate of elimination of Tc-99m labeled red blood cells is a function of the method of labeling. To date, these factors have not been carefully applied to dosimetry calculations.

One factor that affects the dosimetry of blood pool imaging agents is the blood volume of the individual organs. This factor has been applied to dosimetry calculations. In these calculations, the effective half-life is assumed to be equal to the physical half-life, and distribution to the organs is assumed to be solely a function of blood volume. The dosimetry calculated by this method results in higher values than those given from other sources. These data are summarized in Table 2.

Table 2

Radiation Absorbed Dose Estimates for Tc-99m RBC (rads/mCi)		
Organ	**Estimates Using Cell Kinetics Data**	**Estimates Using Organ Blood Volume Data**
Total body	0.016	0.018-0.019
Spleen	0.018	0.039-0.062
Bladder Wall	0.12	-
Testes	0.012	-
Ovaries	0.02	-
Blood	0.052	-
Red Marrow	0.022	-
Liver	-	0.040-0.098
Kidneys	-	0.043-0.066
Lungs	-	0.048-0.064
Heart	-	0.075-0.081

MECHANISMS OF LABELING

It has been shown that in red blood cells labeled with Tc-99m, the majority of radioactivity is associated with hemoglobin. Further investigation has shown that 87% of the activity is associated with the globin portion of the molecule and 10% with the heme. It was therefore concluded that Tc-99m in the lower valence state (probably technetium +4) binds irreversibly with globin, with the highest specific activity found in the beta-chain, most probably by coordinate covalent bond formation.

Various studies have concluded that the process of pertechnetate binding to the red blood cell essentially involves passive diffusion of pertechnetate into the cell. More recently it has been shown that the pertechnetate ion is transported across the red blood cell membrane by the band-3 anion transport system. This system is responsible for maintaining the transmembrane concentrations of chloride and bicarbonate. Since there is no mechanism inside the cell to reduce pertechnetate in the absence of a reducing agent, pertechnetate is readily transported out of the cell by this system when the red blood cells are suspended in a vehicle containing chloride or bicarbonate as exchangers. The role of intracellular reduction of pertechnetate, which results in binding of the Tc-99m to hemoglobin, has been well documented.

FACTORS AFFECTING LABELING EFFICIENCY

Using a method to stop the labeling reaction between Tc-99m and red blood cells at the time of sampling, it has been shown that Tc-99m is incorporated into red blood cells in vivo at a measurable rate, reaching a value of 91.4% at 10 minutes following injection. This suggests that significant amounts of non-red blood cell-bound Tc-99m, probably as pertechnetate, is available for distribution to the extravascular compartments.

The incorporation of Tc-99m pertechnetate into pretinned red blood cells in a system isolated from other body compartments was shown to be affected significantly by the temperature and hematocrit of the reaction mixture, the dose of stannous ion administered and the presence of plasma. The volume of whole blood, activity of Tc-99m, and patient population have no significant effect on the rate or extent of Tc-99m labeling.

Temperature

There is a direct relationship between the temperature of the reaction mixture and rate and extent of labeling. The temperature data suggest that increased labeling and shorter incubation times could be obtained if, in the modified in vivo method, the syringes were maintained at 37ºC rather than allowed to slowly cool during the incubation period. Elevation of the syringe temperature to 49-50ºC for 35 minutes has been shown to sufficiently damage the red blood cells so as to be able to do selective spleen imaging.

Hematocrit

Whole blood hematocrit has a major effect on the rate and extent of red blood cell labeling. Normal values for the hematocrit vary with an individual's age and sex. The normal hematocrit value for adults is 36 to 46% for women and 42 to 52% for men. There is a slight decrease in the hematocrit level after 50 years of age. However, in patients with anemia and in patients with significant blood loss, hematocrit values as low as 12 to 15% can be seen. Thus, these individuals would be expected to show decreased labeling efficiency with resultant increase in extravascular concentration of Tc-99m activity. This effect may be partially overcome by increasing incubation time when patients with known low hematocrit values are studied with the modified in vivo method.

Volume of Whole Blood

Increasing the volume of whole blood from 1.5 to 4.5 ml in the modified in vivo method did not significantly alter the labeling parameters. The normal range of red blood cell count in men is 4.5-6.5E6/uL and in women it is 3.9-5.6E6/uL. Therefore, the number of cells in the whole blood reaction mixture can be varied from approximately 7.5E9-2.2E10 without effects on labeling.

It has been shown that the presence of plasma exerts a competing effect on labeling. The effect of diluting red blood cells with saline has less of an effect on relative labeling than when dilutions are done with plasma. This suggests that the effects of hematocrit on labeling efficiency is due partially to the concentration of red blood cells and partially to the presence of plasma.

Stannous Ion Dose

Changes in blood disappearance of Tc-99m pertechnetate at stannous ion doses as low as 1 μG/kG have been reported. A plateau of labeling efficiency at 10 μG/kG has been reported in several studies, which have also shown this to be the minimum dosage of stannous ion that resulted in satisfactory red blood cell labeling. Decreases in labeling efficiency have been reported at doses in the 35-40 μG/kG range.

Optimal amounts of stannous ion may be decreased in several situations to levels that result in suboptimal radiolabeling of red blood cells. These include oxidation of stannous ion in PYP vials reconstituted with normal saline and not used immediately for in vivo labeling; infiltration of the stannous pyrophosphate injection for in vivo or modified in vivo methods, resulting in inadequate Sn^{+2} delivered into the blood; injection of stannous solution through an IV catheter or tubing for in vivo or modified in vivo methods, resulting in binding of Sn^{+2} to the device; and premature addition of sodium hypochlorite during the in vitro method, thereby oxidizing Sn^{+2} before it enters red blood cells.

SAFETY CONSIDERATIONS

Few disciplines in medicine and pharmacy other than blood banks and nuclear medicine/nuclear pharmacy routinely withdraw blood, take it to a remote site and, after significant manipulation, re-inject it back into the patient minutes to hours later. This procedure presents significant risks to both the operator handling the product and the patient receiving it.

The risks associated with blood pool imaging are a function of the method selected for labeling the red blood cells with Tc-99m. The in vivo and modified in vivo methods carry minimal risks of needle stick to the operator and no risks of misadministration of blood products to the wrong patient.

In vitro kits for radiolabeling have greater risks of needle stick to the operator due to the number of needle and syringe based manipulations required. In addition since the blood is separated from the patient during the labeling procedure, an extraordinary level of safeguards must be present to prevent misadministration of blood products.

Written policies and procedures must be established by nuclear medicine clinics and nuclear pharmacies to define how autologous blood products are to be safely handled. Adequate labeling of all components with patient identification information and the requirement of re-injection of the labeled cells by the same person who withdrew it are the most basic. Various devices and systems of labeling and restricting access to the product have also been developed to improve patient safety.

Life threatening blood born diseases have been reported to have been transmitted by nuclear medicine personnel when using both autologous red cells and white cells. It is the responsibility of all nuclear medicine and nuclear pharmacy personnel to ensure that adequate safeguards are in place with the goal being the elimination of this serious problem.

SUMMARY AND CONCLUSIONS

Radiolabeled red blood cells represent a unique radiopharmaceutical dosage form which allows for the determination of red cell volume and kinetics and the visualization of the intravascular blood pool of organs. While a variety of radiolabels have been used in the past, Tc-99m has gained widespread use for imaging and in vitro measurements.

The selection of the method of labeling red blood cells with Tc-99m depends upon personal preference and the clinical indication being addressed. The modified in vivo method employs some aspects of the in vivo and in vitro methods and results in reproducibly high labeling efficiency without the added efforts of in vitro processing of cells. A readily available, robust kit for the labeling of red blood cells in vitro has increased the utilization of in vitro labeling methods.

The efficiency of labeling red blood cells is affected by temperature, hematocrit, stannous ion dose, mass of technetium, and choice of anticoagulant. Awareness of these factors and control of them, when possible, will result in a highly effective radiopharmaceutical for a variety of nuclear medicine procedures.

Despite risks and technical difficulties associated with the use of autologous blood products as diagnostic imaging agents, they remain in our formularies. Alternative blood pool imaging agents that do not share these problems have been identified and shown to be effective in humans. Unfortunately, high development costs and limited market potential have prevented these novel radiopharmaceuticals from becoming commercially available. For blood pool imaging, it seems autologous red blood cells are here to stay.

BIBLIOGRAPHY

1. Atkins HL, Srivastava SC, Meiken GE, et al. Biological behavior of erythrocytes labeled in vivo and in vitro with 99m-Tc. *J Nucl Med Technol.* 1985; 13:136-139.

2. Callahan RJ, Froelich JW, McKusick KA, et al. A modified method for the in vivo labeling of RBC with 99m-Tc. *J Nucl Med.* 1982; 23:315-318.

3. Callahan RJ. Radiolabeled red blood cells as diagnostic radiopharmaceuticals. In: Fritzberg AR, ed. *Radiopharmaceuticals: Progress and Clinical Perspectives.* Boca Raton, FL: CRC Press; 1986:41-60

4. Chilton H, Callahan RJ and Thrall JH. Radiopharmaceuticals for Cardiac Imaging. In: *Pharmaceuticals in Medical Imaging.* New York, NY: McMillan; 1990.

5. Dewanjee M. Binding of 99m-Tc ion to hemoglobin. *J Nucl Med.* 1974; 15:703-706.

6. Hamilton RG, Alderson PO. A comparative evaluation of technique for rapid and efficient in vivo labeling of red blood cells with 99m-Tc pertechnetate. *J Nucl Med.* 1977; 18:1010-1013.

7. Hladik WB III, Nigg KK, Rhodes BA. Drug-induced changes in the biologic distribution of radiopharmaceuticals. *Semin Nucl Med.* 1982; 12:184-218.

8. Pavel D, Zimmer ZM, Patterson VN. In vivo labeling of red blood cells with 99m-Tc: A new approach to blood pool visualization. *J Nucl Med.* 1977; 18:305-308.

9. Callahan RJ, Rabito CA. Radiolabeling of erythrocytes with Tc-99m: Role of band-3 protein in the transport of pertechnetate across the cell membrane. *J Nucl Med.* 1991; 31:2004-2010.

10. Smith TD, Richards P. A simple kit for the preparation of 99m-Tc labeled red blood cells. *J Nucl Med.* 1976; 17:126-132.

11. Srivastava SC, Chervu LR. Radionuclide labeled red blood cells. Current status and future prospects. *Semin Nucl Med.* 1982; 14:68-82.

12. Srivastava SC, Babich JB, Richards P. A new kit method for the selective labeling of erythrocytes in whole blood with technetium-99m (Abst). *J Nucl Med.* 1983; 24:P128.

13. Thrall JH, Freitas JE, Swanson D, et al. Clinical comparison of cardiac blood pool visualization with technetium-99m red blood cells labeled in vivo and with the technetium-99m human serum albumin. *J Nucl Med.* 1978; 19:796-803.

14. Bogdanov AA, Callahan RJ, Wilkinson RA, Martin C, Cameron JA, Fischman AJ, Brady TJ, Weissleder RJ. Synthetic copolymer kit for radionuclide blood-pool imaging. *J Nucl Med*. 1994; 35:1880-1886.

15. Hambye AS, Vandermeiren R, Vervaet J, and Vandevivere J. Failure to label red blood cells adequately in daily practice using an in vivo method: methodological and clinical considertions. *Eur J Nucl Med.* (1995) 22:61-67.

16. Callahan RJ and Bruce AD: Radiolabeling formed elements of blood: methods and mechanisms. In: Nuclear Medicine. Henkin RE, Boles MA, Dillehay GL, et al. (Editors) Mosby, St. Louis, MO.

QUESTIONS

1. Which of the following radionuclides has NOT been used to label red blood cells?

   A. Rb-81
   B. C-11
   C. K-43
   D. Ga-68

2. The first described use of radiolabeled red blood cells was for:

   A. equilibrium gated cardiac blood pool imaging.
   B. first pass angiocardiography.
   C. detection of gastrointestinal hemorrhage.
   D. measurement of total blood volume.

3. The mechanism of localization of Tc-99m red blood cells for cardiac blood pool imaging is best described as:

   A. active transport.
   B. capillary blockade.
   C. compartmentalization.
   D. extravasation.

4. The correct dosage for tin used in the in vivo labeling of red blood cells is:

   A. 10-20 micrograms stannous ion per kg body weight.
   B. the entire contents of any stannous pyrophosphate kit.
   C. 5 mg stannous pyrophosphate.
   D. 10 mg stannous gluceptate.

5. The role of stannous ion in the labeling of red blood cells with Tc-99m is the:

   A. extracellular reduction of pertechnetate.
   B. intracellular reduction of hemoglobin.
   C. intracellular reduction of pertechnetate.
   D. facilitation of Tc-99m transport into the cell.

6. Which of the following methods has NOT been used to remove extra-cellular stannous ion in labeling red blood cells with Tc-99m?

   A. centrifugation
   B. biological clearance
   C in vivo reduction
   D. oxidation

7. The only commercially available kit for the labeling of red blood cells with Tc-99m is based on the:

    A. modified in vivo method.
    B. in vitro labeling using chemical oxidation.
    C. in vitro labeling using centrifugation.
    D. in vivo method.

8. The pertechnetate ion reaches the intracellular space of the red blood cell via which of the following mechanisms?

    A. Na^+/K^+ ATPase pump
    B. calcium channel transport
    C. band-3 anion transport system
    D. passive diffusion

9. When using the modified in-vivo method for labeling red blood cells with Tc-99m in normal patients, which of the following factors has an inverse effect on labeling efficiency?

    A. hematocrit
    B. temperature
    C. volume of blood
    D. quantity of Tc-99m

10. In radiolabeled red blood cells, intracellular Tc-99m is found in the highest specific activity in:

    A. heme.
    B. alpha chain of globin.
    C. beta chain of globin.
    D. mitochondria.

11. The kinetics of Tc-99m labeling of stannous-treated red blood cells with Tc-99m suggests that the optimum incubation time (in minutes) is:

    A. 1.
    B. 10.
    C. 30.
    D. 60.

12. The temperature (degrees C) needed to damage red blood cells for selective spleen imaging is:

    A. 4.
    B. 37.
    C. 49.
    D. 64.

13. Increasing the dose of stannous ion from 10 micrograms/kg to 40 micrograms/kg has been reported to results in:

A. chemical damage to red blood cells.
B. increase in labeling efficiency.
C. decrease in labeling efficiency.
D hemolysis.

14. Which statement best describes the effect of injecting both stannous pyrophosphate and Tc-99m pertechnetate through a heparinized catheter?

A. decreased labeling efficiency
B. increased urinary excretion
C. labeling of catheter
D. all of the above

15. When all methods of labeling red blood cells with Tc-99m are compared, the method most often found to have the highest labeling efficiency is:

A. in vivo.
B. in vitro kit.
C. modified in vivo.
D. none of the above.

16. When performing gated cardiac imaging, the highest target to background ratios are obtained when using Tc-99m red blood cells labeled by which currently available method:

A. Brookhaven Kit.
B. modified in vivo.
C. in vivo.
D. Ultra-Tag® kit.

17. When ease of labeling and minimizing risk of misadministration are taken into consideration the most inferior of all methods is:

A. in vivo.
B. in vitro kit.
C. modified in vivo.
D. human serum albumin.

18. A drug which decreases the efficiency of labeling red blood cells with Tc-99m by interfering with transport of pertechnetate by the cell membrane is:

A. digoxin.
B. heparin.
C. prazocin.
D. iodinated contrast media.

19. A drug which will form a complex with reduced Tc-99m and thus compete for red blood cell labeling is:

A. doxorubicin.
B. propranolol.
C. heparin.
D. prazocin.

20. When using the modified in vivo method, the 20 minute interval between stannous ion injection and mixing blood with Tc-99m is required in order to allow time for:

A. uptake of tin by the RBC.
B. transport of pertechnetate by RBC membrane.
C. clearance of extracellular stannous ion.
D. equilibration of stannous pyrophosphate with blood.

21. The statement which best describes the whole body clearance of Tc-99m red blood cells is:

A. single exponential with a half-life of 120 hours.
B. single exponential with a half life of 24 hours.
C. bi-exponential with half lives of 2.5 and 75-175 hours.
D. bi-exponential with half lives of 12 and 210 hours.

22. Using the in vivo method, following the intravenous injection of pertechnetate, maximum whole blood activity is reached in _____ minutes(s).

A. 1
B. 10
C. 30
D. 60

23. When using in vitro labeled cells, the most important consideration in patient safety is:

A. minimizing cell lysis when withdrawing blood.
B. minimizing the chance for misadministration of blood products to the wrong patient.
C. limiting the quantity of time between blood draw and patient injection.
D. encouraging frequent voiding to minimize radiation absorbed dose to the bladder.

24. Risks associated with the use of autologous blood products in nuclear medicine / nuclear pharmacy include:

A. risk of needle stick injury to the operator.
B. risk of patient misadministration.
C. risk of transmission of life threatening blood borne diseases.
D. all of the above.

25. Possible causes of interference in RBC labeling in patients having frequent blood transfusions include:

A. crenation.
B. increased circulating free hemoglobin.
C. anti-nuclear antibody formation.
D. all of the above.