The Effect of Cyclosporine Concentration on the Labeling Efficiency of an In Vitro Technetium-99m Red Blood Cell Labeling Procedure

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Objective: The purpose of this study was to determine if the presence of cyclosporine A (CsA) in whole-blood samples decreased labeling efficiency when an in vitro red blood cell labeling procedure is used.

Methods: Red blood cells were labeled by an in vitro method which required sodium pertechnetate, disodium edetate (EDTA), stannous chloride and stopper down centrifugation. Labeling efficiencies were calculated and plotted.

Results: When 2 μg of stannous ion was used to label RBCs, no significant change in labeling efficiency was observed with different concentrations of CsA. Upon increasing the concentration of stannous ion per sample, no improvement in labeling efficiency was observed.

Conclusion: Cyclosporine levels in the blood do not affect the labeling of red blood cells if 2 μg of stannous ion per milliliter of patient whole blood are used. Quality RBC labeling with sodium pertechnetate is possible even when patients have high cyclosporine blood levels.

Key Words: stannous ion; labeling efficiency; cyclosporine A; technetium-99m red blood cells

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Cyclosporine A (CsA) is an immunosuppression drug used in many transplant patients for the control of transplanted organ rejection. In addition to its use in transplantation, it has been given to patients with autoimmune disorders (*1*). Cardiologists rely on the multigated acquisition (MUGA) nuclear medicine study to evaluate the cardiac function of heart transplants. MUGA studies on heart transplant patients are done with labeled red blood cells. These patients have CsA whole-blood levels of more than 250 to 300 μg/l (250 to 300 ηg/ml) during the first postoperative year (*2*).

Several articles have reported the possible interference of CsA with red blood cell (RBC) labeling. A study of a previously available in vitro kit (Brookhaven National Laboratories RBC Kit, Cadema Medical Products, Inc., Middletown, NY) demonstrated diminished RBC labeling in the presence of maintenance levels of CsA (*3*). A group of investigators led by Reisdorff found no labeling inhibition when using a stannous pyrophosphate (PYP) method in vitro (*4*). Yet another group led by Gleue found no labeling inhibition when using the UltraTag® in vitro kit and hypothesized that the amount of stannous ion determines labeling efficiency of CsA concentrated blood (*5*).

Commercial kits in today's market contain different levels of stannous ion. The Brookhaven National Laboratories RBC kit contains 2 μg of Sn^{2+} ion (*6*). The in vitro PYP method provides a maximum of 36 μg of Sn^{2+} ion (*4*). The UltraTag® kit uses 55 μg of Sn^{2+} ion (*7*).

The characteristic shared by CsA and ^{99m}Tc (using Sn^{2+} for ^{99m}Tc reduction and labeling of red blood cells) is that both are attracted to red blood cells. The stannous ion is necessary because pertechnetate freely diffuses in and out of cells. Technetium-99m reduced by stannous ion within the cells is bound to the beta-chain of hemoglobin and is retained by the cell (*8*). Experiments confirm that CsA is preferentially attracted to RBCs with estimates of 50–70% or even up to 90% binding to erythrocytes (*9*). According to Reichel et al., more than 70% of CsA is bound to erythrocytes at whole-blood concentrations of 50–1000 ηg CsA/ml whole blood. The total erythrocyte-binding capacity for CsA is 2.6×10^5 molecules CsA per erythrocyte (*10*).

MATERIALS AND METHODS

Sixty milliliters of whole blood were drawn from three volunteers using a 20-gauge needle. Each was collected in a 60-cc syringe containing 250 units of heparin sodium. Heparin sodium prevents coagulation at a ratio of 3.5–15 units per ml of whole blood (*11*). The individual syringes were kept at room temperature.

Sandimmune® (Sandoz Pharmaceuticals Corp., East Hanover, NJ) is a commercial drug form of cyclosporine A. The drug is available in capsule form, oral solution and intravenous ampules. An ampule containing 250 mg CsA in 5 ml was used. In this study, it was diluted to resemble typical concentrations of CsA in a 1-ml volume of transplant patient's

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blood. A volume of 0.1 ml of Sandimmune® was taken from a 5-ml sterile ampule for intravenous use. It was diluted by adding 99.9 ml sterile saline and mixing thoroughly. This created a concentration of 50 μg CsA/ml solution. The final dilution was made by taking 1 ml of the dilute CsA and adding to it 9 ml of saline and mixing thoroughly. This final concentration became 5 μg CsA/ml solution. Desired concentrations of CsA were pipetted from this final solution into 60 test tubes containing 2 ml of whole blood. Each test tube was gently mixed to ensure equal distribution of CsA without the effect of hemolysis. There were 20 tubes total from each volunteer. These 20 tubes were then divided in 5 sets with each set containing different concentrations of CsA. Each test tube of the first set contained no CsA, test tubes of the second set contained 250 ηg CsA/ml whole blood, and so on until each test tube of the last set contained 1000 ηg CsA/ml whole blood. Concentrations that are >400 ηg CsA/ml of whole blood are considered high CsA levels (*12*).

The process of labeling the red blood cells began after the calculated amount of CsA and 2 ml of whole blood were combined. The procedure used for labeling of the RBCs is the same as that published by Mock and Wellman (*13*). This method required the preparation of a stock stannous chloride solution and an EDTA solution. Stock stannous chloride solution was made by dissolving 2.0 g $SnCl_2 \cdot 2H_2O$ in 8.3 ml concentrated hydrochloric acid and diluting to a total volume of 100 ml with sterile water. Clarifying the solution is done by using 0.45-micron membrane filtration into clean serum vials. To prevent contamination, do not use a filtration unit that contains metal. Stoppers were placed on the vials and autoclaved for 20 min at 121°C. If autoclaving is not available, filter with 0.2-micron membrane filters into sterile vials using a Teflon™ catheter and not a metal needle. The concentration of stock tin solution to HCl is typically 9.5 to 10.5 mg Sn^{2+} per ml of 1 *N* HCl. This stock tin solution was refrigerated and should be discarded after 6 mo (*13*).

A fresh dilution is to be made prior to RBC labeling. This dilution was made due to its high acidity and its adverse affect on small volumes of whole blood. The dilute tin solution was made by adding 0.05 ml of stock tin solution to a 10-ml single-dose vial of sterile preservative-free saline. This dilute tin should be discarded after 30 min (*13*).

Stock EDTA solution was made by dissolving 5.0 g of disodium edetate in 100 ml sterile water and mixing until in solution. Place in sterile vials, autoclave or filter and refrigerate (*13*).

The protocol is as follows and should utilize aseptic techniques:

1. Add 2 ml heparinized blood to a sterile 7-ml vacutainer tube.
2. Using a tuberculin syringe, add 0.05 ml of the dilute tin solution (~2 μg Sn^{2+} ion), mix gently, and allow to stand for 1 min.
3. Add 0.1 ml of the 5% EDTA and 5 ml sterile saline. Use a vent needle to remove any vacuum or positive pressure. Remove vent needle and mix gently.
4. Place in a balanced centrifuge and spin inverted (stopper down) at 1000 *g* for 2 min.
5. Remove tube carefully to maintain the softly packed RBCs. Using a 18- or 20-gauge needle, barely penetrate the stopper, and slowly remove 0.5 ml of the tinned RBCs.
6. Transfer the RBCs to a second syringe containing the desired ^{99m}Tc activity, mix gently and allow 5 min for incubation. If the ratio of pertechnetate volume to RBC volume exceeds 2:1, allow additional incubation time.
7. Perform quality control before patient injection (*13*).

Upon completing the labeling process, this method can give labeling efficiencies as high as 97% (*13*). Protocol suggests using 2 μg Sn^{2+} to achieve a 97% labeling efficiency (*13*). In this study, the amount of stannous ion used to tag the RBCs was methodically increased to levels resembling the amounts of stannous ion in the previously studied kits; i.e. 2 μg, 15 μg, 30μg, and 45 μg. The samples were each labeled with approximately 1.5 mCi of sodium pertechnetate. The generator used to elute the sodium pertechnetate had a 24-hr ingrowth time. Of course, this activity of pertechnetate is not as high as a suggested clinical activity of 30 mCi. This lower activity was used to maintain the researcher's exposure to as low as reasonably achievable. Percent labeling efficiency was then calculated for each test tube by the following method currently in use at Indiana University Medical Center:

1. Using a dose calibrator to measure activity, place more than 250 μCi of the ^{99m}Tc RBCs in a separate test tube.
2. Record this activity.
3. Wash gently with 5 cc of sterile saline.
4. Give this tube containing the ^{99m}Tc RBC and 5 cc saline a hard spin (3000 rpm for 5 min) in a balanced centrifuge to separate free TcO_4 from the RBCs. Any free pertechnetate will travel away from the RBC pellet and into the saline.
5. Carefully remove the saline and free pertechnetate with a spinal needle attached to a 5-cc syringe and discard.
6. Place the test tube containing the RBC pellet back into the dose calibrator and record its activity.
7. The ratio of the primary activity to the secondary activity times 100% gives percent labeling efficiency (*13*).

RESULTS AND DISCUSSION

The percent labeling efficiencies obtained are given in Table 1. EDTA washing removes extracellular Sn2+ ion which, if left in the trapped plasma of the RBC pellet, can be reduced prior to penetrating the RBC membrane. This, in turn, can cause impurities like Sn:Tc colloid and/or labeled plasma proteins (*13*). Recent publications suggest that CsA will interfere with ^{99m}Tc RBC labeling if a low amount of Sn^{2+} is used. It has been suggested that CsA and its metabolites cause an interference to effective labeling of RBCs and that high concentrations of Sn^{2+} are necessary in this situation (*5*). Their hypothesis

TABLE 1
Average Percent Labeling Efficiencies and Standard Deviations Obtained from Blood Samples of Three Volunteers

	0 ηg CsA/ml whole blood	250 ηg CsA/ml whole blood	500 ηg CsA/ml whole blood	750 ηg CsA/ml whole blood	1000 ηg CsA/ml whole blood
2 μg Sn^{2+}	91 ± 2.0	91 ± 2.0	93 ± 1.2	93 ± 1.2	94 ± 1.9
15 μg Sn^{2+}	89 ± 2.0	89 ± 1.9	89 ± 2.1	91 ± 1.7	86 ± 1.9
30 μg Sn^{2+}	82 ± 1.6	82 ± 1.9	81 ± 1.9	82 ± 1.9	81 ± 2.2
45 μg Sn^{2+}	80 ± 1.6	75 ± 2.1	75 ± 1.7	72 ± 3.7	78 ± 3.1

was based on a study of the UltraTag® kit (Mallinckrodt Medical Inc., St. Louis, MO) which uses an oxidizing agent to remove this extracellular Sn^{2+} (7). With this in mind, refer to Figure 1 which shows that the averaged labeling efficiencies were not affected by CsA in whole blood samples when only 2 μg of Sn^{2+} were used.

Such a finding is significant. It nullifies the hypothesis that a decreased amount of stannous ion, in the presence of increasing concentrations of CsA, will inhibit effective red blood cell labeling with sodium pertechnetate. Figure 2 shows that increasing Sn^{2+} ion concentration does not increase labeling efficiency when high levels of CsA are present. In fact, the drop in labeling efficiency is associated to the increase in Sn^{2+} ion itself. Figure 3 shows this effect. This information shows the importance of using 2 μg of tin in this method to keep labeling efficiency at a suggested level of >90%. A labeling efficiency above 90% results in good image quality and less free pertechnetate (TcO_4) uptake in the thyroid and stomach. Technetium-labeled red blood cells are also used in upper gastrointestinal bleed localization. Therefore, a low percent labeling efficiency of RBCs leads to an increased amount of free pertechnetate and an increased amount of stomach uptake. This free TcO_4 uptake in and near the stomach could be mistaken for an upper gastrointestinal bleed.

It is interesting to point out that the best percent labeling efficiency found in this experiment does not reach the 97%

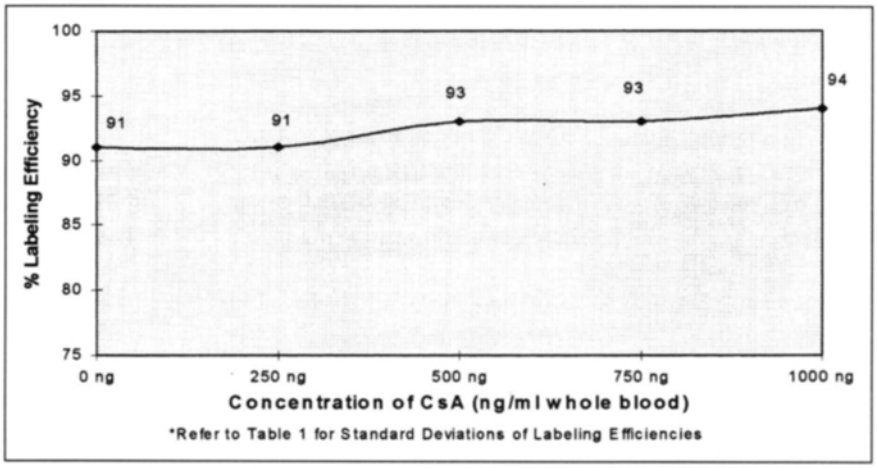

FIGURE 1. The average labeling efficiency* when using 2 μg of stannous ion and increasing the concentration of CsA.

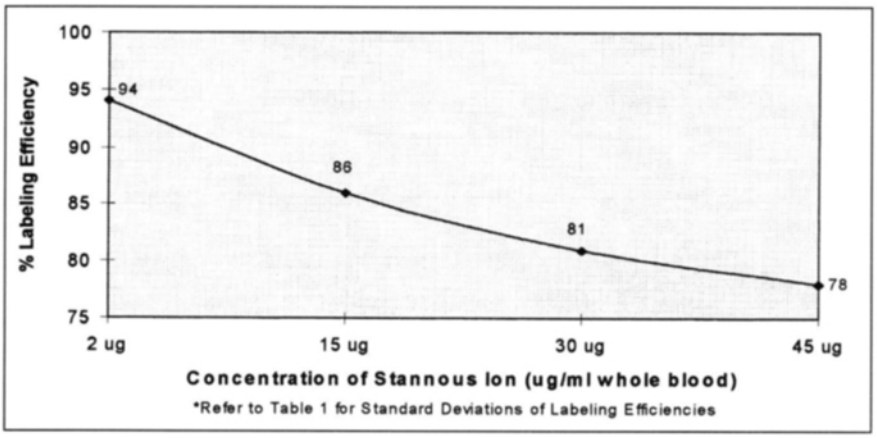

FIGURE 2. The average labeling efficiency* of RBCs after increasing stannous ion in samples containing 1000 ng CsA/ml whole blood.

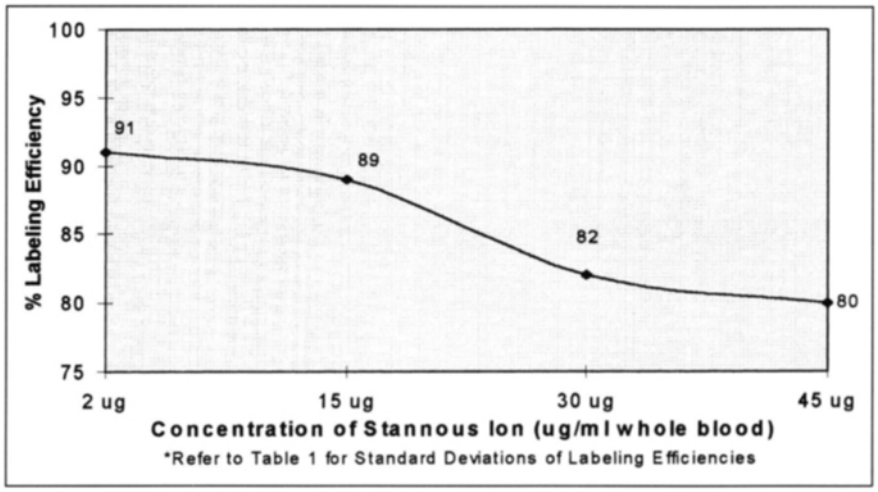

FIGURE 3. The average labeling efficiency* of RBCs by increasing stannous ion in CsA free samples.

that is obtainable. Indiana University Medical Center routinely obtains a high labeling efficiency. This could be due to a low amount of technetium atoms present in 1.5 mCi, requiring a longer period of incubation than the protocol suggests and what was performed.

CONCLUSION

Cyclosporine levels in the blood do not affect the labeling of red blood cells if the technologist uses 2 μg of stannous ion per milliliter of patient whole blood as recommended by this in vitro labeling method. Therefore, data obtained with this study's increments of 250 ηg CsA/ml of whole blood allow for conclusions to been drawn about the effects on labeling efficiency given normal concentrations of CsA or high concentrations of CsA. Data was collected within the relevant range of CsA blood levels typically found in patients who might receive a nuclear medicine MUGA study. Ultimately, quality is the goal of most nuclear medicine departments. This research shows that quality RBC labeling with sodium pertechnetate is possible even when patients have high cyclosporine blood levels.

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