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


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 cyclosporine blood levels (2).

The drug is available in capsule form, oral solution and intravenous ampules. An ampule containing 250 mg CsA in 5 ml whole blood. The total erythrocyte-binding capacity for CsA is 2.6 × 10^9 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 20 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
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 cre-
ated 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 concen-
tration became 5 μg CsA/ml solution. Desired concentra-
tions 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 ng CsA/ml whole blood, and so on until each
test tube of the last set contained 1000 ng CsA/ml whole blood.
Concentrations that are >400 ng 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 SnCl₂ · 2H₂O 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
with 0.2-micron membrane filters into sterile vials using a

VOLUME 24, NUMBER 3, SEPTEMBER 1996

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 ⁹⁹ᵐTc 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 Sn²⁺ 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,
30 μg, and 45 μg. The samples were each labeled with approx­
imately 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 rea­
sonably achievable. Percent labeling efficiency was then calcu­
ated 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 ⁹⁹ᵐTc 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 ⁹⁹ᵐTc RBC and 5 cc saline
a hard spin (3000 rpm for 5 min) in a balanced centrifuge
to separate free TcO₄⁻ 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:Te colloid and/or labeled plasma proteins
(13). Recent publications suggest that CsA will interfere with
⁹⁹ᵐTc RBC labeling if a low amount of Sn²⁺ is used. It has
been suggested that CsA and its metabolites cause an interfer­
ence to effective labeling of RBCs and that high concentrations
of Sn²⁺ are necessary in this situation (5). Their hypothesis
TABLE 1
Average Percent Labeling Efficiencies and Standard Deviations Obtained from Blood Samples of Three Volunteers

<table>
<thead>
<tr>
<th>Concentration (ng CsA/ml)</th>
<th>0 ng</th>
<th>250 ng</th>
<th>500 ng</th>
<th>750 ng</th>
<th>1000 ng</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 μg Sn²⁺</td>
<td>91 ± 2.0</td>
<td>91 ± 2.0</td>
<td>93 ± 1.2</td>
<td>93 ± 1.2</td>
<td>94 ± 1.9</td>
</tr>
<tr>
<td>15 μg Sn²⁺</td>
<td>89 ± 2.0</td>
<td>89 ± 1.9</td>
<td>89 ± 2.1</td>
<td>91 ± 1.7</td>
<td>86 ± 1.9</td>
</tr>
<tr>
<td>30 μg Sn²⁺</td>
<td>82 ± 1.6</td>
<td>82 ± 1.9</td>
<td>81 ± 1.9</td>
<td>82 ± 1.9</td>
<td>81 ± 2.2</td>
</tr>
<tr>
<td>45 μg Sn²⁺</td>
<td>80 ± 1.6</td>
<td>75 ± 2.1</td>
<td>75 ± 1.7</td>
<td>72 ± 3.7</td>
<td>78 ± 3.1</td>
</tr>
</tbody>
</table>

was based on a study of the UltraTag® kit (Mallinckrodt Medical Inc., St. Louis, MO) which uses an oxidizing agent to remove this extracellular Sn²⁺ (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 Sn²⁺ 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 Sn²⁺ 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 Sn²⁺ 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 (Tc⁰⁴) 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 Tc⁰⁴ 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%
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 ng 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.

**ACKNOWLEDGMENTS**

The author thanks Bruce Mock, Henry Wellman, Robert Burt, Judy Kosegi, Jim Winther, Tom Kuster, Doug Roller, Ian Secoff, Greg Watts, Amanda Guillermo and David Gibson for their contributions to this research.

**REFERENCES**