

The Effect of Cyclosporine on Technetium-99m Red Blood Cell Labeling Using the Ultratag® RBC In Vitro Kit

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Objective: The purpose of this investigation is to determine if cyclosporine (CsA) interferes with Technetium-99m red blood cell (^{99m}Tc-RBC) labeling when using the Ultratag® in vitro RBC kit.

Methods: The investigators set up a dilution study using increasing cyclosporine concentrations with whole blood samples. Whole blood samples from four heart transplant patients on maintenance CsA medication were also radiolabeled to investigate labeling efficiencies.

Results: Cyclosporine did not interfere with ^{99m}Tc-RBC radiolabeling as labeling efficiencies were found to be ≥95% in both the dilution and patient studies.

Conclusions: Although CsA does not inhibit ^{99m}Tc-RBC radiolabeling of whole blood when using the Ultratag kit, the investigators hypothesize that CsA may interfere with the label if kit levels of stannous ion were to be decreased.

Key Words: cyclosporine; technetium-99m red blood cell labeling; Ultratag® kit

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To prevent rejection of a transplanted organ, patients take immunosuppressive drugs. One of the most widely prescribed agents is cyclosporine, a third generation immunosuppressive medication (1). Cyclosporine (molecular weight 1202.63) is a cyclic endecapeptide derived from the fungus *Tohyopocladium inflatum* Gams (2). In addition to its use in transplantation, it has been given to patients with autoimmune disorders (3). Cyclosporine acts predominantly on T-helper cells, specifically and reversibly inhibiting their ability to produce interleukins and lymphokines, thereby preventing T- and B-cell maturation (4).

Although up to 25 variants of cyclosporine exist (5) (identified by letters: cyclosporine A, B, C, etc.), cyclosporine A (CsA) has the greatest pharmacological potency (3). The orally administered compound is absorbed by the gastrointestinal tract and eliminated by the liver with only 6% of the

dose found in the urine (6). Cytochrome P-450 enzymes metabolize CsA (7) into at least 20 metabolites (8,9). Several of these metabolites demonstrate in vitro immunosuppressive activity, but none at the level seen in the parent drug (10).

We follow a number of cardiac transplant patients in our nuclear medicine department. To assess the functional vitality of their transplanted hearts, noninvasive multi-gated acquisition (MUGA) scans and first-pass studies are performed on these patients. To accomplish these studies, red blood cells (RBCs) are labeled with ^{99m}Tc. RBCs are labeled by using in vitro, in vivo, or modified in vivo methods (11). For our cardiac transplant patients, the in vitro method is selected at our institution because it gives higher and more reproducible labeling efficiencies (11), offers labeled cells with good in vivo stability (12) and gives higher heart-to-background ratios of radioactivity (13,14). With the availability of an in vitro self-contained RBC labeling kit (Ultratag®, Mallinkrodt, Inc., St. Louis, MO), the labeling procedure has become more convenient and less time consuming when contrasted with older in vitro labeling kits.

The labeling efficiency of RBCs can decrease in the presence of certain types of medication. Anticoagulants such as heparin and certain drugs such as methyl dopa, hydralazine, and prazosin may interfere with the tagging mechanism (15). Antibiotics and anti-inflammatory agents may also alter the labeling efficiency (11,16). Two studies have previously examined the role of CsA in the labeling efficiency of ^{99m}Tc labeled RBCs (17,18). Use 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 (17). Another group of investigators used an in vitro stannous pyrophosphate method and found no labeling inhibition (18). In our follow-up study, we utilized an Ultratag RBC labeling kit to analyze the effect of CsA on ^{99m}Tc-RBC labeling efficiency.

MATERIALS AND METHODS

To determine if CsA interfered with RBC labeling, an initial dilution study was set up using incremental increases

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DISCUSSION

of CsA concentrations. Blood was drawn from a volunteer with a 19-gauge needle and 10 ml of whole blood was equally divided into five heparinized test tubes. In the first sample (control tube) no CsA was added. Incremental CsA concentrations were added to the remaining 2-ml samples (500, 1000, 1500, and 2000 ng/ml), and all five tubes were incubated at room temperature for three to four hours. These CsA concentrations were chosen because, under normal in vivo circumstances, peak and trough levels typically fall within this range (1,6,18). The incubation time reflects the peak concentration of CsA in the bloodstream at 3.5 hr post-oral dosing (2). After incubation, we completed the RBC labeling using the Ultratag kit instructions (19). The authors calculated the labeling efficiency (LE%) using the following formula: $LE\% = \{Activity\ RBC / (Activity\ RBC + Activity\ Plasma)\} \times 100\%$. This dilution study was completed with whole blood obtained from a total of three volunteers who were on no medications.

After the dilution study, we obtained six whole blood samples from four cardiac transplant patients and also labeled these samples with the Ultratag in vitro tagging kit. These patients were at least one year post-transplant to ensure stable maintenance levels of blood CsA and metabolites. Whole blood was drawn from these patients at their convenience from 45 min to 4 hr after they had taken their usual CsA morning dose. Approximately two ml of whole blood were labeled, using the Ultratag instructions (19). Labeling efficiencies were calculated in the same manner as for the dilution study. We did not measure patient whole blood CsA levels as our investigational endpoint was labeling efficiency.

RESULTS

Results from the controlled dilution study demonstrated that CsA levels up to 2000 ng/ml have no effect on ^{99m}Tc -RBC labeling efficiencies when the Ultratag kit is used. From Table 1, our mean labeling efficiencies never fell below 97%. Additionally, patient data reflected the same results (Table 2). The Ultratag kit product insert notes that RBC labeling efficiencies of $\geq 95\%$ are typically obtained (19).

TABLE 1
Whole Blood RBC Labeling Efficiencies (LE%) for Incrementally Increasing Cyclosporine A Concentrations

Volunteer	Cyclosporine A Concentration (ng/ml)				
	0	500	1000	1500	2000
A	98	99	99	99	100
B	96	99	96	99	100
C	99	98	99	100	100
mean	97.7	98.7	98.0	99.3	100.0

For optimal results an in vitro kit should provide an RBC labeling efficiency of $\geq 95\%$ (11). Image quality quickly degrades as tagging efficiencies drop. High labeling efficiencies produce images with lower background noise and ultimately provide better test conclusions. Our results concur with those obtained by Reisdorff et al. (18), in that CsA does not inhibit ^{99m}Tc -RBC labeling in an in vitro dilution test, and there was no labeling interference from CsA or its metabolites in vivo.

However, the results contrasted with those obtained by Allen et al. (17), who found that CsA did inhibit ^{99m}Tc -RBC labeling. In all three studies, whole blood was tagged using in vitro labeling kits or similar methods. All three techniques followed the three general steps of first "tinning" the RBCs with stannous ions, then removing, chelating, or oxidizing extracellular stannous ion, and finally adding the pertechnetate to the tinned RBCs (15). We examined each step in detail to determine why these three studies produced differing results.

Stannous ion (Sn^{+2}) is present in the kit to reduce intracellular pertechnetate ($^{99m}TcO_4^{-1}$) and prepare it for binding to hemoglobin (20). Theoretically, $^{99m}TcO_4^{-1}$ moves into the RBC through the band-3 anion exchange transport system (21) replacing either chloride and/or bicarbonate (22). The ^{99m}Tc in the pertechnetate molecule (in the +7 oxidation state) is not reactive intracellularly (15), and it will exchange freely between the RBC and plasma unless it is reduced to a lower oxidation number.

Tin is introduced into the labeling kits usually as stannous chloride or in a chelated form such as stannous pyrophosphate. The stannous pyrophosphate chelate is very unstable in vivo (23) providing a source of free stannous ions. Although the mechanism for Sn^{+2} entry into the RBCs is not perfectly clear, it seems to be transported intracellularly through some diffusion system (24). There may be Sn^{+2} exchange with Na^{+1} or K^{+1} using the sodium pump and/or an exchange with divalent cations along the calcium pump.

Before the $^{99m}TcO_4^{-1}$ is added to the tinned blood, any extracellular Sn^{+2} must be removed, chelated, or oxidized to prevent any extracellular reduction of $^{99m}TcO_4^{-1}$ (15). This can be done through centrifugation to physically separate the

TABLE 2
Whole Blood RBC Labeling Efficiencies (LE%) in Cardiac Transplant Patients

Patient	Time (hr) post Cyclosporine A dosage	LE%
1	0.75	97
2	0.75	99
	2.5	99
3	3.0	99
	4.0	98
4	3.0	99

intra- and extracellular Sn^{+2} , or chemically with chelating agents such as ethylenediamine tetraacetic acid (EDTA) or oxidizing agents like sodium hypochlorite. In the previous CsA- $^{99\text{m}}\text{Tc}$ -RBC labeling studies (17,18), and in our study, extracellular Sn^{+2} was removed through chelation or oxidation. After this step, the $^{99\text{m}}\text{TcO}_4^-$ is added initiating the radioactive labeling of the RBCs.

Experiments confirm that CsA is preferentially attracted to RBCs with estimates of 50–70% (25) or even up to 90% binding to erythrocytes (26). Additionally, several CsA metabolites also show strong affinity for RBCs (6). CsA and its metabolites are not bound to the erythrocytic membrane (26), but are present only in the cytoplasm of the cell. Although it was once thought that CsA may bind to hemoglobin (27), it now appears that CsA and its metabolites enter the RBC passively (28) and bind to a cyclosporine-binding protein identified as cyclophilin (3,28,29). This experimental data would suggest that there is no competition for hemoglobin among intracellular reduced pertechnetate, CsA, and/or its metabolites.

Although CsA and its metabolites may not interfere with reduced pertechnetate intracellularly, they may have a role in compromising the availability of Sn^{+2} to reduce $^{99\text{m}}\text{TcO}_4^-$ within the red blood cell. As noted earlier, $^{99\text{m}}\text{TcO}_4^-$ freely moves in and out of the RBC and will firmly bind to hemoglobin when it is reduced. Experimental data indicate that CsA can disturb the RBC membrane by accelerating K^{+1} efflux through activation of K^{+1} channels (30). Even low doses of CsA therapy may have profound effects on the movement of cations in and out of the RBC, through alterations in both the RBC $\text{Na}^{+1}/\text{K}^{+1}$ pump and $\text{Na}^{+1}/\text{K}^{+1}$ cotransport system (31).

The discussion earlier described the lack of understanding about the mechanism(s) by which Sn^{+2} may enter and leave the RBC; there may be exchange with Na^{+1} and/or K^{+1} along the erythrocytic monovalent pumps or with divalent cations along the calcium pump. If this is the case, it follows that CsA and/or its metabolites may perturb the erythrocytic membrane by either inhibiting Sn^{+2} from entering the RBCs or allowing the ions to leak back into the plasma. This would have significant deleterious effects on the ability to complete the $^{99\text{m}}\text{Tc}$ -RBC labeling without enough intracellular Sn^{+2} to reduce the $^{99\text{m}}\text{TcO}_4^-$ anions.

We hypothesize that CsA and its metabolites will inhibit $^{99\text{m}}\text{Tc}$ -RBC labeling by interfering with Sn^{+2} labeling only if the amount of tin provided by the kit is not adequate to account for this interference. The kit used by Allen et al (17) provided 2.0 μg of tin per procedure (32). Reisdorff et al used a Sn^{+2} -pyrophosphate kit in their study providing a maximum of 36 μg of tin (18). With the Ultratag kit used in this study, the manufacturer places 55 μg maximum of Sn^{+2} in the kit (19). The authors hypothesize that in the two latter preparations (36 μg and 55 μg Sn^{+2}), enough tin was added to counteract the effects of CsA and its metabolites on the RBC membrane and cation transport systems. This hypothesis supports both the results of Reisdorff et al (18) and our study, as both groups of investigators found no CsA inter-

ference of RBC in vitro tagging. It also supports the results obtained by Allen et al (17) that demonstrate decreasing RBC labeling efficiencies in the presence of CsA. Future research may disclose the threshold Sn^{+2} concentration which results in CsA labeling interference, and the mechanism by which CsA and its metabolites interfere with Sn^{+2} movement across the erythrocytic membrane.

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