Comparison of In Vitro RBC Labeling with the UltraTag® RBC Kit Versus In Vivo Labeling

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This study compared cardiac-gated equilibrium blood-pool imaging studies using in vitro technetium-99m- (^{99m}Tc) labeled red blood cells (RBCs) prepared with the UltraTag® RBC kit to in vivo labeling with stannous (pyro- and trimeta-) phosphates. The in vitro labeling procedure takes ~ 25 min and does not require centrifugation to separate free from bound ^{99m}Tc. Imaging studies were performed in 30 patients using the in vitro labeling procedure and in 30 patients with in vivo labeling. Regions of interest were placed over the center of the left ventricle, inferior and lateral to the left ventricle (background), and over the right midlung. The mean ± s.e. in vitro RBC labeling efficiency was 98.5 ± 0.2%. The heart-to-background ratios were significantly higher with in vitro labeling. The heart-to-background ratios, averaged among two blinded reviewers, were 4.6 and 3.4 for the in vitro and in vivo methods, respectively. The heart-to-lung ratio was generally higher with the in vitro procedure (3.6) than that observed with the in vivo method (3.2) but failed to attain statistical significance ($p = 0.059$). These results demonstrate the superiority of the in vitro labeling procedure over in vivo labeling for gated equilibrium blood-pool imaging.

J Nucl Med 1991; 32:242–244

The past 20 yr have witnessed the development of many methods for the in vitro labeling of red blood cells (RBCs) with technetium-99m (^{99m}Tc) (*1,2*). Techniques have been modified in an effort to decrease damage to RBCs and reduce the preparation time, while increasing the labeling efficiency. Based on these efforts, several authors have suggested the superiority of in vitro labeling over in vivo labeling (*1–5*).

The UltraTag® RBC kit (Mallinckrodt Medical, Inc., St. Louis, MO) is a modification of the current Brookhaven RBC kit (*1,2*). The kit is a sterile closed system that does not require centrifugation, washing, or separation of the labeled RBCs. The kit is simple to use and labeling takes ~ 25 min with a typical labeling efficiency of >95%.

Radionuclide ventriculography has been used extensively as an effective, noninvasive means to determine left ventricular volume, wall motion, and ejection fraction. The ability to calculate these parameters accurately is a function of the target (left ventricle) to background (surrounding tissue) ratio, which is affected by the labeling efficiency. The purpose of the present study was to compare cardiac-gated equilibrium blood-pool imaging (MUGA) studies done with the in vitro kit to in vivo labeling with stannous (pyro- and trimeta-) phosphates.

Received Apr. 20, 1990; revision accepted Aug. 14, 1990.

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MATERIALS AND METHODS

Patients

Sixty patients (1 female and 59 males) had MUGA studies performed on a Siemens Basicam gamma camera, 30 with in vitro labeling and 30 with in vivo labeling. Inpatients or outpatients 18 yr of age or older requiring radionuclide gated cardiac blood-pool imaging were eligible candidates for the in vitro study. The in vitro protocol excluded patients who had previously entered into this study or had received an investigational drug within 30 days, pregnant or nursing women, women of childbearing potential unless the possibility of pregnancy could be ruled out, patients whose immediate (1 wk) prognosis was questionable, and patients known to have sickle cell disease or trait. Informed written consent was obtained from all patients prior to enrollment in the in vitro study.

Patient demographics are summarized in Table 1. The 30 in vivo patients, chosen retrospectively, were selected so that their left ventricular ejection fractions were similar (±1%) to the patients in the in vitro group. The mean hematocrits ± s.e. were 39.1 ± 1.2 and 40.6 ± 1.4 for the in vitro and in vivo groups, respectively. One patient in the in vitro group and four patients in the in vivo group were on cyclosporine therapy. One patient in each group received subcutaneous heparin treatment. No other patients were on concomitant medications known to affect RBC labeling. All other patient characteristics were similar in the in vitro and in vivo groups.

In Vitro Labeling of RBCs

For in vitro patients, 1.0–3.0 ml of blood was collected with a large-bore needle using heparin or ACD as an anticoagulant. Neither ethylenediaminetetraacetic acid (EDTA) nor oxalate was used for this purpose. The whole blood was then transferred to the 10-ml closed-system sterile RBC kit lyophilized reaction vial containing a maximum of 105 μg total tin with a minimum of 50 μg in the form of stannous chloride dihy-

TABLE 1
Patient Demography for the In Vitro and In Vivo Labeling Procedures

	In vitro	In vivo
Mean age ± s.e. (yr)	63 ± 2	60 ± 2
Mean weight ± s.e. (lb)	185 ± 5	187 ± 7
Mean height ± s.e. (in.)	70 ± 1	71 ± 1
Sex (M/F)	29/1	30/0
Race (white/black)	29/1	30/0

drate as the active ingredient. The contents of the vial were gently mixed, allowing the lyophilized material to dissolve and react for 5 min. The stannous ion is used as a reducing agent and enters the RBC during the 5-min incubation time.

After incubation with the stannous ion, 0.6 ml of a 0.1% solution of sodium hypochlorite was added and mixed by inverting the vial four to five times. The hypochlorite served to oxidize the extracellular tin before ^{99m}Tc incubation. A 1.0-ml quantity of an ACD solution was then added to the vial and mixed by inverting the vial four to five times. The ACD is added to sequester the remaining extracellular stannous ion and make it accessible to the sodium hypochlorite (*1*). Previously, in vitro kits used EDTA as an anticoagulant and chelating agent (*1,6–8*). EDTA lowers blood pH (*5*) and is suspected of causing cell damage and reducing RBC viability (*9,10*).

The reaction vial was then placed in a lead shield and 1110 MBq (30 mCi) of sterile sodium pertechnetate were added to the vial. The mixture was allowed to react for 20 min with occasional mixing by inversion of the vial as described above. Technetium moves in and out of RBCs freely as pertechnetate. However, when reduced by tin (II) within the cell, technetium does not cross the cell membrane and is believed to bind predominantly to the β-chain of the globin part of hemoglobin (*1,11,12*).

A dose of ~ 740 MBq (20 mCi) of ^{99m}Tc-labeled RBCs was then aseptically transferred to a syringe for administration to the patient. The syringe was assayed immediately prior to injection.

In Vivo Labeling of RBCs

In vivo RBC labeling was performed by injection of one vial of Pyrolite® (E.I. du Pont de Nemours & Co., North Billerica, MA), which contained a minimum of 0.95 mg stannous chloride dihydrate, 10 mg sodium pyrophosphate, and 30 mg sodium trimetaphosphate followed by injection of 925 MBq (25 mCi) of sodium pertechnetate 30 min later.

Labeling Efficiency

The labeling efficiency of the in vitro labeled RBCs studies was calculated by the centrifugation technique. This consisted of removing an aliquot of 0.2 ml of labeled RBCs and dilution to 2.0 ml with saline. This solution was centrifuged at 2,000 × g for 2 min and the supernatant pipeted off and assayed. The RBC pellet was also assayed and the labeling efficiency determined using the following formula:

$$\left(\frac{\text{RBC activity } (\mu\text{Ci})}{\text{RBC + supernatant activity } (\mu\text{Ci})}\right) \times 100\% = \begin{matrix}\text{\% Labeling} \\ \text{Efficiency}\end{matrix}$$

Data Collection and Image Analysis

Anterior (ANT), left anterior oblique (LAO), and left lateral views were obtained in a 64 × 64 matrix, 16 frames per cycle. An ADAC computer (ADAC Lab., Milpitas, CA) was used for data acquisition and analysis. The ADAC software interpolates the 64 × 64 image to a 256 × 256 image prior to analysis. In the LAO view, square 20 × 20 pixel regions of interest (ROIs) were placed over the center of the left ventricle and over the background area inferior and lateral to the left ventricle A square 20 × 20 pixel ROI was also placed over the left midlung in the ANT view. The heart-to-lung and heart-to-background ratios were calculated.

Statistical Analyses

Mean and standard error (s.e.) values for heart-to-lung and heart-to-background ratios were calculated for each group. A two-way analysis of variance and covariance with repeated measures (across observers) was performed using the program BMDP2V (BMDP Statistical Software, Inc., Los Angeles, CA) to determine the significance between in vivo and in vitro values. A value of $p < 0.05$ was used to determine statistical significance.

RESULTS

The mean ± s.e. in vitro RBC labeling efficiency was 98.5% ± 0.16% with a range from 95.6% to 99.5%. The mean ± s.e. left ventricular ejection fraction was 43.5% ± 3.3% for the in vivo patients and 43.5% ± 3.4% for the in vitro patients. Both groups had a range of left ventricular ejection fractions from 8% to 71%.

Two investigators (STP, JVG) analyzed the images independently. The mean ± s.e. for the heart-to-background ratios and heart-to-lung ratios for both the in vivo and in vitro patients are presented in Tables 2 and 3. There was no significant difference between observers in calculating the ratios. The in vitro labeling method provided a significantly improved heart-to-background ratio ($p < 0.001$). The heart-to-lung ratio was also generally higher with the in vitro labeling method, but failed to attain statistical significance ($p = 0.059$).

TABLE 2
Heart-to-Background Ratios

Observer	In vivo labeled RBCs	In vitro labeled RBCs
STP	3.38 ± 0.14	4.71 ± 0.19
JVG	3.35 ± 0.18	4.53 ± 0.19

Values represent the mean ± s.e. (n = 30/group). The pooled p value for both observers is <0.001. No significant difference was noted between observers.

TABLE 3
Heart-to-Lung Ratios

Observer	In vivo labeled RBCs	In vitro labeled RBCs
STP	3.24 ± 0.17	3.62 ± 0.22
JVG	3.24 ± 0.17	3.63 ± 0.17

Values represent the mean ± s.e. (n = 30/group). The pooled p value for both observers is 0.059. No significant difference was noted between observers.

DISCUSSION

Many studies have been performed over the past 20 yr using the in vitro labeled RBCs. These studies have been primarily concerned with evaluating labeling efficiency and measuring the stability of the label (*1,2,5–7,10,13,14*). Some have also centered on clinical applications (*3,4,8,9,15*). This study focused on the comparison of RBCs labeled with the Ultratab RBC™ kit to in vivo labeled RBC in radionuclide ventriculography.

The patients used in this study were not selected according to cardiac disease and as a result had a wide variety of ejection fractions. Thus, the study demonstrated that the in vitro kit is useful for both healthy and diseased patients. Two studies have compared in vitro labeling to in vivo labeling in the same patients (*3,4*). The MUGA studies reported in the present study were done as part of a Phase II/III noncomparative study to assess the efficacy and safety of the UltraTag® RBC kit. Thus, for comparison, patients who had MUGA studies using in vivo labeled RBCs were retrospectively chosen. As has been shown previously (*3, 15*), the present study demonstrated that in vitro labeling improves heart-to-background ratios significantly.

CONCLUSION

Our data demonstrate that in vitro RBC labeling with the UltraTag® RBC kit is superior to in vivo RBC labeling in radionuclide ventriculography. This technique should enable clearer visualization of the heart in MUGA studies. Since both methods of labeling require two venipunctures and take ~ 25 min to execute, the in vitro labeling procedure may be the preferred method of radionuclide ventriculography.

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