In Vivo Comparison of Anticoagulant Citrate Dextrose Versus Heparin for Use as an Anticoagulant with the UltraTag® Red Blood Cell Kit

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Objective: The package insert for the UltraTag® RBC kit recommends the use of either heparin or anticoagulant citrate dextrose (ACD) as an anticoagulant. A comparison study between heparin and ACD solution was done to assess image quality.

Methods: Fourteen patients' red blood cells (RBC) were labeled with the UltraTag® RBC kit and ^{99m}Tc using 10 units of heparin or 0.15 ml of ACD solution-A per ml of blood. The labeling efficiency (LE) of the ^{99m}Tc-labeled RBC was determined before and after reinjection, and multigated acquisition (MUGA) and whole-body images were subsequently obtained.

Results: The images were analyzed by comparing the heart-to-background ratio, heart-to-lung ratio and kidney-to-background ratio. Both heparin and ACD gave similar LE and ratios of regions of interest.

Conclusion: There was no significant difference between ACD and heparin groups in overall image quality either on MUGA or on visual inspection of whole-body images.

Key Words: technetium-99m; red blood cells; UltraTag® RBC kit; anticoagulant

J Nucl Med Technol 1994; 22:178–181

The UltraTag® RBC kit (Mallinckrodt Medical, Inc., St. Louis, MO) is a simple in vitro procedure for the preparation of ^{99m}Tc-labeled red blood cells (RBC) requiring approximately 30 min to perform. This RBC labeling procedure uses a modification of the Brookhaven National Laboratory RBC labeling method (*1,2*). Red blood cells labeled with the UltraTag® RBC kit can achieve an RBC labeling efficiency (LE) of greater than 95% (*3*).

The LE of ^{99m}Tc-labeled RBC prepared with the in vitro method is usually higher than the other two radiolabeling methods; the in vivo labeling method (LE: 70%–80%) (*4*) and the modified in vivo method (LE: ~90%) (*5*). The high LE of ^{99m}Tc-labeled RBC can increase the image quality of a study, reduce unnecessary radiation exposure to nontarget organs and result in lower background activity. Low background activity is desirable in the localization of a small gastrointestinal (GI) bleed and in multigated acquisition (MUGA) studies.

The package insert of the UltraTag® RBC kit recommends the use of either heparin or anticoagulant citrate dextrose (ACD) as an anticoagulant when collecting the patient's blood for radiolabeling (*3*). Hegge et al. (*6*) and Porter et al. (*7*) have shown that increased renal and urinary activity may occur with the use of heparin and a pyrophosphate kit in the preparation of ^{99m}Tc-labeled RBC in vivo. Porter et al. demonstrated that superior image quality can be obtained when using ACD as the anticoagulant in vivo (*7*). However, a recent study by Bonacorrsi et al. shows that when using the UltraTag® RBC kit, heparin gives a better target-to-background ratio and image quality than ACD (*8*). Their study only evaluated the difference between the two anticoagulants in the heart region and not in the GI area. Therefore, it is not clear whether heparin also shows superior image quality and less renal and bladder activity than ACD, which would be important in the detection of sites of GI bleeding.

The purpose of this study was to use MUGA and whole-body images to compare heparin and ACD solution-A as the anticoagulant when using the UltraTag® RBC kit to determine the agent of choice for the preparation of ^{99m}Tc-labeled RBC.

MATERIALS AND METHODS

Patient Groups

Fourteen patients were entered into this study who were scheduled for an exercise MUGA for various clinical reasons. After consent was granted from the patient, they were randomly entered into one of the two anticoagulant groups (i.e., heparin or ACD). Both anticoagulant groups had seven patients each. Extremes regarding patient age, height and weight were avoided so as to select the *average* patient.

Preparation of ^{99m}Tc-Labeled RBC

Three milliliters of blood were drawn from each patient and labeled with ^{99m}Tc as per the package insert instructions (*3*). The amount of heparin used was 10 units per milliliter of

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TABLE 1
Labeling Efficiencies of ^{99m}Tc-Labeled RBC Prior to Reinjection

Anticoagulant	n	LE (%)
Heparin	7	97.3 ± 0.5
ACD solution-A	7	96.7 ± 2.6
p-value		0.6

TABLE 3
Key Ratios in Determination of Image Quality

Anticoagulant	n	Heart/ Bkg	Heart/ Lung	Kidney/ Bkg
Heparin	7	2.3 ± 0.2	2.1 ± 0.1	2.1 ± 0.1
ACD solution-A	7	2.3 ± 0.4	2.1 ± 0.4	1.9 ± 0.3
p-value		0.7	0.8	0.2

whole blood or a total of 30 units, whereas the amount of ACD solution-A was 0.15 ml per milliliter of whole blood or a total of 0.45 ml of ACD solution-A. The amount of ^{99m}Tc used for each patient was based upon the patient's sex, height and weight according to a previously established table (*9*).

LE Determination

The LE prior to the reinjection of ^{99m}Tc-labeled RBC was determined by a modified centrifugation method prior to and 30 min after reinjection (*10*) as recommended by the package insert (*3*). A 0.2-ml blood sample was taken from 3.0 ml of labeled blood. The 0.2-ml sample was diluted to 2.0 ml with physiological saline. This sample was then spun at 2,000 g for 5 min in a centrifuge. The activity of both the RBC precipitate and the supernate was then assayed with a dose calibrator. The percentage of bound ^{99m}Tc-labeled RBC was calculated by the following equation:

$$\%^{99m}\text{Tc-labeled RBC} = \frac{\text{RBC activity}}{\text{RBC activity} + \text{Plasma activity}} \times 100.$$

A 10-ml blood sample was taken from the patient 30 min after the injection of labeled RBC. This sample was used to determine if either anticoagulant affected the LE of the ^{99m}Tc-labeled RBC after reinjection. The sample was centrifuged and separated, and the percentage of bound ^{99m}Tc-labeled RBC was calculated as above.

MUGA Images

Upon reinjection of ^{99m}Tc-labeled RBC, each patient underwent a full rest and exercise MUGA. The resting portion of the MUGA consisted of three views: anterior, left lateral and left anterior oblique.

MUGA images were acquired with a low-energy, all-purpose collimator, 300 K counts per frame, in a 64 × 64-word mode for a total of 20 frames. The resting left anterior oblique composite view was used for computer analysis to determine the heart-to-background ratio which was used to determine the image quality. In determining the heart-to-background activity, the entire left ventricle was outlined and the heart activity was compared to an area inferior and lateral to the heart as the background activity.

TABLE 2
Labeling Efficiencies of ^{99m}Tc-Labeled RBC 30 Minutes Postreinjection

Anticoagulant	n	LE (%)
Heparin	7	97.6 ± 1.0
ACD solution-A	7	97.4 ± 1.1
p-value		0.8

Whole-Body Images

Following the MUGA study, a whole-body scan was performed approximately 80 min after the reinjection of ^{99m}Tc-labeled RBC. A computer-linked dual-headed system with low-energy, high-resolution collimation was used at 10 cm/min to acquire the images and formulate a geometric mean image which was then analyzed to determine the heart-to-lung and kidney-to-background ratio.

The activity within the left and right ventricle was included in determining the heart-to-lung ratio. The activity of the right lung excluding the heart was used for the lung activity.

Statistical Analysis

The comparison of the ratios between the two anticoagulant groups were analyzed with the Student's t-test.

Visual Inspection

The views from the MUGA and whole-body imaging were visually inspected by an experienced observer using a double blind approach. The images were rated on a scale of 1 to 5 in half-point increments; 1 being poor quality and 5 being excellent quality.

RESULTS

LE of ^{99m}Tc-Labeled RBC

Tables 1 and 2 indicate the LE of both groups of patients'

TABLE 4
Experienced Observer Rating of Image by Visual Inspection

Anticoagulant	n	MUGA	Whole-body
Heparin	7	4.6 ± 0.5	4.5 ± 0.7
ACD solution-A	7	4.4 ± 0.8	4.3 ± 0.6
p-value		0.8	0.7

*A scale of 1 to 5 was used; 1 = poor; 5 = excellent.

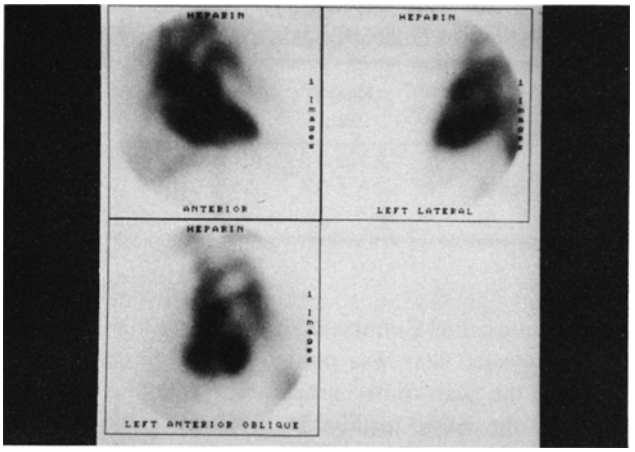

FIGURE 1. Composite MUGA images with heparin as the anticoagulant showing various images of the heart.

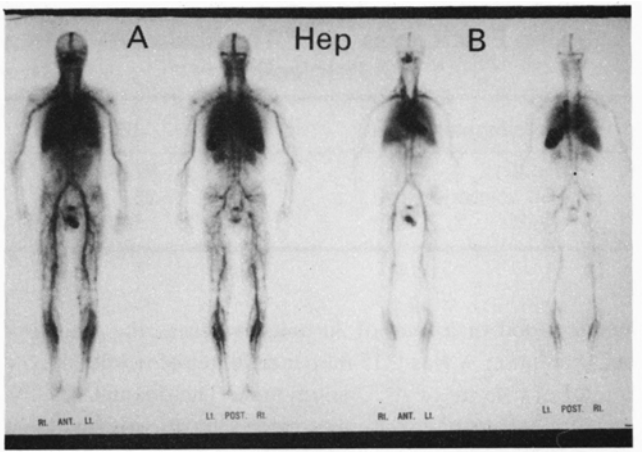

FIGURE 3. Whole-body images with heparin as the anticoagulant showing two different intensity images of the same patient.

blood samples prior to (Table 1) and at 30 min after reinjection of ^{99m}Tc-labeled RBC (Table 2). Both tables show a comparably high LE of the anticoagulants when used with the UltraTag® kit with no significant difference between the two groups.

Image Ratios

Table 3 demonstrates that there was no significant difference between the heparin and ACD groups in three ratios (i.e., heart-to-background, heart-to-lung and kidney-to-background) determined from the images obtained from the MUGA and the whole-body scan.

Table 4 shows the results of the visual inspection of the images from MUGA and whole-body studies. The p-value shows there was no significant difference between any of the ratios.

Image Quality

The experienced observer found no significant difference between the images from either anticoagulant as demonstrated in Figures 1 and 2 (MUGA images) and Figures 3 and 4 (whole-body images).

DISCUSSION

The main objective of this work was to determine if either anticoagulant, heparin or ACD solution-A, gave a higher LE and better image quality when used with the UltraTag® RBC labeling method. The in vitro method of ^{99m}Tc-RBC labeling achieved an LE of nearly 98% with the UltraTag® RBC kit (Tables 1 and 2). As shown in Tables 1 and 2, when the package insert instructions are followed, an LE approaching 98% and excellent image quality with lower background activity will be achieved (Tables 3 and 4, Figs. 1–4).

Past studies have shown that when heparin is used as an anticoagulant, renal and bladder activity may occur (*6,7*). Although the exact mechanism of this adverse effect is not clear, it may be explained if a large amount of heparin is used in the heparinized catheter and syringe (i.e., 100 ± 16 units) (*7*). It has been reported that heparin can be successfully labeled with ^{99m}Tc in the presence of stannous ions, and this may contribute to the major localization of ^{99m}Tc in the kidneys (*11*). Our results indicated no increased kidney and bladder activity when the recommended amount of heparin was used (*3*).

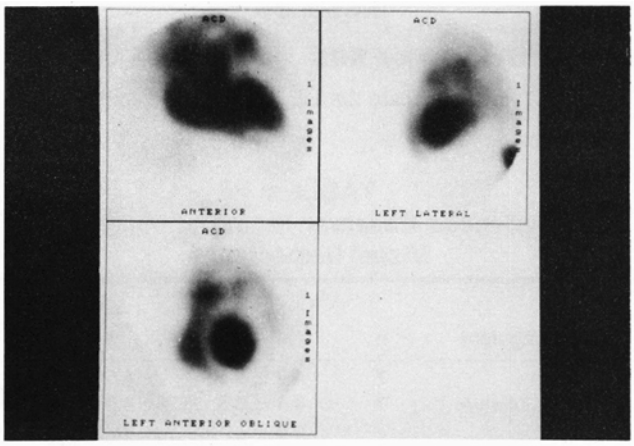

FIGURE 2. Composite MUGA images with ACD solution-A as the anticoagulant showing various images of the heart.

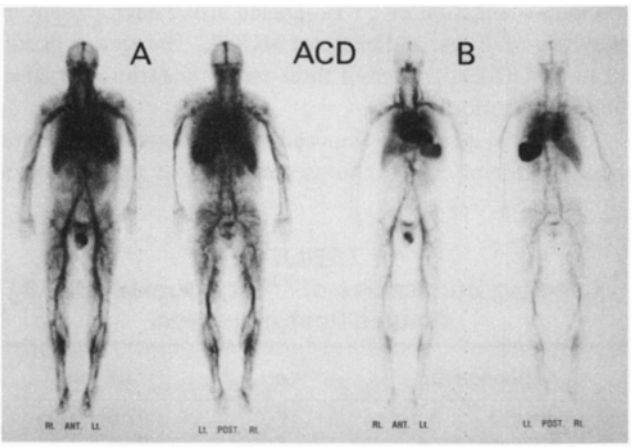

FIGURE 4. Whole-body images with ACD solution-A as the anticoagulant showing two different intensity images of the same patient.

Our study showed that there was no significant difference in the LE (Tables 1 and 2) and image quality (Tables 3 and 4, Figs. 1–4) of either anticoagulant when used with the UltraTag® RBC kit. Therefore, other factors must be considered to determine the anticoagulant of choice between ACD and Heparin. Heparin comes in a multidose vial whereas ACD solution-A does not. A multidose vial allows many heparin doses to be withdrawn without the sterility being affected as a bacteriostatic agent (i.e., benzyl alcohol) is contained in each heparin vial. ACD solution-A does not contain any bacteriostatic agent, so questions about its sterility could be raised if it is re-used with the UltraTag® RBC kit during the RBC labeling procedure. It is possible to divide the ACD solution-A into several unit doses. This, however, would mean the time-consuming pyrogenicity and sterility testing being performed to assure the safety of the ACD unit doses. A more practical method is to use the ACD solution as a single dose and waste the remainder.

Another factor which one must consider when deciding which anticoagulant to use is the ingrowth time of the generator from which the ^{99m}Tc came. If the ^{99m}Tc is from a generator that has an ingrowth time greater than 72 hr, it should be noted that ACD solution-A will affect the LE and image quality of a scan. Wilson and Hung reported that a poor tag of RBCs with the UltraTag® RBC kit method occurs when ACD solution-A is the anticoagulant used with sodium pertechnetate ^{99m}Tc which came from a generator with a 72-hr ingrowth time (*12*). The problem of the low LE with ACD is that the anticoagulant could reduce the stannous ion uptake into the RBC during the tinning process due to citrate in the UltraTag® RBC kit, as well as any excess citrate in the ACD solution. This would therefore reduce the tolerance for the large amount of the ^{99}Tc in the long ingrowth time eluate (*13*). Heparin has no such effect on RBC labeling performed with the UltraTag® RBC kit (*12*). This concern is important especially for the first elution of a generator which often has an ingrowth time of ≥72 hr. This elution needs to be used immediately for RBC labeling with the UltraTag® RBC kit if the ACD is used, because the LE will start to fail after 2 hr (*12*). Heparin should be the anticoagulant of choice if eluate from a long ingrowth time generator could possibly be a problem on the LE of ^{99m}Tc-labeled RBC with the use of the UltraTag® RBC kit.

If one decides to use heparin, the concentration of the commercial heparin preparation varies. Different companies produce heparin in different concentrations which can cause confusion. The nuclear medicine technologist must know the amount of heparin units per milliliter so as not to exceed the amount stated in the package insert (*3*). A simple mistake on the amount of heparin one uses may result in a poor image and excessive activity in the kidneys and bladder (*6, 7*).

CONCLUSION

Our study shows that one can use either heparin or ACD solution-A as the anticoagulant when labeling RBC by the UltraTag® method. The UltraTag® RBC kit is a simple method for RBC labeling which gives superior image quality, although one needs to decide which anticoagulant works best in their own department.

ACKNOWLEDGMENTS

The authors thank Ms. Vicki S. Krage and Mrs. Rose M. Busta for their help in preparing this manuscript. This paper was presented at the 40th Annual Meeting of the Society of Nuclear Medicine in Toronto, Canada, on June 9, 1993.

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JNMT Journal of NUCLEAR MEDICINE TECHNOLOGY

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J. Nucl. Med. Technol. 1994;22:178-181.

Journal of Nuclear Medicine Technology is published quarterly.
SNMMI | Society of Nuclear Medicine and Molecular Imaging
1850 Samuel Morse Drive, Reston, VA 20190.
(Print ISSN: 0091-4916, Online ISSN: 1535-5675)

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