In Vivo Red Blood Cell Compatibility Testing Using Indium-113m Tropolone-Labeled Red Blood Cells

Gary J. Morrissey, Denis Gravelle, Glen Dietz, Albert A. Driedger, Martin King, and Trevor D. Cradduck

Departments of Nuclear Medicine and Clinical Pathology, Victoria Hospital, and Department of Nuclear Medicine, University Hospital, University of Western Ontario, London, Ontario, Canada

In vivo radionuclide crossmatch is a method for identifying compatible blood for transfusion when allo- or autoantibodies preclude the use of conventional crossmatching techniques. A technique for labeling small volumes of donor red blood cells with [^{113m}In]tropolone is reported. The use of ^{113m}In minimizes the accumulation of background radioactivity and the radiation dose especially so when multiple crossmatches are performed. Labeling red cells with [^{113m}In]tropolone is faster and easier to perform than with other radionuclides. Consistently high labeling efficiencies are obtained and minimal ^{113m}In activity elutes from the labeled red blood cells. A case study involving 22 crossmatches is presented to demonstrate the technique. The radiation dose equivalent from ^{113m}In is significantly less than with other radionuclides that may be used to label red cells.

J Nucl Med 29:684–688, 1988

Radionuclide labeling of red blood cells has found various applications in hematology. A number of radionuclides and red cell labeling methods have been used to measure such indices as normal or shortened red cell life span, red cell sequestration, red cell mass, clinical significance of blood group antibodies (*1*), and to identify compatible blood for transfusion when conventional crossmatching fails to do so (*1*).

The use of radioactively labeled donor red blood cells to determine in vivo compatibility was first described by Mollison using phosphorus-32 (^{32}P) (*2*). Since then, various radionuclides and techniques have been evaluated for this purpose including chromium-51 (^{51}Cr) as sodium chromate (*3*) and technetium-99m (^{99m}Tc) (*4*, *5*). Phosphorus-32 is an unacceptable radionuclide in current practice because of the high associated radiation dose. The method of labeling red blood cells with ^{51}Cr has been widely validated (*6*,*7*) but the label is not ideal in all circumstances: when many units of blood need to be identified in a short time the radiation dose from ^{51}Cr becomes unacceptably high. In this application it is preferable to have a choice of radiotracers that can be counted in separate energy windows and/or a short lived tracer that will decay rapidly enough to minimize accumulating background radioactivity. While ^{99m}Tc is satisfactory, one may in current practice encounter patients who concurrently require both in vivo crossmatch and other diagnostic studies with ^{99m}Tc-labeled radiopharmaceuticals. Indium-111 as an oxine or tropolone complex is now commonly used for leukocyte labeling and the methodology is capable of wider applications although the 2.7 day half-life of the nuclide is longer than is required for cross match purposes. The purpose of this communication is to describe our current procedure of red cell labeling with the shorter lived nuclide, indium-113m (^{113m}In), as the tropolone complex, and to illustrate clinical experience using a case study.

Received Aug. 24, 1987; revision accepted Nov. 12, 1987.

For reprints contact: Gary J Morrissey, MSc, Victoria Hospital, Nuclear Medicine, 375 South St., London, Ont., Canada, N6A 4G5.

MATERIALS AND METHODS

Preparation of In-113m Tropolone

The [^{113m}In]tropolone complex was prepared by the addition of 50 μl of tin-113 (^{113}Sn)/^{113m}In generator eluate (0.04 N HCl) to 50 μl of tropolone (4.4 m*M* in 0.2*M* HEPES buffer, pH 6.0) in a sterile microcentrifuge tube. Twenty-five micro-

liters of 0.1 N NaOH was added to the tube, the solution mixed and incubated. The effects of tropolone concentration, of incubation time and of temperature on the efficiency of the complex formation were evaluated relative to efficiency of labeling red blood cells.

Labeling of Red Blood Cells

One milliliter of whole blood was added to the [^{113m}In] tropolone complex, mixed by inversion and incubated. The effect of incubation time with [^{113m}In]tropolone on labeling efficiency was evaluated at 5, 10, 20, and 30 min and the effect of temperature at 22°C and 37°C. The radiolabeled blood was then transferred to a sterile 15-ml centrifuge tube and 3.0 ml of cell free donor plasma were added. After centrifugation for 5 min at 500 *g*, the supernatant was decanted into another tube and both supernatant and cell pellet were assayed in a dose calibrator. The labeling efficiency was calculated and recorded.

Injection Technique

A peripheral intravenous site was set up to facilitate blood sampling. This was especially important when many units of blood were anticipated to be required. A pre-injection blood sample (2 ml) was obtained. The labeled red blood cells were injected at a site remote from the sampling site. A 4-ml blood sample was drawn into a heparin coated tube at 3, 10, 20, and 60 min after injection.

Blood Sample Counting

Three 1.0-ml volumes of freshly mixed blood samples were pipetted into gamma counting tubes and counted in a gamma well counter. The sample counts were corrected for decay and background and the average counts determined for each time. The 3-min sample was assumed to represent 100% recovery and survival at each successive time point was calculated relative to that specimen.

Quality Control

A separate 0.5-ml volume from each blood sample was pipetted into gamma counting tubes and 1.0 ml of saline added to each. After centrifugation for 8 min at 500 *g*, the supernatants were carefully decanted into separate gamma counting tubes. The pellets were resuspended in 1.0 ml of saline to preserve geometry for gamma counting and all samples were counted as above. After correction for decay and background the percentage of free ^{113m}In in the supernatant was calculated relative to total counts in the sample.

Estimate of Effective Dose Equivalent Ratio

An estimate of the ratios of effective dose equivalents for ^{99m}Tc/^{113m}In and ^{111}In/^{113m}In was made from a single test on a consenting normal adult male. One-milliliter samples of autologous whole blood were labeled with similar doses for each of ^{99m}Tc, ^{111}In, and ^{113m}In. The labeled cells were reinjected, the 60-min cell survivals were calculated for each radionuclide and the effective half-life of each radionuclide was calculated. Together with these data and the Oak Ridge MIRD Dose Calculation program, incorporating the total body as the source organ and the sum of the red and yellow bone marrow as the target organ, estimates of dose equivalents were calculated for each radionuclide. These data allowed ratios of the estimated dose equivalents, a measure of relative risks, to be made.

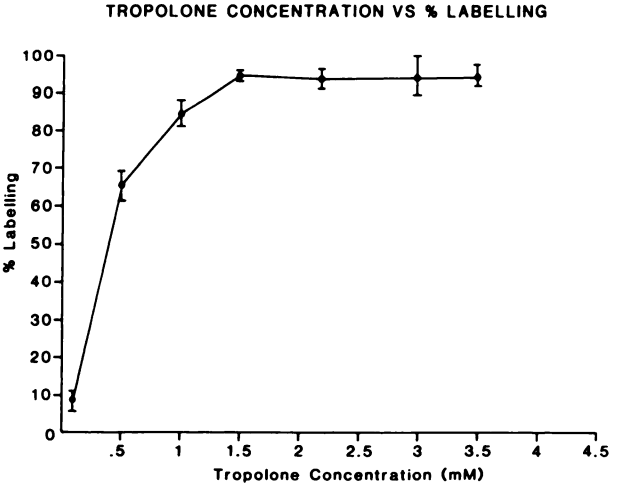

FIGURE 1
The labeling efficiency of 1.0 ml of whole blood with [^{113m}In] tropolone is plotted as a function of tropolone concentration.

RESULTS

Indium-113m Tropolone Red Blood Cell Labeling Efficiency

Various parameters that might influence labeling red blood cells with [^{113m}In]tropolone were evaluated. The effect of tropolone concentration on complex formation as shown by labeling efficiency is shown in Figure 1. Labeling efficiency plateaued at tropolone concentrations >1.4 m*M*. The tropolone concentration of 2.0 m*M* was determined from this curve to be optimal.

The time of incubation had no effect on the complex at intervals between 5 and 30 min (Fig. 2). Incubation temperature (22°C versus 37°C) did not affect the formation of the [^{113m}In]tropolone complex (Fig. 3).

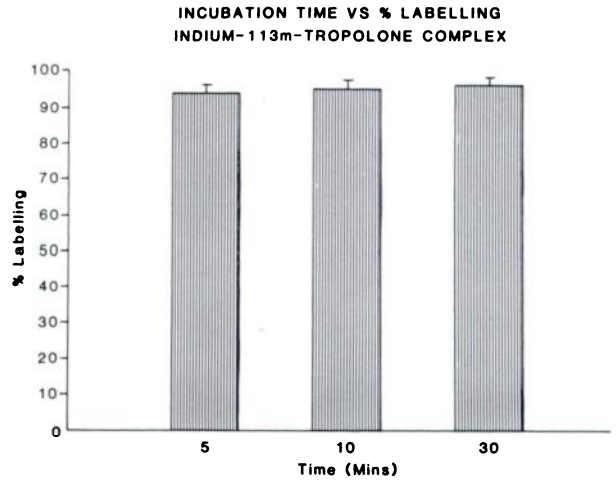

FIGURE 2
The labeling efficiency of 1.0 ml of whole blood is plotted as a function of the incubation time of the [^{113m}In]tropolone complex.

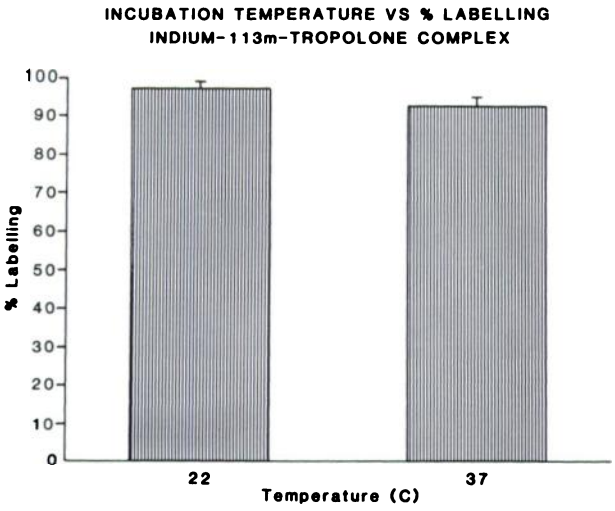

FIGURE 3
The labeling efficiency of 1.0 ml of whole blood is plotted against the temperatures of incubation for the [^{113m}In] tropolone complex.

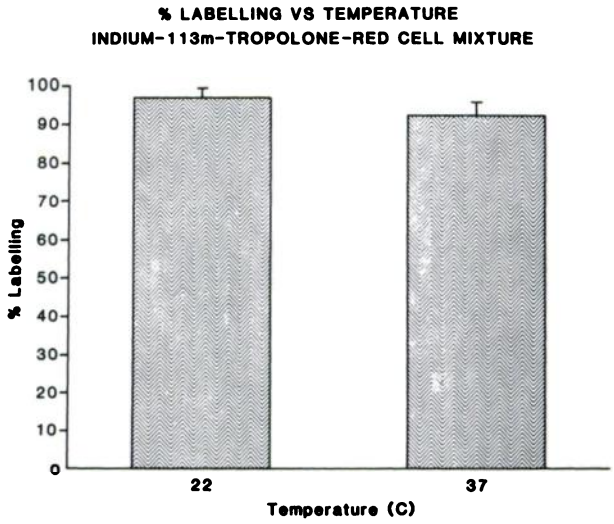

FIGURE 5
The labeling efficiency of 1.0 ml of whole blood is plotted against the temperature of incubation for the [^{113m}In]tropolone complex and the 1.0 ml whole blood sample.

The effects of incubation time and temperature on the red cell labeling efficiency were also evaluated and found to have no effect (Figs. 4 and 5).

The estimated effective dose equivalent ratios are summarized in Table 1. The estimates of the dose equivalent ratios show that the radiation risk from an in vivo radionuclide crossmatch with ^{113m}In is one-half that of ^{99m}Tc and 1/60th that of ^{111}In or ^{51}Cr.

CASE PRESENTATION

A 29-yr-old female was admitted with full thickness burns to 50% of her body. By Day 3, her hemoglobin had fallen from 141 g/l to 95 g/l. She was A Rh positive. The antibody screening test and direct antiglobulin test were negative. Four units of packed red cells were transfused on Day 4, one on Day 5, and another on Day 6.

A blood specimen on Day 10 gave a positive direct antiglobulin test and a positive antibody screening test. IgG and complement were present on the patient's red cells and anti-E was present in the serum. Anti-E was also present in an eluate made from her red cells. Only one of the six units of packed red cells which had been transfused on Days 4 to 6 was shown to have been positive for the E antigen.

On Day 14, the direct antiglobulin test was positive and again only anti-E was present in an eluate prepared from her red cells. The patient's serum reacted with all of the panel cells and donor cells tested. There was evidence of hemolysis in that her hemoglobin fell to 75 g/l by Day 16 and to 60 g/l by Day 22 without any signs of blood loss.

In vivo crossmatches were performed using [^{113m}In]tropolone-labeled red cells. Between Days 21 and 24, 22 studies were performed using red cells from 18 donors. The radionuclide dose for injection was 1.1 MBq/test. Compatible donor cells were signified by a cell survival of 90% or greater in the 60-min sample. Table 2 shows sample identification number, blood grouping, radionuclide cell survival at 60 min and percent free radioactivity in the plasma of the 60 min

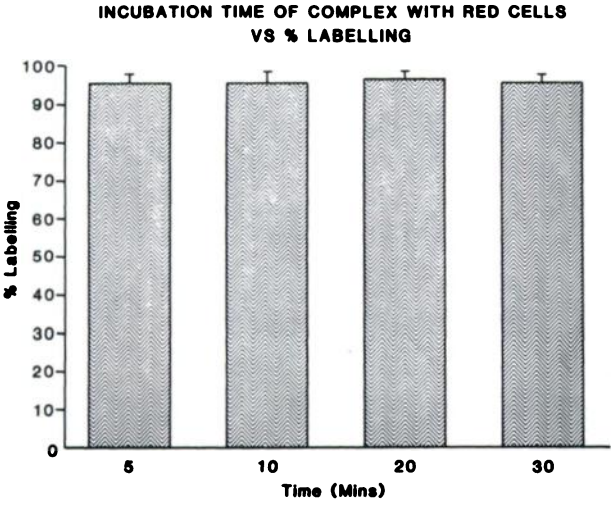

FIGURE 4
The labeling efficiency of 1.0 ml of whole blood is plotted against the time of incubation of the [^{113m}In]tropolone complex and the 1.0 ml whole blood sample.

TABLE 1
Summary of Estimated Effective Dose Equivalent Data

Radionuclide	$ET_{½}$* (hr)	Dose (mSv/MBq)	Dose (X/^{113m}In)
^{113m}In	1.59	0.0035	1.0
^{99m}Tc	4.1	0.006	2.0
		0.007†	
^{111}In	64.1	0.21	60.0
^{51}Cr	—	0.21†	60.0

* $ET_{½}$ = Effective half-life.

† Johansson L, et al. Effective dose equivalents of radiopharmaceuticals. *Eur J Nucl Med* 1984; 9:485–489.

TABLE 2
Crossmatch Study Data*

Unit	ABO	S	C.S	% L	% F
12-07-86					
53,230	A+	1[2]	97.0	98	5.3
73,537	A+	1[2]	86.0	95	
84,215	A+	1[2]	100.0	95	2.9
73,506	A+	2	99.0	93	2.0
77,833	A+	2[2]	94.4	94	1.7
53,221	A+	2	91.6	99	2.7
77,867	A+	2[2]	84.0	98	8.5
48,969	O+	2	89.0	99	1.7
53,229	A+	2[2]	95.4	97	4.0
36,660	O+	2	98.7	99	5.6
85,393	A+	vw	102.0	98	2.0
16-07-86					
48,969	O+	2	89.0	99	—
36,793	O+	vw	105.0	98	3.2
36,839	O+	wk	93.5	96	3.4
37,242	O+	wk	88.5	97	1.3
73,537	A+	wk	—		
32,066	O+	2	92.2		
37,042	O+	wk	103.0		
77,867	A+	nc	87.0		
85,393	A+	nc	105.0		
48,969	O+	nc	89.0		
73,537	A+	nc	88.0		

* ABO = Blood Group: S = Strength on DAT (vw = very weak, wk = weak, nc = no change); C.S. = Radionuclide Cell Survival @ 60 min; % L = [^{113m}In]Tropolone labeling efficiency; % F = Percent free tracer in plasma @ 60 min.

sample. The mean red cell labeling efficiency was 97% ± 1.9 s.d. The mean percent free tracer in the 60-min sample was 3.9% ± 2.5 s.d. Thirteen of the 18 units studied had 60-min survivals of >90% and she received seven of these units. Her hemoglobin increased to 135 g/l.

DISCUSSION

The interaction of host circulating antibody with a foreign antigen on the surface of the donor red cells is usually responsible for a hemolytic reaction following transfusion. Immunohematologic testing will in most cases predict these incompatibilities. However, when a blood bank is unable to provide compatible blood using routine serological tests a radionuclide in vivo crossmatch will identify compatible blood for transfusion. Two situations arise where an in vivo compatibility test may be required; (a) serologic testing cannot identify an antibody, or (b) the antibody's specificity and/or clinical significance cannot be determined.

Chromium-51 historically has been the reference tracer for in vivo compatibility testing and red cell survival studies (*6*). Technetium-99m-labeled red blood cells have also been used for in vivo compatibility testing (*4*). In our experience, there may be a requirement for many units to be tested over a short period of time. Chromium-51, because of its long half-life (27.5 days), is suitable for long-term red blood cell survival measurements, but it is not practical for short-term survival studies because persisting circulating activity requires progressive escalation of injected dose in successive tests. A nuclide of choice for this purpose is one that has a short half-life in the range of the time span of the study, all other factors being equal. Indium-113m is preferable to ^{32}P, ^{51}Cr, ^{111}In, and ^{99m}Tc because of its physical half-life of 1.7 hr. The labeling methodology is simple and practical. It is also readily available as a generator product and small volumes of blood can be efficiently labeled. Thus, ^{113m}In fulfills all of the prerequisites.

In our institution over the past 5 years, an in vivo radionuclide crossmatch study has averaged 4 units of blood (n = 13) with a maximum of 28 units over 1 wk. In Table 3, the total administered activity for a crossmatch study involving the testing of 4 units for each potential radionuclide as well as the ratios of effective dose equivalents between each radionuclide and ^{113m}In are shown. Because of the half-lives of ^{99m}Tc, ^{111}In, and ^{51}Cr on red cells, the radioactive doses of these nuclides given per crossmatch require to be increased on every second match so as to maintain counting statistics of the sample above accumulating background. Thus, the radiation doses are increased. In the case of the patient who required 28 units to be matched, ^{111}In was used and the total dose administered was 86 MBq.

The obvious prerequisite of this protocol is the ^{113}Sn/^{113m}In generator. In an individual institution there would be a substantial cost if the sole purpose was to supply ^{113m}In for an infrequently performed in vivo crossmatch. From our experience, it is feasible to share a generator among investigators and thereby reduce the unit cost of the procedure. In summary, ^{113m}In tropolone is the optimal radionuclide for red blood cell labeling for short term studies from the perspective of radiation dose limitation. The technique is practical provided that local arrangements can be made to offset the cost factor associated with generator maintenance.

TABLE 3
Summary of Estimated Dose Equivalent Data for an In Vivo RBC Compatibility Study Involving Four Crossmatches

Radionuclide X	No. of units	Administered dose (MBq)	MBq dose ratio factor (X/^{113m}In)	Dose ratio
^{113m}In	4	4.4	1.0	1
^{99m}Tc	4	6.6	1.5	3
^{111}In	4	11.0	2.5	150
^{51}Cr	4	11.0	2.5	150

REFERENCES

1. Mollison PL. The transfusion of red cells. In: Mollison PL, ed. *Blood transfusion in clinical medicine, second edition.* Oxford: Blackwell: 1983.
2. Mollison PL, Cutbush M. Use of isotope-labelled red cells to demonstrate incompatibility in vivo. *Lancet* 1955; 1:1290–1295.
3. Silvergleid AJ, Wells RF, Hafleigh EB, et al. Compatibility test using ^{51}chromium-labelled red blood cells in crossmatch positive patients. *Transfusion* 1978; 18:8–13.
4. Holt JT, Spitalnik ST, McMilan AE, et al. A technetium-99m red cell survival technique for in vivo compatibility testing. *Transfusion* 1983; 23:148–151.
5. Benedetto AP, Harrison CR, Blumhardt R, et al. Tc-99m red blood cells for the study of rapid hemolytic processes associated with heterologous blood transfusions. *Clin Nucl Med* 1984; 9:561–564.
6. International Committee for Standardization in Haematology. *Br J Haemat* 1980; 45:659–666.
7. Mollison PL. Survival curves of incompatible red cells. *Transfusion* 1986; 26:43–49.