High RBC Labeling Efficiency By Controlling Pretinning with the Modified In Vivo/In Vitro Labeling Method

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Objective: The image quality for gastrointestinal bleeding studies depends on the efficiency of red blood cell labeling. The in vitro technique has been used widely because of its high labeling efficiency. New data for the modified in vivo/in vitro method are lacking. This study reports on the high labeling efficiency that can be obtained with the modified in vivo/in vitro method and the pitfalls to avoid.

Methods: A consecutive series of 91 labeling studies was analyzed. Different amounts of tin, red blood cell concentrations, and infusions of interfering substances also were studied.

Results: A mean efficiency of red blood cell labeling of 97% (80%–99%) was obtained. Only 3 cases showed unacceptable results. Suboptimal results were obtained with amounts of tin below 10 μg $SnCl_2$/kg body weight, a reduced hematocrit, and blood transfusion or infusion of HES solution during pretinning.

Conclusion: The modified in vivo/in vitro red blood cell labeling technique is performed easily, is useful and is comparable to the gold standard in vitro method, provided that the pretinning period is controlled carefully.

Key Words: technetium-99m labeling of red blood cells; gastrointestinal bleeding studies; quality control; labeling purity; interfering substances

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The quality of gastrointestinal bleeding imaging studies, with prolonged acquisition times, depends on high-efficiency red blood cell (RBC) labeling with [99m]Tc. A significantly higher image contrast has been demonstrated with in vitro labeling as compared to in vivo labeling (*1*).The inadequacy of the modified in vivo/in vitro technique may be due to suboptimal protocols (*2,3*) or to interference by drug interactions (*4–6*).

To optimize the standard protocol for in vitro/in vivo labeling of RBCs, we studied the relationship between the in vivo tin dose and the in vitro labeling yield. A supermaximum tin dose has been reported to give suboptimal labeling results (*7*).

Binding kinetics were performed applying 3 times the normal amount of tin to duplicate this finding.

In clinical settings of gastrointestinal blood loss, the diagnostic procedure could be affected by dilution effects from the body's attempts to restore plasma volume. It is known that a low hematocrit diminishes the labeling yield (*8,9*). We tried to determine the minimum hematocrit that still provides acceptable labeling quality.

The pretinning step defines the essential preparation difference between the modified in vivo/in vitro method and the pure in vitro technique. The goal of this study was to find out whether volume replacement interferes with the labeling yield during this crucial reaction step. No published data are available, so we performed in vivo experiments combined with in vitro labeling to study this topic.

MATERIALS AND METHODS

A consecutive series of all bleeding studies and liver hemangioma examinations was evaluated for RBC labeling efficiency. Ninety-one labeling procedures performed on 77 patients were analyzed, and the records of those patients with substandard labeling efficiencies were reviewed for interfering factors.

The following experiments were performed on the same normal subject with different levels of variables tested as indicated below. A kinetic study design was used to show trends (see below).

Pretinning was performed using a commercial pyrophosphate kit (15 mg pyrophosphate decahydrate, 2 mg stannous chloride dihydrate [Pyroscint; DuPont Pharma S.A., Brussels, Belgium]) after reconstitution with sterile isotonic saline to give 1 mg $SnCl_2$/mL. The stannous ion solution was injected through a metal needle, and tagging of the RBCs by the tin was allowed over 20 min. During this period tin diffused from the vascular compartment. Eight milliliters of blood were drawn into a 12-mL syringe prepared with 1.2 mL solution of acid citrate dextrose (ACD) as an anticoagulant. The RBCs were labeled by adding 0.8 GBq ^{99m}Tc into a volume of not more than 1 mL of the daily generator eluate. The mixture was incubated while gently rotating the syringe at room temperature. One-milliliter
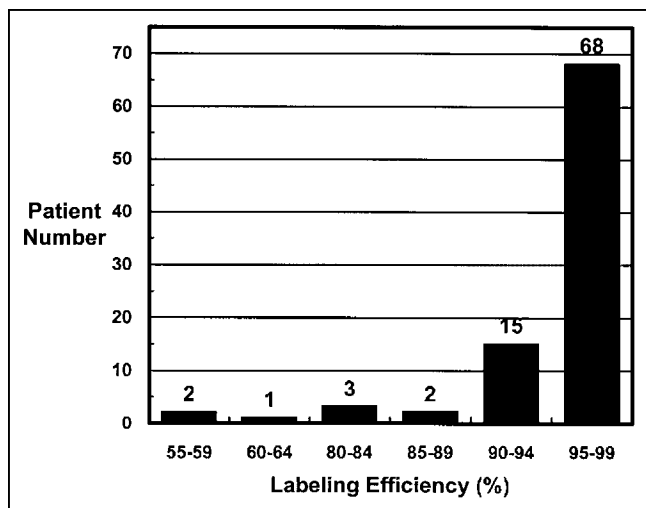
FIGURE 1. Distribution of labeling yields in a clinical series of studies for gastrointestinal bleeding and for liver hemangioma. Unacceptable results were obtained in 3 of 77 patients; 2 of these unacceptable results were caused by transfusion during pretinning.

samples were removed at 2, 5, 10, and 20 min. The binding reaction was stopped by adding 0.5 mL pyrophosphate solution containing 25 µg $SnCl_2$/mL as a quenching agent. After centrifugation at 5,000 rpm for 10 min, the radioactivity was measured in a dose calibrator before and after the removal of the supernatant. Labeling efficiency was calculated according to the formula:

% Efficiency = (activity [pellet]/activity [total]) × 100.

Variable amounts of $SnCl_2$ were injected intravenously to evaluate the optimal tin dose. Increasing doses were given to the same normal subject at intervals of at least 3 d.

In a second set of experiments, different in vivo hematocrit conditions were simulated by progressive dilutions of a concentrated blood sample. After pretinning with 10 µg $SnCl_2$/kg body weight, 30 mL of anticoagulated blood were centrifuged at 1,000 g for 10 min. The supernatant was removed and, after a second vigorous spin, it was used as a dilutant to obtain additional samples with lower hematocrits. After labeling, the hematocrit of each of the concentrated and diluted samples was measured in a Coulter counter. Each preparation was analyzed as described above.

To study the consequences of volume expanders during pretinning, experiments were performed with and without infusion of 0.5 L isotonic saline or 0.5 L of a 6% hydroxyl ethyl starch solution (HES) in the same subject on separate days. The infusions started immediately after injections of 10 µg $SnCl_2$/kg body weight and were completed within 10 min. Ten minutes later, blood samples were taken and labeled as described above.

RESULTS

Eighty-eight of 91 RBC preparations had acceptable labeling efficiencies (defined as 80%–99%). Eighty-three preparations had efficiencies from 91%–99% (Fig. 1). Three patients had efficiency values below 80% (low efficiency). Two of these patients received blood transfusions during the pretinning period. One patient also was given a colloid solution (plasma expander) during the pretinning period. The labeling efficiencies for these 2 patients were 57% and 64%. One patient without transfusion or elevated creatinine had an unexplained low labeling efficiency of 55%. In contrast, 3 patients who had blood transfusions in the pretinning period had yields of 80% and 81%. One patient with renal insufficiency had a yield of 88%.

Figure 2 shows the effects of tin doses ranging from 0–43 µg $SnCl_2$/kg body weight. Without any pretinning, the spontaneous
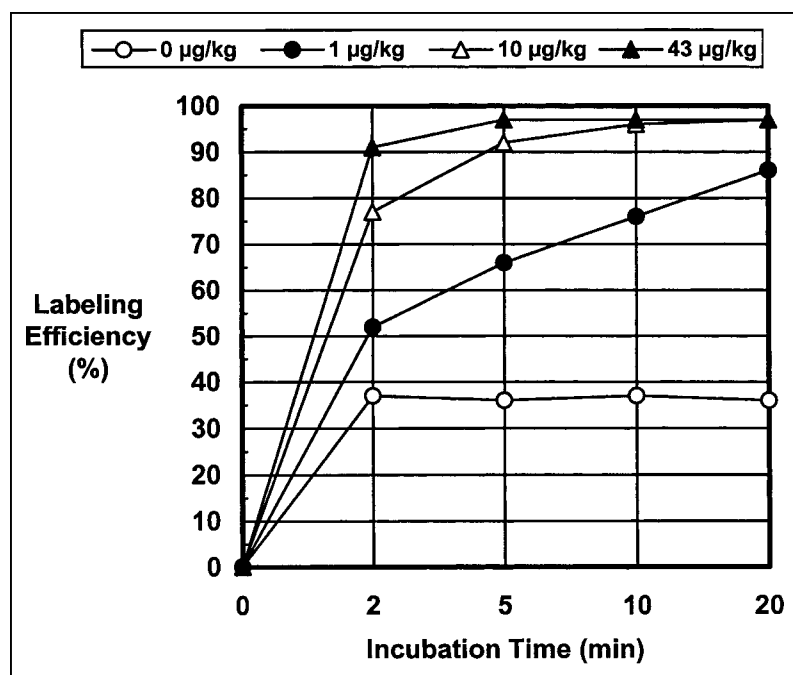

FIGURE 2. Relationship between binding efficiency and tin doses over time. Increasing doses ranging from 0–43 µg $SnCl_2$/kg body weight (corresponding to 27 µg stannous ion) are shown. Sufficient yield was achieved above 10 µg $SnCl_2$/kg body weight. The shortest time period to achieve equilibrium is observed at the maximum dose.

binding of ^{99m}Tc to the RBCs was 36%. A tin dose of 1 µg SnCl$_2$/kg body weight was clearly suboptimal. After application of a dose of 10 µg SnCl$_2$/kg body weight, which corresponds to 6.3 µg stannous ion, a 97% labeling efficiency was achieved. The maximum dose of 43 µg SnCl$_2$/kg body weight (27 µg stannous ion) increased the reaction velocity, but did not reduce the labeling yield in our system.

Maximum binding was obtained with the 52% hematocrit blood sample (Fig. 3). As the addition of 2.2 mL cell-free solution (ACD solution and generator eluate) to 8 mL of native blood reduces the hematocrit value to 22%, the maximum yield obtained in this preparation was 94%, due to the additional centrifugation step in this set of experiments.

The sample diluted to a hematocrit of 31% resulted in an acceptable efficiency of 89% after a 20-min incubation. A substandard yield of 69% was observed in the diluted sample with a hematocrit of 17% (equivalent to 22% in a native blood sample).

A severe reduction of the labeling efficiency occurred in the case of the 6% HES infusion (Fig. 4) in accordance with the observations on the effects of rapid infusions during the pretinning period. Conversely, no reduction of the labeling yield was obtained when isotonic saline was infused in the identical manner.

DISCUSSION

Various efficiencies of RBC labeling with ^{99m}Tc have been published using 3 different labeling methods. The in vivo technique has been reported to result in lower efficiency values (60%–90%) (10) and results in substandard imaging (4). This is not acceptable in studies where prolonged acquisition is manda-

tory, as in bleeding studies. Highly effective labeling is achieved by the in vitro method with a low frequency (2%) of substandard results (11). The modified in vivo/in vitro method has been investigated elsewhere with respect to the tin dose and reaction times in vivo, blood volume, hematocrit, reaction temperature, and binding times of the sample (8,9). The influence of generator ingrowth time, teflon catheters, heparin, chemotherapeutic agents, and iodinated contrast media are beyond the scope of this study.

Typical % efficiency for the modified in vivo/in vitro method range from 82–95% (9,12), with better results using ACD solution as the anticoagulant instead of heparin (93% versus 87%) (5). The typical binding value was 97% in our series with substandard results (below 90%) observed in 8 of 91 (9%) preparations. Three cases (3%) had unacceptable results. Despite the application of a strict standard protocol, 5% unacceptable preparations have been reported from a large series of patients (4). In our series, subnormal labeling yield was related to renal insufficiency, transfusion, or infusion during the pretinning period, when urgent therapeutic intervention was required. Almost without exception high binding values can be expected under stable clinical conditions, as shown in the subgroup of patients who had hemangioma studies.

In an average adult patient, approximately 0.5 mg stannous ion corresponding to 6 µg stannous ion/kg body weight (10 µg SnCl$_2$/kg body weight) is the minimum effective tin dose. Increasing the tin dose to 4 times the normal may reduce RBC binding efficiency from 90% to 82% (7), possibly due to increasing the fraction of stannous ions bound to serum proteins, and reducing the amount available for inducing ^{99m}Tc receptivity in the structural proteins of the RBC (13). In patients with renal insufficiency, even normal tin doses could be
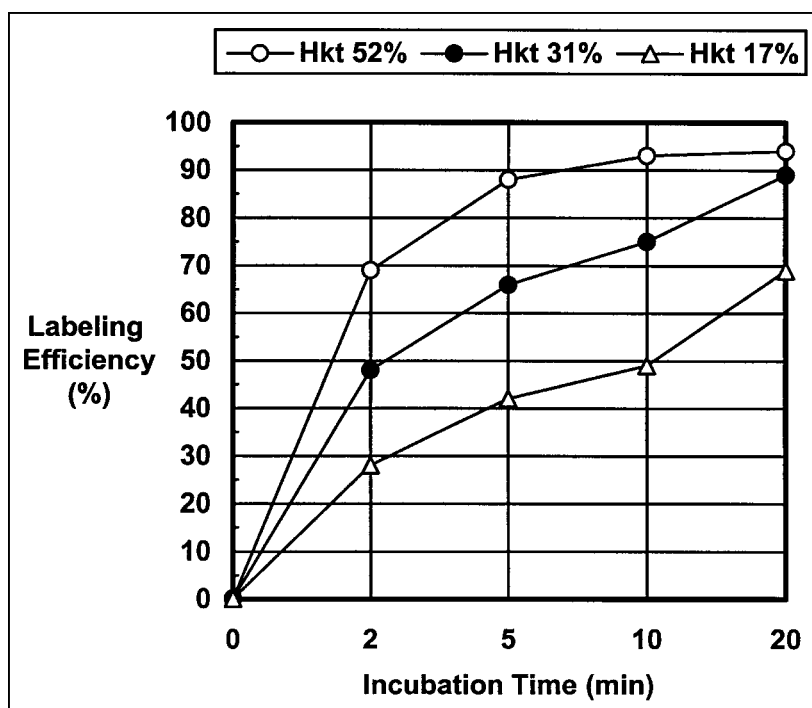

FIGURE 3. Dependence of the labeling yield on the number of RBCs in the reaction mixture during a 20-min incubation period. No serious decrease in efficiency was observed above a hematocrit of 30% (This value corresponds to 40% in a native blood sample after correction for added ACD solution and generator eluate.).
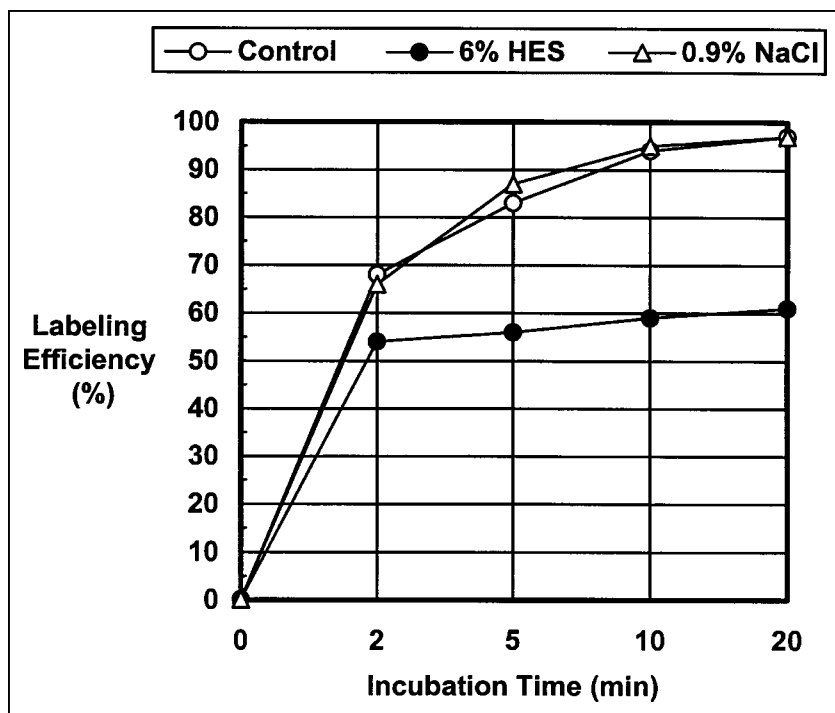
FIGURE 4. Interaction of a 6% HES solution with labeling efficiency. The labeling yield only reached a maximum of 64% when HES was given during the pretinning period. This phenomenon did not occur with isotonic saline.

responsible for this phenomenon. This was observed in 3 of the 77 patients, resulting in a moderate reduction of labeling efficiency. Our observations do not confirm a decreased labeling in patients with normal kidney function up to 43 μg SnCl₂/kg body weight. Conversely, this maximum tin dose increased binding velocity and helped shorten the preparation time.

Transfusion of RBCs during the 20-min pretinning period affects the binding of stannous ions to all RBCs present. In addition, incompatibility of RBC antibodies has been shown to alter the labeling quality (*14*).

After gastrointestinal blood loss has occurred, volume replacement by infusion decreases the hematocrit, which then can influence the binding efficiency. The consequences of this decreased hematocrit value in the incubation mixture are described elsewhere (*9*). Blood loss plus volume replacement up to a hematocrit of 22% led to a moderate decline in the labeling efficiency.

Infusion of HES, but not isotonic saline, produced marked reduction in labeling. This effect could be due to changes in charge distribution of the RBC, which has been shown to be closely related to labeling efficiency in an experimental system using neuramidase (*15*).

CONCLUSION

RBC labeling with ^{99m}Tc using the modified in vivo/in vitro method yields a high labeling efficiency, comparable to that of the in vitro technique. The in vivo pretinning of the RBC is the essential difference between the 2 techniques. This step should be strictly controlled. An adequately high stannous ion in the normal patient, restriction of the amount of tin to the optimum

(but not the maximum) dose at elevated creatinine serum concentrations, high specific activity eluate, increase in blood volume of the incubation mixture when the patient's hematocrit is low, and restriction of blood transfusion or infusion of HES during the 20-min pretinning period all are important factors that must be considered in good clinical practice.

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