Indium-111 Platelet Labeling Using Homologous Platelets

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Objective: This paper describes an ^{111}In-labeled platelet procedure using substituted donor platelets (homologous) in acutely thrombocytopenic patients.
Methods: In two patients, we used packed donor platelets which were centrifuged once. Once the platelets were isolated, they were incubated with ^{111}In-oxine in an anticoagulated solution of normal saline for 5 min. Upon resuspension in the plasma, the platelets were injected into the patients who were imaged with a dual-headed SPECT camera the following day.
Results: We successfully labeled packed donor platelets to produce clinically acceptable images by modifying the current standard protocol for platelet labeling.
Conclusion: Indium-111 platelet labeling of donor platelets is a feasible alternative to autologous labeling in acutely thrombocytopenic patients.
Key Words: platelet labeling; indium-111-oxine; thrombocytopenic patients

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The objective of this investigation was to evaluate the feasibility of labeling homologous packed platelets rather than autologous patient platelets with ^{111}In-oxine and achieving an adequately diagnostic scan in thrombocytopenic patients. It is often difficult to obtain a sufficient number of platelets from platelet-deficient patients. A readily available source of platelets is the local blood bank where packed platelets may be obtained in the concentration of 5.5×10^{11} platelets per unit (55 cc).

Using packed platelets instead of whole blood required some modification of a standard labeling procedure already in use in our pharmacy. Packed donor platelets easily give adequate numbers for labeling and provide for the opportunity of performing a ^{111}In-oxine-labeled platelet procedure in thrombocytopenic patients who might not otherwise have this diagnostic option. To our knowledge this is the first report of using ^{111}In-labeled homologous platelets in the acutely thrombocytopenic patient.

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MATERIALS AND METHODS

Our protocol for ^{111}In-labeling of homologous platelets (Table 1) is a modification of the procedure described by Bauman et al. (*1*). The primary difference is that we used packed donor platelets instead of platelets isolated from an autologous blood sample. We obtained one unit of HLA-matched donor platelets from our hospital blood bank for each patient. Because of the high concentration of platelets, it was necessary to centrifuge only once to obtain an adequate sample for labeling.

Upon isolation of the platelets, they were incubated with 500–600 μCi of ^{111}In-oxine in an anticoagulated solution of normal saline. The high concentration of platelets allowed us to incubate them for only 5 min rather than the 15 min called for in the original procedure. Upon resuspension in plasma, the platelets were injected into the patients who were then scanned the following day.

All patients were imaged based on our standard protocol. A dual-headed SPECT camera was used with a medium-energy collimator. Anterior and posterior images from head to pelvis were acquired. A 20% window was used for energy of 173 and 246 keV. Each image was acquired with a 1.0 zoom and 200–300K for approximately 5 min each. The nuclear medicine physician would specify the portion of the body to be imaged and if any additional delays were needed.

The labeling efficiency of the preparation was calculated based on:

$$\%\ \text{labeling efficiency} = \frac{\text{Activity of resuspended platelets}}{\text{Platelet Activity + Supernatant Activity}} \times 100.$$

RESULTS

The labeling efficiencies were generally 80%–90% (n = 2, 92% ± 5%, 88%–95%) with this modified protocol. We reviewed two acutely thrombocytopenic patients following

TABLE 1
Procedure for Labeling a Homologous Platelet Sample with ^{111}In-Oxine

1. Transfer approximately 25–45 ml of homologous packed platelets directly from the transfusion bag through a polypropylene transfer set without a needle into a 50-ml conical polypropylene centrifuge tube.
2. Prepare a balance tube and centrifuge the packed platelet sample at 225 × g for 15 min.
3. Separate the platelet poor plasma (PPP) with a new sterile plastic pipette and store in a sterile centrifuge tube at room temperature.
4. Add 2 cc of 1:6 ACD-to-normal saline which is buffered to a pH not greater than 6.5. Place the tube aside and let stand at room temperature for 10 min. Thereafter, the button may be agitated gently, without frothing, to completely resuspend the cells.
5. When the button is completely resuspended, place the tube in the water bath, add 500–600 μCi of ^{111}In-oxine and incubate at 37°C for an additional 5 min.
6. Prepare a balance tube and centrifuge the resuspended platelets at 180 × g for 15 min. (NOTE: Although this gentle spin may not completely remove all platelets from suspension, it does allow rapid resuspension of the platelet button. If complete separation is desired, this spin should be accomplished at 225 × g.)
7. Decant radioactive supernatant (the empty balance tube may be used for this). Gently layer 2 cc of 1:6 ACD-to-saline solution over the button and decant to the same tube used previously. Add 6 cc of PPP to the pellet as soon as possible and let stand for at least 10 min at room temperature. Thereafter, the button may be agitated gently, without frothing, to resuspend the cells.
8. When the platelets are completely resuspended, draw the labeled platelets into a syringe through an 18-gauge angiocatheter. Remove plastic catheter and replace with a 21-gauge or larger needle for reinjection. Measure the syringe activity in the dose calibrator.

injection and scanning with ^{111}In-labeled homologous platelets. Patient 1 was a 5-yr-old female without a functioning spleen presenting for evaluation following a bone marrow transplant. Patient 2 was a 52-yr-old female presenting for evaluation following a renal transplant. Both patients were scanned 24 hr postinjection with the following results obtained.

In Patient 1, whole-body images revealed concentration of the radionuclide within the liver and enlargement of the liver following the administration of platelets labeled with 0.25 mCi of ^{111}In-oxine. No other areas of focal increase in uptake were noted. This scan was read with the impression of hepatomegaly. The radioactive platelets were sequestered within the liver (Fig. 1).

In Patient 2, whole-body images were obtained, as well as localized images of the spleen and feet following the administration of platelets labeled with 0.5 mCi of ^{111}In-oxine. The images revealed increased activity in the left femoral region, highly suggestive of thrombus. Localized views of the spleen revealed that it was 11.7 cm in size. The renal transplant was faintly seen in the right iliac fossa. This scan was read with the impression of a normal-sized spleen and increased activity in the femoral vessels suggestive of thrombosis (Fig. 2).

DISCUSSION

Our modified procedure for labeling homologous platelets has several advantages over using an autologous sample from a patient with thrombocytopenia. The primary advantage is that a homologous sample contains 5.5×10^{11} platelets per unit (55 cc) which is many more than could be collected from 45–60 cc of whole blood from a nonthrombocytopenic patient (normal = $2.0–4.0 \times 10^5$). Inadequate platelet numbers will result in very few labeled platelets and a poor scan, thus, decreasing the diagnostic utility of the study.

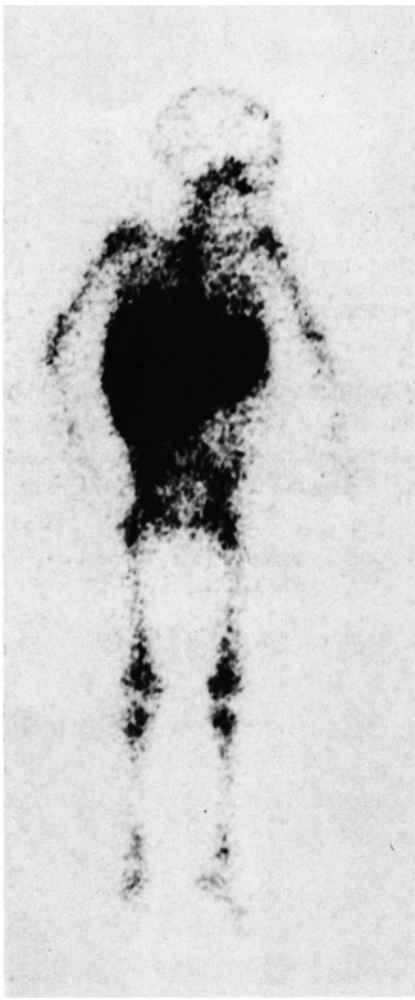

FIGURE 1. Anterior view of the 5-yr-old female with hepatomegaly.

FIGURE 2. Anterior view of the 52-yr-old female with increased activity in the femoral region.

Another advantage for using a homologous sample is that the cells are not subjected to the physical trauma to which an autologous sample is subjected during the isolation process (*1,2*). Trauma to the platelets is reduced by decreasing the amount of sample manipulation, specifically centrifugation. Damage is further decreased by handling the sample in a manner that minimizes its contact with any metal, such as an injection needle. Frothing and foaming of the sample should also be kept to a minimum as this will also cause platelet damage. Any such damage to only a few platelets could initiate the platelets' natural coagulation cascade causing aggregation and negate the procedure. Platelet integrity and functionality are better maintained and platelet death is reduced by decreasing the incubation time. Because plasma proteins compete with platelets for ^{111}In-oxine labeling, it is necessary to incubate in a normal saline environment. Outside of their natural plasma medium, platelets become dormant and, generally, the longer they remain outside the body the longer their recovery time upon injection.

We believe that this procedure for labeling homologous platelets with ^{111}In-oxine is safe and effective. Its main disadvantage is the risk associated with any transfusion of a blood product, such as blood-borne disease or the introduction of antigens that could cause the formation of anti-platelet antibodies. With the acutely thrombocytopenic patient, however, these risks may already be realized due to the probability that the patient is already receiving regular transfusions of platelets. Because of these risks, we recommend this procedure only to be used as an alternative to autologous platelet labeling in patients who are not likely to give a sufficient number of platelets for the labeling procedure.

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