Splenic Sequestration of Tc-99m Labeled
Heat Treated Red Blood Cells

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ABSTRACT

The rate of blood clearance and spleen uptake as well as the total spleen uptake of heat damaged red blood cells labeled with Tc-99m was determined in eight patients, six of whom had chronic lymphatic leukemia, one had polycythemia vera and one had eosinophilia of unknown origin. Spleen uptake at 2 hours was 72.0 ± 18.5%. Approximately 82.6% of the initial radioactivity was cleared from the blood by 2 hours with a rapid T½ component of 6.3 ± 4.7 minutes. The T½ of splenic uptake was 8.3 ± 4.6 minutes with a plateauing of splenic radioactivity by 30 minutes. The preliminary results indicate that the method of preparation is reliable but the usefulness of the method for evaluating spleen function remains to be determined.

Key Words: spleen, Tc-99m heated red blood cells, spleen imaging
Radionuclide imaging of the spleen is useful for a number of clinical situations including trauma, investigation of left upper quadrant masses or pain, evaluation of spleen size, the diagnosis of splenic infarcts and space occupying disease, and in the search for accessory spleens (1). The spleen is usually readily visualized following the administration of Tc-99m sulfur colloid and this is the most common method of radionuclide imaging of the spleen today.

A more specific agent is desirable because portions of the spleen may be obscured by the liver and because of the difficulty in obtaining lateral views of the spleen. In addition, since the major portion of Tc-99m sulfur colloid is localized in the liver, a great deal of unnecessary irradiation is delivered to the patient. In the past Cr-51 has been used as a label with splenic specificity increased by damaging the labeled cells by heating at 49-50°C or through the use of chemical or immunological methods (2,3,4). Mercury, in the form of Hg-197 bromo-mercuri-hydroxypropane has also been used (5). None of these methods is in common use today for several reasons such as high radiation dose, low photon yield and the relative ease in using Tc-99m sulfur colloid.

We have had considerable experience with a simple kit for the labeling of red blood cells with Tc-99m (6) and have used such cells, heated for 15 minutes at 49.5°C, for imaging the spleen. It is possible, with this kit, to label a very small volume of cells with high levels of radioactivity. Quantitative data on the rate and level of splenic uptake using Tc-99m as a label for heated red cells are not available. The present investigation was undertaken to assess the reliability of the method, to determine biodistribution in patients and to determine the rates of blood clearance and splenic uptake of the label.
Method

Eight patients (4 males and 4 females) were examined. Six of the patients (3 males and 3 females) had chronic lymphocytic leukemia in good control. One male had polycythemia vera in a stable state and one female was under investigation for an unexplained eosinophilia and pulmonary emphysema.

Blood was obtained for labeling as follows: a) 16 ml blood was drawn into a 20 ml syringe containing 4 ml ACD (acid-citrate-dextrose) mixture and labeled with 50 μCi Cr-51 by incubation for 30 minutes. Labeling was terminated by addition of 50 mg ascorbic acid. Four to 5 ml of normal saline were added, 20 ml were taken into a syringe for administration to the patient and 2 ml were retained as a standard and further diluted to 250 ml. b) About 6 ml of blood was drawn into a heparinized syringe and emptied into an evacuated vial containing 2 μg stannous ion and 3.67 mg sodium citrate. After mixing for 5 minutes about 4 ml normal saline was added as a plasma diluent. The tube was centrifuged with the rubber top down for 5 minutes at 1300 g. One and a half to 2 ml of packed red cells were carefully removed and added to a vial containing about 4 mCi (147.9 MBq) of Tc-99m pertechnetate in 2 ml normal saline. The vial was then heated to 49.5°C in a gently shaking water bath for 15 minutes. Approximately 1 ml (1 mCi or 37 MBq) was removed for administration to the patient and an equal amount was removed and diluted to 100 ml. One-half of this was put into a Lucite spleen phantom and 10 ml were diluted to 250 ml for a standard.

One patient (AG) did not receive Cr-51 labeled cells. In this patient an estimate of the initial volume of distribution of the Tc-99m labeled cells was made by extrapolation of the computed blood clearance curve to \( T_0 \).

The patients were positioned supine with a large field gamma camera underneath to view the spleen. The Cr-51 labeled red cells were injected into an
antecubital vein in one arm followed by the heated Tc-99m labeled cells. Sequential images were obtained over the next 2 hours at 1 minute intervals and stored on the magnetic disc of a medical computer system. Blood samples were obtained from the opposite arm at 1, 2, 3, 4, 5, 10, 20, 40, 60, 90, and 120 minutes.

At the end of two hours the spleen was imaged in the anterior, posterior and left lateral projections for 2 minutes each. Immediately thereafter the 50% Tc-99m standard was inserted into a paraffin slab phantom which was adjusted to simulate the calipered size of the patient and anterior and posterior 2 minute views were obtained.

At completion of imaging the patients bladder was emptied and the urine assayed for total radioactivity along with the blood samples. Technetium-99m activity was determined the next day and Cr-51 activity one to two days later following decay of the Tc-99m. Curves were generated from the Tc-99m blood clearance data and the sequential spleen images (corrected for radioactive decay). These were then best fit to two component exponential curves by an iterative technique on a large computer system. The loss of Tc-99m activity from the blood was determined by comparing the 2 hour sample to the equilibrium Cr-51 activity in blood.

An estimation of extracellular radioactivity was made by assuming the concentration in the extracellular fluid to be the same as the plasma Tc-99m radioactivity and estimating the extracellular space according to body weight.

Spleen radioactivity was determined by comparing the spleen counts (less background) to standard counts using the geometric mean of the anterior and posterior views and correcting for decay and attenuation.
Results

Although the splenic uptake of Tc-99m labeled heat-treated cells showed considerable variability in different patients overall the uptake was excellent in all patients (Table 1). The lowest value determined was .42%. The mean was 72.0 ± 18.5%.

Residual blood activity at 2 hours ranged from 3.8% to 46.2% with a mean of 17.4 ± 14.6%. Urinary excretion was 4.7 ± 1.1% and the estimated extracellular space activity was 11.3 ± 2.8%. The remaining unaccounted for activity correlated reasonably close to the spleen uptake as determined by the direct measurement compared to the standard.

The short T½ component of the blood disappearance and spleen uptake curves were very close in most instances (Table 2). The T½ for the blood clearance was 6.3 ± 4.7 minutes. For the splenic uptake rate the T½ was 8.3 ± 4.6 minutes. By about 30 minutes spleen radioactivity had reached a plateau and changed very little thereafter. Characteristic curves for spleen uptake and blood clearance are shown in Figure 1.

On the computer display the liver could be faintly visualized in most patients when sensitivity was turned up very high. This uptake was not visible on the polaroid or transparency scintiphotos. While accurate estimation is not possible it is unlikely that the liver accumulated as much as 5% of the administered radioactivity. Spleen images in a patient with a splenic infarct (JA) are seen in Figure 2.

Discussion

These preliminary results indicate that heating at 49.5° is sufficiently reliable for inducing splenic sequestration of Tc-99m labeled red blood cells. Previous studies using Cr-51 as a label required the use of relatively large vol-
umes of labeled cells in order to obtain the necessary activity for imaging. Two factors may affect the reliability of the method: a) Heating of the small volume required in this study (less than 4 ml) is more uniform than with larger volumes so that adequate preparation of the cells is achieved in a short time. With the larger volumes of Cr-51 labeled cells used it may not have been possible to achieve uniformity of heat-induced changes in a reasonable period of time, b) an additional factor is the possibility, in certain clinical situations, of overloading the spleen's sequestering ability with relatively large volumes of heated cells. This has been demonstrated by some workers in the past (7).

The rapid blood clearance (T½ of 6.3 minutes) and plateauing of splenic activity by 30 minutes means that spleen imaging can be accomplished a relatively short time after radionuclide administration. The high level of splenic uptake reduces the quantity of radioactivity that must be administered and thereby lowers the radiation dose to the patient. Sufficient counts can be achieved by administration of 0.5-1.0 mCi (18.5-37 MBq) for rapid imaging in multiple views. This results, in a normal sized spleen, in a radiation dose of 0.8-2.9 rads.

More patients with a variety of clinical conditions must be studied with this method before one can define any differences in rate or degree of splenic uptake of Tc-99m labeled red blood cells associated with a specific hematological entity. It would not be surprising if no differences could be delineated unless one were to stress the spleen with larger quantities of heated cells.

The authors are grateful for the assistance provided by Howard Pate in the computer analysis of data.
Table 1

Estimation of Spleen Uptake of Tc-99m RBC

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>Residual Blood Activity of $^{99m}$Tc-RBC</th>
<th>Urinary Excretion, %</th>
<th>Estimated ECF Activity, %</th>
<th>% Unaccounted</th>
<th>Spleen Uptake %</th>
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<tr>
<td>AG</td>
<td>CLL</td>
<td>3.8</td>
<td>6.6</td>
<td>10.2</td>
<td>79.4</td>
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<tr>
<td>CR</td>
<td>CLL</td>
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<td>58.0</td>
<td>57.6</td>
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<tr>
<td>VV</td>
<td>CLL</td>
<td>5.8</td>
<td>3.9</td>
<td>10.1</td>
<td>80.2</td>
<td>78.1</td>
</tr>
<tr>
<td>AS</td>
<td>CLL</td>
<td>23.0</td>
<td>4.3</td>
<td>8.3</td>
<td>64.4</td>
<td>72.9</td>
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<td>EW</td>
<td>CLL</td>
<td>4.9</td>
<td>4.5</td>
<td>12.0</td>
<td>78.6</td>
<td>91.1</td>
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<tr>
<td>RY</td>
<td>CLL</td>
<td>46.2</td>
<td>5.6</td>
<td>16.3</td>
<td>31.9</td>
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<tr>
<td>JA</td>
<td>PCV</td>
<td>8.8</td>
<td>4.0</td>
<td>11.9</td>
<td>75.3</td>
<td>100.7</td>
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<tr>
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<td>Eos.</td>
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<td>5.4</td>
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<td>64.7</td>
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Table 2

Rate of Spleen Uptake and Blood Clearance of Tc-99m RBC

<table>
<thead>
<tr>
<th>Patient</th>
<th>Spleen Uptake $T^1_2$, min.</th>
<th>Blood Clearance $T^2_3$, min.</th>
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<td>3.8</td>
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<tr>
<td>CR</td>
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<tr>
<td>VV</td>
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<tr>
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<td>17.3</td>
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Illustrations

Figure 1. Spleen uptake and blood clearance of Tc-99m RBC in two patients. EW had a spleen uptake greater than 90% and RY had an uptake of 50%.

Figure 2. Posterior and left lateral views of the spleen in patient JA. This patient had splenic infarction 4 years before. In addition to the large round defect posteriorly a wedge-shaped defect is seen anteriorly.
References


POSTERIOR

L. LATERAL
Tc-99m HEAT TREATED RBC

% ACTIVITY REMAINING IN BLOOD

RELATIVE RADIOACTIVITY IN SPLEEN

TIME (min)

% ACTIVITY REMAINING IN BLOOD

RE CE S TEM U IND PR IN ERM  IN J E E I E T B

TIME (min)