Dependence of Technetium-99m Red Blood Cell Labeling Efficiency on Red Cell Surface Charge

David W. Seldin, Shlomoh Simchon, Kung-Ming Jan,
Shu Chien, and Philip O. Alderson

Department of Radiology, and Department of Physiology and Cellular Biophysics, Columbia University, New York, New York

The mechanisms by which [^{99m}Tc]pertechnetate becomes attached to stannous-primed red blood cells are not known in detail. To study the problem further, the effect of red cell surface charge on labeling efficiency was evaluated. Red cell surface charge was reduced by using the enzyme neuraminidase to remove the terminal charge-bearing sialic acid moiety of the membrane glycoprotein. Forty-five blood samples from six volunteers were treated with neuraminidase for varying lengths of time, resulting in the removal of from 11% to 99% of the normal negative surface charge, as determined from electrophoretic mobility measurements. There was excellent linear correlation between labeling efficiency and the remaining red cell surface charge for values down to 20% of normal ($r = 0.89$). When surface charge was less than 20% of normal, labeling efficiency was constant at 30%. Eleven blood samples from three donors were divided into two groups that were treated with neuraminidase either before or after they were labeled. The labeling efficiency was independent of the order in which the steps were performed. No evidence for shifting of the radiolabel from the cell membrane to hemoglobin was found. The results suggest that clinical conditions associated with a reduction of sialic acid on the erythrocyte membrane may be one cause of decreased red blood cell labeling efficiency, and that increased membrane permeability for reduced technetium species may be responsible for the decrease.

J Nucl Med 29:1710–1713, 1988

The ability of technetium-99m (^{99m}Tc) pertechnetate to become attached to red blood cells (RBCs) that have been exposed to stannous ion has been known for almost two decades (*1*). While the details of the labeling process are not known, it has been shown that most of the radioisotope seems to be bound to the hemoglobin within the red cell (*2*). The efficiency with which tracer is incorporated into the red cell is dependent upon the details of the labeling procedure (*3,4*). Labeling efficiency also is known to be diminished by the presence of many commonly used medications, radiographic contrast agents and circulating antibodies (*5–8*). While conducting a series of experimental studies of abnormal red blood cell distribution in vivo, we observed that cells with reduced surface charge also showed reduced efficiency of labeling with pertechnetate. We investigated this phenomenon in more detail and herein present our results.

Received Dec. 17, 1987; revision accepted May 19, 1988.

For reprints contact: David W. Seldin, MD, Div. of Nuclear Medicine, 622 West 168th St., New York, NY 10032.

MATERIALS AND METHODS

Eleven blood samples were obtained from six healthy adult male donors. These 40-ml samples were collected in heparinized syringes. Neuraminidase solution (1 unit in 10 ml of normal saline) was added to six of these samples (from five donors) in order to remove terminal charge-bearing sialic acid residues from the erythrocytes (*9,10*). One-milliliter aliquots were removed from each sample at times of 0, 5, 10, 15, 20, 30, 40, and 60 min following neuraminidase addition. Ten milliliters of cold normal saline were added to each aliquot as soon as it was removed in order to stop the action of the neuraminidase. The samples were next centrifuged at 3,000 rpm for 10 min and the red blood cell fraction was resuspended in 10 ml of cold normal saline. This procedure was repeated two more times. A total of 45 such aliquots were produced. Electrophoretic mobility of cells from each of the aliquots was measured and expressed as a percent of normal,

using a previously described technique (*11*). The reduction in mobility served as a quantitative marker of the degree of surface charge removed by the neuraminidase (*9*). The remaining cells from each aliquot were then incubated at room temperature for 20–30 min with 2.5 ml (2.2 mg stannous ion) of a solution prepared by adding 5 ml of normal saline to the contents of a commercial kit (TechneScan PYP—Mallinckrodt, Inc., St. Louis, MO). The cells were subsequently incubated at room temperature with 0.5–2 mCi [^{99m}Tc]pertechnetate for an additional 10 min. The cells then were spun down, washed, and resuspended once as described above. Activity in the cell samples and in the washings was then measured using a well counter and/or dose calibrator.

In order to determine if the order in which neuraminidase treatment and radiolabeling of the cells were performed influenced our results, three blood samples from different donors were each divided into two groups of four. The first group in each set was incubated for 60 min with neuraminidase solutions of varying concentrations calculated to remove ~0, 30, 60, and 100% of the red cell surface charge. These cells then were washed, labeled with ^{99m}Tc, washed again, and measured for activity as described above. The second group was first labeled with ^{99m}Tc, then washed and similarly incubated with the neuraminidase solutions and washed again. These washings and cells were also assayed. The labeling efficiencies of these groups were then compared. Electrophoretic mobility data could not be compared, however, because technetium-labeled cells were found not to migrate in the measurement device.

The remaining two blood samples from two different donors were labeled as above, washed, and then incubated at room temperature for 15 and 60 min in either 10 ml of 8 g-% hemoglobin solution or in this hemoglobin solution plus 2.5 ml of the stannous solution previously described. The hemoglobin was obtained from cells taken from the same samples that were mechanically lysed by centrifugation with glass beads. Following incubation, the cells were washed and both cells and washings measured for activity. This was done to investigate whether extracellular hemoglobin would alter the ^{99m}Tc distribution.

For all samples the RBC labeling efficiency was calculated as the percent of activity with the red blood cell fraction as compared to the combined activity of the red cell and final washing fractions.

RESULTS

Red blood cell labeling efficiencies (n=45) are plotted against the electrophoretic mobilities in Figure 1. Inspection of the graph suggested a linear relationship between mobility and labeling efficiency in the range between 100% and 20% of normal surface charge and constant labeling efficiency below 20% described the data better than a straight line over the entire range. Subsequent analysis was performed accordingly. For the eight samples with mobility values <20% of normal, the labeling efficiency was 30.4 ± 3.9%. Linear regression analysis of the remaining points showed an excellent correlation between the labeling efficiency and electrophoretic mobility (r = 0.89, s.e.e. = 9.0, n = 37).

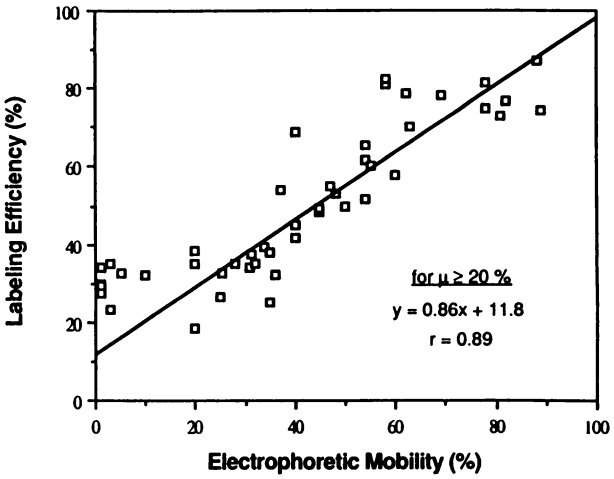

FIGURE 1
RBC labeling efficiency as a function of electrophoretic mobility (μ) in neuraminidase treated red cells. The mobility is directly proportional to the membrane surface charge remaining on the RBC following treatment. When <20% of the original surface charge remains, labeling efficiency is constant at ~30%.

Correlation between labeling efficiency of cells that were treated with neuraminidase before being labeled and cells that were first labeled with ^{99m}Tc and subsequently treated with neuraminidase (n = 11) was excellent (Fig. 2). There was no significant difference between the two groups (paired t-test), with near identical values for the measured labeling efficiencies (Eff) ($Eff_{label,treat} = 0.92 \times Eff_{treat,label} + 5.9$; r = 0.997; s.e.e. = 2.3%).

The four samples that were labeled and then incu-

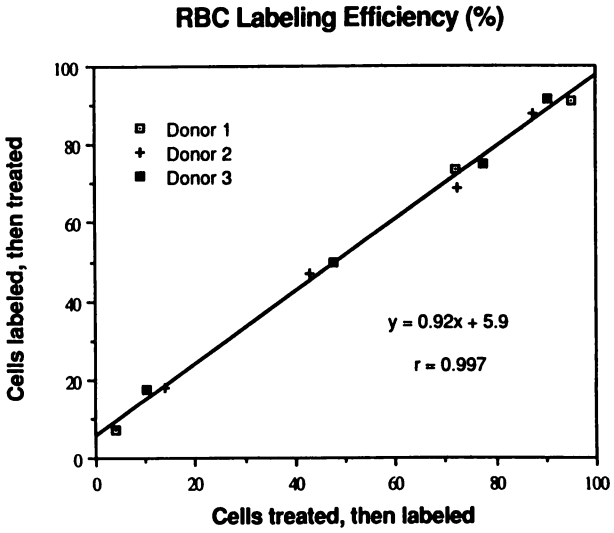

FIGURE 2
Comparison of labeling efficiency between cells that have been treated with neuraminidase before ^{99m}Tc labeling with cells treated with neuraminidase after ^{99m}Tc labeling. There is excellent correlation with the regression line close to the line of identity.

bated with either hemoglobin or hemoglobin primed with stannous ion showed no significant difference in the amount of ^{99m}Tc remaining with the cell from the corresponding samples incubated with saline. The mean difference in labeling efficiency was 0.6 ± 2.6%.

DISCUSSION

Technetium-99m-labeled red blood cells are easily prepared (*12,13*) and are widely used for cardiac ventricular function studies, radionuclide venography, and for detection of gastrointestinal bleeding and hepatic hemangiomas. A possible mechanism by which ^{99m}Tc becomes attached to RBCs has been previously described (*14*). First, stannous ion freely diffuses into the RBC but due to intracellular binding cannot escape. Next, pertechnetate ion diffuses freely into the RBC, but within the cell is likely to be reduced by the Sn^{++}. This reduced technetium is not transported across the cell membrane—that portion reduced intracellularly remains inside the RBC. Any technetium reduced by extracellular tin(II) will remain outside.

While attempting to study the biodistribution of RBCs that had been treated with neuraminidase, we observed that this treatment interfered with the ability of the pertechnetate to label the cells. Neuraminidase has long been used as a tool to study the erythrocyte cell membrane and erythrocyte aging. This enzyme, produced by the bacterium *Vibrio cholerae*, cleaves the terminal sialic acid residue of the red cell membrane glycoprotein (*9,10*). This residue is responsible for most of the RBC negative surface charge, and its removal can be monitored by measuring the electrophoretic mobility of the cells.

Our results demonstrate a close relationship between the amount of surface charge removed from the red cell membrane and the efficiency of red cell labeling. The relationship was linear following the removal of up to 80% of the initial surface charge; cleavage of additional sialic acid residues, to the point of virtual removal of all surface charge, did not seem to further diminish the amount of technetium bound to the cell. These results were seen regardless of whether the neuraminidase treatment occurred before or after the cell labeling process.

Prior studies of the distribution of technetium within labeled RBCs have suggested that *most* of the activity is associated with the beta-chain of globin (*2,14*). Our results suggest, however, that the majority of RBC-bound ^{99m}Tc is not associated with hemoglobin. Neuroaminidase treatment is known to affect the red cell membrane, but has no known effect on hemoglobin. In particular, neuraminidase treatment of red cells does not interfere with chromium-51 labeling of hemoglobin (*15*). Nevertheless, neuraminidase treatment was able to reduce ^{99m}Tc RBC activity by as much as 80%, while this activity was not altered by incubation with hemoglobin [which is known to have a high affinity for ^{99m}Tc (*16*)] or stannous-primed hemoglobin. Perhaps the 20% of the ^{99m}Tc activity that remained with the RBCs despite further reduction in surface charge is fixed to the beta-chain of hemoglobin. The remaining 80% seems to be in a more labile intracellular pool, perhaps in the cytoplasm or on the red cell membrane itself.

Cleavage of the sialic acid following cell labeling would result in corresponding proportional losses of both surface charge and cell-bound technetium activity if technetium were bound to the sialic acid moiety on the red cell membrane. When the order of labeling and neuraminidase treatment is reversed, however, removal of a given fraction of the binding sites would result in the same reduction in the fraction of ^{99m}Tc bound to the RBC *only* if the amount of ^{99m}Tc present *exactly* filled the number of binding sites initially available. Removal of all or part of any initial excess binding capacity would not be expected to reduce the amount of ^{99m}Tc that could be bound. We observed the same proportional losses in surface charge and activity regardless of the ^{99m}Tc concentration used. The membrane binding hypothesis thus fails to explain our results.

Alteration in the ability of stannous or pertechnetate ion to enter or leave the cell as a result of prior neuraminidase treatment might be considered as the cause of the reduced labeling efficiency in treated cells. Cells that are first labeled with ^{99m}Tc, however, presumably have all their pertechnetate reduced and thus trapped within the cell before treatment. Post-treatment movement of stannous or pertechnetate ions alone would not affect the amount of activity in this reduced intracellular ^{99m}Tc pool. Therefore this hypothesis, too, cannot be correct.

A possible explanation for our results is that the increase in erythrocyte membrane permeability associated with the cleavage of terminal sialic acid residues permits increased loss of *reduced* technetium species from the cells. Such loss of label from the red cells normally occurs and can be modeled by a two component exponential (*4*). Removal of surface charge and unmasking of interior portions of the cell membrane due to neuraminidase treatment (*10*) may be associated with increased membrane permeability. The importance of membrane electrostatic forces in the labeling process is suggested by the observed loss of electrophoretic mobility of labeled RBCs. The mechanism might be via a direct membrane effect or through changes in the intracellular charge distribution. The residual activity that remains with the cell even when all surface charge is removed may be attributed to technetium bound to large molecules such as hemoglobin unaffected by a change in membrane permeability.

The decrease in efficiency of technetium RBC labeling associated with the loss of sialic acid residues on the RBC surface may have clinical implications. Antigenic sites that are revealed as the aging red cell loses surface sialic acid lead to antibody accumulation and removal of the cell from the circulation (*10*). The loss of surface charge with age is not large (≈15%) and is accompanied by a corresponding decrease in surface area, but it may mean that there is a preferential ^{99m}Tc labeling of younger cells. Decreased surface charge also is seen in patients following stroke (*11*) and acute myocardial infarction (*17*), and in patients with diabetes mellitus (*18*), sickle-cell disease (*19*), and thalassemia (*20*). No severe problems in ^{99m}Tc blood-pool imaging have been recognized in these patient groups, but our findings suggest that labeling efficiency in these patients may be reduced. In addition, if it is true that alterations in RBC membrane permeability may lead to reductions in labeling efficiency, such alterations might represent a generalized mechanism by which some drugs interfere with standard red cell labeling techniques.

REFERENCES

1. Eckelman W, Richards P, Hauser W, et al. Technetium-labeled red blood cells. *J Nucl Med* 1970; 12:22–24.
2. Rehani MM, Sharma SK. Site of Tc-99m binding to the red blood cell: concise communication. *J Nucl Med* 1980; 21:676–678.
3. Hamilton RG, Alderson PO. A comparative evaluation of techniques for rapid and efficient in vivo labeling of red cells with [^{99m}Tc] pertechnetate. *J Nucl Med* 1977; 18:1010–1013.
4. Mock BH, Wellman HN. Stoichiometric Tc-99m RBC labeling using stable kit solutions of stannous chloride and EDTA: concise communication. *J Nucl Med* 1984; 25:881–886.
5. Hladik WB III, Nigg KK, Rhodes BA. Drug-induced changes in the biologic distribution of radiopharmaceuticals. *Semin Nucl Med* 1982; 12:184–218.
6. Lee HB, Wexler JP, Scharf SC, et al. Pharmacologic alterations in Tc-99m binding by red blood cells: concise communication. *J Nucl Med* 1983; 24:397–401.
7. Tatum JL, Burke TS, Hirsch JI, et al. Pitfall to modified in vivo method of technetium-99m red blood cell labeling—iodinated contrast media. *Clin Nucl Med* 1983; 8:585–587.
8. Leitl GP, Drew HM, Kelly ME, et al. Interference with Tc-99m labeling of red blood cells (RBCs) by RBC antibodies [Abstract]. *J Nucl Med* 1980; 21:P44.
9. Eylar EH, Madoff MA, Brody OV, et al. The contribution of sialic acid to the surface charge of the erythrocyte. *J Biol Chem* 1962; 237:1992–2000.
10. Gattegno L, Fabia F, Bladier D, et al. Physiological ageing of red blood cells and changes in membrane carbohydrates. *Biomedicine* 1979; 30:194–199.
11. Chien S, Cooper GW Jr, Jan K, et al. N-acetylneuramic acid deficiency in erythrocyte membranes: biophysical and biochemical correlates. *Blood* 1974; 43:445–460.
12. Pavel DG, Zimmer AM, Patterson VN. In vivo labeling of red blood cells with ^{99m}Tc: a new approach to blood pool visualization. *J Nucl Med* 1977; 18:305–308.
13. Callahan RJ, Froelich JW, McKusick KA, et al. A modified method for the in vivo labeling of red blood cells with Tc-99m: concise communication. *J Nucl Med* 1982; 23:315–318.
14. Srivastava SC, Chervu LR. Radionuclide-labeled red blood cells: current status and future prospects. *Semin Nucl Med* 1984; 14:68–82.
15. Jancik J, Schauer R. Sialic acid—a determinant of the life-time of rabbit erythrocytes. *Hoppe-Seyler's Z Physiol Chem* 1974; 355:395–400.
16. Callahan RJ, Froelich JW, McKusick KA, et al. Studies of red blood cell (RBC) labeling: rate of binding of Tc-99m to hemoglobin (Hgb) in the intact cell and in Hgb solution [Abstract]. *J Nucl Med* 1981; 22:P70.
17. Hanson VA Jr, Landaw SA, Flashner M, et al. Sialic acid-depleted red cells following acute myocardial infarction. *Am Heart J* 1980; 99:483–486.
18. Baba Y, Kai M, Setoyama S, et al. The lower levels of erythrocyte surface electric charge in diabetes mellitus. *Clin Chim Acta* 1978; 84:247–249.
19. Riggs MG, Ingram VM. Differences in erythrocyte membrane proteins and glycoproteins in sickle cell disease. *Biochem Biophys Res Comm* 1977; 74:191–198.
20. Kahane I, Polliack A, Rachmilewitz EA, et al. Distribution of sialic acids on the red blood cell membrane in β thalassemia. *Nature* 1978; 271:674–675.