Survival of Human Sickle-Cell Erythrocytes in Heterologous Species: Response to Variations in Oxygen Tension

(sickle-cell anemia/¹⁴C)erythrocyte survival/heterologous transfusions/hypoxia/hemoglobin S)

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ABSTRACT Survival characteristics, after transfusion, of erythrocytes from patients with homozygous sickle-cell disease were studied in rats. The study was made possible by previous injection of the animals with ethyl palmitate, which depressed reticuloendothelial system function, and with a factor in cobra venom that inactivated complement. This treatment prevented the rapid phagocytosis and intravascular hemolysis of donor erythrocytes that usually follow a heterologous transfusion. Both immediate after-transfusion recovery and survival of ¹⁴C-labeled sickle erythrocytes were decreased in comparison to the values obtained for control erythrocytes from individuals without sickle-cell anemia. Survival of sickle erythrocytes was improved during exposure of the animals to 100% O2. Hypoxia (7-10% O2) resulted in the abrupt removal of 35-60% of sickle erythrocytes from the rats' circulation. Variations in oxygen tension did not affect survival of control erythrocytes. The usefulness of this convenient animal model for the study of sickle-cell anemia is suggested by the similarities between our results and the behavior of sickle erythrocytes in humans. The system may also be suitable for studying a wide variety of other human erythrocyte disorders.

Significant prolongation of human erythrocyte survival in a heterologous species was first demonstrated in 1970 (1). Rapid removal of the erythrocytes transfused to rats was prevented through the use of ethyl palmitate, which blocked the reticuloendothelial system and induced acute splenic necrosis (2, 3), and a factor in cobra venom (CVF), which inactivated the third component of complement resulting in profound inhibition of intravascular hemolysis (4).

The present investigation involves the survival of ¹⁴C-tagged erythrocytes from patients with sickle-cell anemia (sickle erythrocytes) in rats that have been previously treated with ethyl palmitate and CVF. The study demonstrates that sickle erythrocytes have a shorter circulating life span than that of control human erythrocytes (sickle erythrocytes). It also demonstrates that exposure of the recipient animals to 100% O2 improves the survival of sickel erythrocytes and that subsequent animal exposure to low oxygen tension results in rapid loss of 35-60% of sickle erythrocytes. This response to hypoxia was not observed with control erythrocytes.

Analogy between these results and observations on the behavior of sickle erythrocytes in humans suggests that this animal model may be of considerable value in the study of the pathogenesis and therapy of human sickle-cell disease.

Abbreviation: CVF, cobra venom factor.

MATERIALS AND METHODS

Studies were performed on eight patients with homozygous sickle-cell disease, which was proven by analysis of hemoglobin by acrylamide gel electrophoresis. The hematologic and clinical characteristics for each patient are shown on Table 1. None of the patients had received blood transfusions within the previous 3 months. The 12 control subjects were healthy volunteers of comparable age in whom hemoglobin electrophoresis showed an AA pattern. 4- to 8-ml Aliquots of sterile venous blood was removed from each individual and anticoagulated with acid-citrate-dextrose (Vacutainer tubes no. 4786, Becton-Dickinson, Rutherford, N.J.) solution in a ratio of blood to anticoagulant of 4:1. 5 ml of the anticoagulated blood was incubated with 200 µCi of ⁴¹Cr (sodium Chromate, E. R. Squibb & Sons, New Brunswick, N.J.) for 30 min at room temperature (23°) followed by 10 min at 37° with frequent mixing. The ⁴¹Cr-tagged erythrocytes were then washed twice in normal saline and suspended in saline to a concentration of 30-33%.

The transfusion recipients for these experiments consisted of male Sprague-Dawley rats weighing between 150 and 250 g, 18-24 h before transfusion each rat was given 3 g/kg of body weight of ethyl palmitate (Eastman Kodak Co., Rochester, N.Y.) as an emulsion by the tail vein (3). The animals were also injected intravenously 30-60 min before transfusion with 10-20 anticomplementary units of CVF (Cordis Laboratories, Miami, Fla.). Each transfusion was done by the administration of a suspension of ⁴¹Cr-tagged human erythrocytes in the amount of 1 ml of packed erythrocytes per kg through the dorsal vein of the penis. A minimum of two animals was injected with each of the donor samples. Rats were anesthetized with ether for all of their intravenous injections, but no anesthesia was used while the survival experiments were in progress. Blood samples for radioassay were taken 15 min after injection and thereafter at hourly intervals for at least 6-8 h. Each sample consisted of 10 µl of capillary blood obtained from the cut surface of the tail. The blood-filled microcapillary tube was then placed in the bottom of a disposable plastic test tube, and the radioactivity was counted in a well-type scintillation counter for a period of time sufficiently long to reduce the counting error to less than 5%. The net radioactive counts were plotted on semilogarithmic paper against time in order to determine the T½, and erythrocyte survival characteristics.

The immediate recovery of human erythrocytes after transfusion was estimated from the counts injected in com-
parison to those recovered from an extrapolation of the initial portion of the survival curve to time 0. The rat blood volume used for these calculations was 4.98 ml/100 g of body weight.

In several experiments, rats were placed in a large plastic chamber through which oxygen and nitrogen were infused in variable proportions at a constant rate. The usual procedure was to determine erythrocyte survival for 2–3 hr while the rats were in 100% O₂, after which the concentration of oxygen was rapidly reduced to 7–10% for the remainder of the observation. Oxygen concentration was carefully monitored at frequent intervals by means of a Beckman Oxygen Analyzer, model D2.

In order to be certain that differences in the ⁵¹Cr survival of control and sickle erythrocytes were not due to increased in vivo elution of ⁵¹Cr from sickle erythrocytes, concomitant differential agglutination (Ashby) (6) and ⁵¹Cr/erythrocyte survivals were performed in duplicate in one of the patients studied (J.G.). Her erythrocytes were of blood group A so that anti-B typing serum was used for agglutination of the rat cells, which are of type AB.

RESULTS

The results of the recovery and survival, after transfusion, of sickle erythrocytes in rats in room air in comparison to those of erythrocytes from the control subjects are shown in Table 1. T½ for controls ranged from 7 to 23.5 hr (mean 13.3 ± 4.77 hr). The T½ for the sickle erythrocytes was between 1.2 and 4.5 hr in seven of the eight subjects and averaged 7.0 hr in the other patient. The mean T½ for the sickle erythrocytes group was 3.5 ± 1.40 hr. Survival was essentially exponential with first-order kinetics for most of the sickle erythrocytes (Fig. 1). The erythrocyte survival for patient E.M. followed the control range for about 6 hr, after which there was a definitely more rapid loss of erythrocytes during the remainder of the observation period.

A total of 36 experiments with blood of the eight patients with sickle-cell disease and of seven control subjects was performed in which the recipient rats first were exposed to 100% O₂ for 2–3 hr and then switched abruptly to 7–10% O₂. In five of the eight patients (14 experiments) there was significant prolongation of the ⁵¹Cr T½, to a mean of 5.34 ± 2.35 hr during hyperoxygenation. An abrupt loss of 35–60% of the blood radioactivity occurred during the 30–60 min after exposure to hypoxia (Fig. 2). 12 Experiments with erythrocytes from the three remaining patients demonstrated inconstant prolongation of ⁴¹Ca/erythrocyte survival in two patients (1 or 6 and 3 or 5) and no prolongation in survival in the other (1 experiment). Only those studies in which the ⁴¹Ca T½ could be prolonged beyond 3 hr with 100% O₂ showed a significant accelerated loss of erythrocytes after exposure to hypoxia.

Recovery of sickle erythrocytes immediately in rats was significantly lower than that for the controls (Table 1). In both groups there was a wide range of recovery values.

![Graph](image_url)

**Table 1. Recovery and survival of human sickle cells in rats after transfusion**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (y)</th>
<th>Hb S (%)</th>
<th>Hb F (%)</th>
<th>Hematocrit (%)</th>
<th>Recovery after transfusion (%)</th>
<th>Erythrocyte survival ⁴¹Ca T½ (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mean ± Range</td>
</tr>
<tr>
<td>S.B.</td>
<td>F</td>
<td>22</td>
<td>87.5</td>
<td>10.5</td>
<td>27-30</td>
<td>85.2(4)</td>
<td>68-96</td>
</tr>
<tr>
<td>E.O.</td>
<td>F</td>
<td>28</td>
<td>95.9</td>
<td>3.1</td>
<td>22-27</td>
<td>3.3(4)</td>
<td>10-4.2</td>
</tr>
<tr>
<td>M.C.</td>
<td>F</td>
<td>16</td>
<td>88.6</td>
<td>8.2</td>
<td>22-26</td>
<td>3.3(6)</td>
<td>2.0-4.5</td>
</tr>
<tr>
<td>S.E.</td>
<td>F</td>
<td>23</td>
<td>92.9</td>
<td>5.5</td>
<td>21-25</td>
<td>2.7(9)</td>
<td>1.2-4.5</td>
</tr>
<tr>
<td>J.G.</td>
<td>F</td>
<td>33</td>
<td>93.4</td>
<td>3.7</td>
<td>20-25</td>
<td>2.0(8)</td>
<td>1.2-4.5</td>
</tr>
<tr>
<td>E.M.</td>
<td>F</td>
<td>6</td>
<td>85.0</td>
<td>15.0†</td>
<td>24-28</td>
<td>56.0(6)</td>
<td>46-64</td>
</tr>
<tr>
<td>S.M.</td>
<td>F</td>
<td>25</td>
<td>93.0</td>
<td>5.3</td>
<td>21-24</td>
<td>36.2(11)</td>
<td>13-66</td>
</tr>
<tr>
<td>S.T.</td>
<td>M</td>
<td>5</td>
<td>90.8</td>
<td>9.2</td>
<td>25-28</td>
<td>61.0(3)</td>
<td>57-63</td>
</tr>
</tbody>
</table>

Controls

(Means of 12 AA individuals)

<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
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<th>Mean ± Range</th>
<th>Mean ± Range</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>79.3(20)</td>
<td>13.3(28)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>49-118</td>
<td>7-23.5</td>
</tr>
</tbody>
</table>

* Number of studies performed, in parentheses.
† At 8 months of age.
Fig. 2. Effect of exposure of recipient animals to varying oxygen tension on the survival of sickle erythrocytes. (●—●) sickle erythrocytes (〇—〇) control erythrocytes. Points represent means and bars represent standard deviation for each group (see text).

No apparent relationship was observed between [51Cr]-erythrocyte T1/2 and the recovery immediately after transfusion of either of the study groups.

The results of simultaneous 51Cr and Ashby survival studies are shown in Fig. 3. Virtually no difference was noted in either shape or slope of the two survival curves, as demonstrated by the 51Cr and differential agglutination methods. This was interpreted as meaning that there was no excessive elution of 51Cr from sickle erythrocytes to account for their shortened survival. These erythrocytes could be easily identified in rat capillary blood (Fig. 4) during the early period after transfusion by means of sodium metabisulfite hemoglobin reduction (7).

**DISCUSSION**

The behavior of homozygous sickle-cell erythrocytes in rats injected with CVF and ethyl palmitate closely resembles their behavior in man. In humans, the 51Cr T1/2 of sickle erythrocytes is in the range of 1:7 to 1:3 that of control erythrocytes (8). This relationship is maintained in rats despite the relatively short survival of all human erythrocytes in this species. This strongly suggests that the factors responsible for the accelerated removal of homozygous sickle cells in humans are also operative in rats. Presumably, the main determinant of survival of sickle erythrocytes in both species is intravascular sickling with small vessel trapping of the sickled erythrocytes. This assumption also is supported by the behavior of sickle erythrocytes in rats in states of hyperoxygenation and hypoxia. The mean T1/2 of sickle erythrocytes when the animals breathed 100% O2 was substantially prolonged in comparison to that of the animals breathing room air with rapid decline in the number of tagged cells in circulation after the introduction of marked hypoxia. These results are consistent with the relationship of oxygen tension to the sickling and unsickling of sickle erythrocytes.

The hypoxia effect on sickle erythrocytes was evident only if the 51Cr T1/2 approached the control range. In most instances this was achieved through the use of 100% O2. This observation suggests that hyperoxygenation in rats resulted in recruitment or accumulation of an increased number of sickle erythrocytes that were extremely sensitive to hypoxia. This population of cells probably would have been removed at the usual faster rate had the animals been left in room air.

An explanation for the peculiar survival characteristics of E.M.'s erythrocytes is not apparent. The substantial fall with hypoxia after a near normal survival in room air indicates that hyperoxygenation is not always necessary for production of the hypoxic phenomenon.

Fig. 3. Similarity between the survival of sickle erythrocytes as measured by 51Cr (●—●) and differential agglutination (Ashby) (〇—〇) techniques.

Fig. 4. Wet preparations from capillary blood of rats 2 hr after transfusion with human sickle erythrocytes. Sodium metabisulfite was added to the blood. Upper (×400 magnification), typical holly leaf deformity of sickle erythrocytes. Lower (×1000 magnification), obvious sickle forms.
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It is fully appreciated that one should be very careful in extrapolating results concerning the behavior of human sickle cells in rats to humans. Modification of reticuloendothelial system function and immune mechanisms in rats results in an unphysiologic state. These manipulations, however, modify the undesirable side effects of massive hemolysis and accentuate the purely vascular effects on the donor erythrocytes. Also, exposure to widely divergent oxygen tensions is not physiologic, but again this manipulation serves to exaggerate conditions that may be constantly occurring in certain tissues in humans. If the sudden removal of sickle erythrocytes from the hypoxic rat's blood were analogous to the changes that occur in humans during sickle-cell crises, our observations might also imply the following: sickle crises could result or be enhanced through (a) transient periods of reduced erythrocyte destruction with accumulation of a population of erythrocytes exquisitely sensitive to hypoxia-induced sickling, and (b) a sickling triggering event such as hypoxia. If the sensitive population of erythrocytes was small, very little change in the patient's blood values would be noted during crisis and the crisis itself would cease spontaneously after the susceptible population had been removed.

Hypoxia resulted in the rapid clearance of only a certain proportion of sickle erythrocytes (between 35 and 60% of the number present before hypoxia). The remaining cells were destroyed at a lower rate despite continuation of the hypoxic stimulus in the animal. This observation strongly suggests that sickle erythrocytes are heterogenous in their susceptibility to small vessel trapping and removal from circulation in the presence of hypoxia. The nature of the factors responsible for this erythrocyte variability is uncertain, but erythrocyte age, variations in concentration of erythrocyte hemoglobin F, and membrane damage through previous reversible sickling may play a role.

This animal model could be useful in the evaluation of various therapeutic modalities directed at extending the survival of sickle erythrocytes. Preliminary experiments in our laboratory, for example, indicate that carbamylation of sickle erythrocytes with potassium cyanate results in significant improvement of their survival in the rat at normal oxygen tension. Carbamylation of sickle erythrocytes, however, did not prevent their accelerated removal in hypoxic animals. The major advantages of such studies are that they can be conducted in a rapid and inexpensive fashion without the hazards of human testing. It is hoped that additional research with this model will greatly accelerate progress in understanding the pathogenesis of sickle-cell anemia. The possible usefulness of this model in the investigation of other erythrocyte defects remains to be determined.

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