CONCISE REPORT

The Storage of Hard-Packed Red Blood Cells in Citrate-Phosphate-Dextrose (CPD) and CPD-Adenine (CPDA-1)

By Ernest Beutler and Carol West

The preservation of red cells "hard packed" to a hematocrit of over 80% from blood collected in citrate-phosphate-dextrose (CPD) or CPD-adenine (CPDA-1) has been investigated. After 21 days of storage, cells that had been collected in CPD solution had consumed most or all of the available glucose and manifested markedly impaired viability after reinfusion into the normal donor. In contrast, red cells prepared from blood collected in CPDA-1, a medium containing supplementary adenine and an increased amount of glucose, maintained higher glucose and adenosine triphosphate levels and, in most instances, manifested satisfactory posttransfusion viability. We emphasize that in addition to providing longer shelf life of stored blood, CPDA-1 provides a better hard-packed red cell concentrate for transfusion at 21 days.

HUMAN PLASMA has become an increasingly valuable blood resource. Although plasma expressed from whole units of blood serves as a major source of components such as albumin, factor VIII (antihemophilic globulin), and immunoglobulins, plasma obtained by pheresis of paid donors is required to supply sufficient amounts of various plasma components. Fortunately, many physicians have learned to use red cell concentrates rather than whole blood when appropriate, thus increasing the quantity of plasma available from single units. Traditionally, red cells are packed to a hematocrit of approximately 70% prior to storage. Under these circumstances, if 450 ml of blood with a packed cell volume (PCV) of 45% is collected into 63 ml of citrate-phosphate-dextrose (CPD) solution, one-half of the plasma has been left behind to be transfused with the erythrocytes. Although this is desirable from the point of view of providing a red cell concentrate with relatively low viscosity, the flow properties of red cell concentrates can also be restored by resuspending hard-packed erythrocytes in saline.

Understandably, then, the potential of nearly doubling the yield of plasma from units of blood collected in blood banks promotes a trend toward tighter packing of erythrocytes prior to storage at 4°C. However, little is known of the viability of the red cells stored in this manner. We have now studied the effect of storage on hard-packed red cells collected in CPD and in the newly licensed preservative CPD-adenine (CPDA-1). We have found, rather surprisingly, that red cells packed to a hematocrit of over 80% survive poorly when collected in CPD solution, but quite satisfactorily when CPDA-1 is used.

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MATERIALS AND METHODS

Studies were carried out in 14 adult men and women. All investigations were performed after obtaining informed consent from the volunteers and with the approval of the Institutional Review Board in accordance with NIH guidelines.

Four hundred fifty milliliters of blood (by weight) were collected into a triple-pack. The primary bag contained 63 ml of CPD or CPDA-1 solutions (Fenwal). One liter of CPD contains 3.27 g of citric acid·H2O, 26.3 g of sodium citrate·3H2O, 25.5 g of glucose·H2O, and 2.22 g of NaH2PO4·H2O. CPDA-1 contains 1.25 times the concentration of glucose in CPD, and in addition it contains 2 mM adenine, so as to provide a 0.25-mM concentration of adenine in the blood-preservation mixture. After being allowed to equilibrate at room temperature for 20-40 min, the units of freshly collected blood were centrifuged either at 1000 g for 9 min (to simulate centrifuging for platelet recovery) or at 3000 g for 9 min, and all of the visible plasma was expressed. After thorough mixing and removal of 9 ml of the packed cells for biochemical studies, the remainder of each red cell concentrate was stored at 4°C for 3 wk. The stored concentrates were undisturbed except for removal of an aliquot for culture 3 days prior to reinfusion. After 21 days of storage the packed cells were carefully mixed, and 7 ml were removed and mixed with 4-6 μCi of Na251CrO4 (Mallinkrodt Nuclear). After 20 min at room temperature, the cells were mixed with seven volumes of preservative-free sterile saline, and the suspension was centrifuged at 1000 g for 10 min. After a second identical washing, the red cells were resuspended in one volume of sterile saline and were reinfused into an antecubital vein of the original donor. Samples were drawn from the opposite arm at 5 min, 10 min, 15 min, 20 min, and 24 hr. The zero-time level of blood radioactivity was estimated by back-extrapolation of the first four points using a least-squares fit of a semilogarithmic plot. The viability of the red cells was then computed directly from percentage of label remaining in the blood at 24 hr.

Estimations of glucose and adenosine triphosphate (ATP) were carried out through the hexokinase-linked technique. The packed cell volumes were measured after centrifuging for 5 min in an International microhematocrit centrifuge.

RESULTS

Red cell concentrates with PCV of greater than 80% prepared from blood collected in CPD solution had consumed virtually all of the glucose at the end of 21 days. Similar concentrates prepared from blood collected in CPDA-1, a preservative with a higher glucose content, fared better, but in samples packed to a hematocrit of over 95%, glucose was also exhausted in 21 days. The relationships between the PCV and glucose and ATP concentrations after 21 days of storage are summarized in Fig. 1. Cells stored in CPDA-1 consistently maintained higher glucose and ATP levels than did samples stored in CPD.

The results of viability studies are summarized in Fig. 2. Of six units of concentrates collected in CPD packed with a cell volume of 80% or above, five failed to achieve 70% viability. In the case of one unit of packed cells collected in CPD and stored at a hematocrit of 92.5%, only 41% of the cells survived for 24 hr in the circulation. In sharp contrast, of seven units of red cells collected in CPDA-1 and packed at hematocrits ranging from 83% to 96%, only one, a unit with a PCV of 95.5%, failed to achieve 70% 24-hr viability.

The viability of the seven units of red cells collected in CPD (average hematocrit 86.9%) was only 63.5 ± 14.0% (mean ± SD). In spite of a higher average hematocrit of 91.4%, the viability of the packed cells collected in CPDA-1 was 76.5 ± 7.4%. This difference is statistically significant (t = 2.2, p < 0.05). An even greater difference is apparent when only those units with a hematocrit of greater than 85% are taken into consideration. The viability of the four such units stored in CPD (average hematocrit 91.5%) was 53.8 ± 8.6%. The six units of packed cells prepared from blood collected in CPDA-1 and stored at a hematocrit of over 85%
(average hematocrit 92.9%) had viability of 75.5 ± 7.5%. This difference was statistically highly significant ($t = 4.1, p < 0.01$).

**DISCUSSION**

In a recent survey of a few Red Cross blood centers storing packed cells from blood collected in CPD solution, 33% of the packed cells were stored at a

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**Fig. 1.** Relationships between glucose and ATP concentration and PCV in 21-day-stored red cell concentrates. The residual glucose and red cell ATP levels were consistently higher in concentrates prepared from CPDA-1 than in those prepared from CPD.

**Fig. 2.** Relationship between 24-hr viability and PCV in 21-day-stored red cell concentrates. Viability was consistently better in concentrates prepared from CPDA-1 than in those prepared from CPD.
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hematocrit of over 80%. In one center, 35% of the units were stored at hematocrits of 80%–85% and 10% at hematocrits of 85% or above.\(^3\) The trend toward storage of hard-packed red cells may well accelerate as increasing amounts of plasma are needed for fractionation.

In view of the massive quantity of data currently required for licensure of new drugs and blood preservatives, it is extraordinary to contemplate how little information is available regarding the efficacy of hard-packed red cells after collection of blood in CPD solution. The general assumption that a 21-day dating period is appropriate for packed cells stored in CPD, no matter what the hematocrit of the concentrate, is probably based in part on some relatively scanty data concerning concentrates stored in acid-citrate-dextrose (ACD) solution. However, these data\(^4,5\) are not applicable, since blood collected in ACD consumes glucose more slowly because of its lower pH and phosphate content. Its initial glucose content is also slightly higher than that of CPD-collected blood. In only one study\(^6\) were packed red cells with hematocrit over 85% that had been prepared from CPD blood investigated. Only two such units were examined after 21 days of storage; one had a reported viability of just over 70% and one just under 70%.

We now find that although the viability of red cells packed to a hematocrit of over 80% from CPD blood may sometimes be satisfactory, this is by no means always the case. As the PCV of the stored cells approaches 90%, the preservation of CPD-collected red cells seems to be uniformly quite unacceptable. The poor preservation of red cells under these circumstances seems to be largely related to the inadequate amount of glucose present, but other factors may be operative as well.

In designing an adenine-containing preservative solution, it seemed desirable to take into account the fact that a preservative used for the collection of whole blood should be suitable for the storage of packed red cells as well.\(^7\) For this reason the glucose content of the new preservative solution CPDA-1 was increased. CPDA-1 has been shown to be suitable for the storage of whole blood and red cells for 35 days.\(^8\) One of the advantages of this new preservative is that the longer shelf life of stored blood should decrease outdating in many blood banks and should provide the flexibility needed to ensure availability of blood during a period of decreased blood collection. A second advantage of CPDA-1 that has not received much attention is that it should improve the quality of blood given near the outdating period of CPD. This study dramatically demonstrates that the viability of hard-packed CPD-stored red cells is much less satisfactory than had previously been suspected. In contrast, satisfactory viability is maintained when the primary preservative is CPDA-1. However, it is apparent that even in this glucose-fortified medium, hard-packed cells consume most of the available glucose by the end of 21 days. It is unlikely that these cells can survive for much longer.

Studies of preservative solutions containing larger amounts of glucose are needed and are being undertaken.

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