Erythrocyte Creatine in Cord Blood

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ABSTRACT

Erythrocyte creatine (EC) content and reticulocyte counts were compared in normal adults, pediatric patients with hemolytic anemia, and cord blood. A good correlation between reticulocyte count and EC content was found in normal subjects and patients with hemolysis, thus confirming the usefulness of creatine as an estimate of mean red cell age in these populations. No significant correlation (p > 0.1) was observed between the two measurements in cord blood. While reticulocyte counts were significantly elevated (p < 0.001) in cord blood when compared to normal adults (indicating the presence of a young mean red cell age), EC concentrations in most samples were not correspondingly high. These results may indicate that creatine is not well synthesized by the neonatal red blood cell.

Introduction

Erythrocyte creatine (EC) has been found to be an accurate indicator of mean red cell age in normal adults and in patients with various types of anemia prior to therapy and during treatment.1,2,4,6,7,11 It correlates with other methods of determining red cell age including reticulocyte counts,51 Chromium erythrocyte half life (T50Cr), hexokinase activity, and aspartate aminotransferase activity.4,11 Erythrocyte creatine has been used to determine the relative age of cells which have been separated by density gradient centrifugation, a technique which concentrates in the upper layer those cells having the highest reticulocyte count and creatine level.4 With increasing density or increasing red cell age, the creatine content decreases to a lower but measurable level while the reticulocytes disappear almost completely. In cases of mild hemolysis (T50Cr ≥ 11 days), a better correlation has been noted between T50Cr and EC than between T50Cr and reticulocyte count.4 On the basis of these findings, it has been suggested that EC might prove to be a sensitive indicator for the assessment of hemolytic disease.4

The neonate has an erythrocyte population that is biochemically and physiologically unique. In comparison to erythrocytes and reticulocytes of older individuals, the newborn's red cells contain more fetal hemoglobin and methemoglobin, exhibit a unique membrane structure, possess different levels of activity of glycolytic enzymes, and have a less efficient energy metabolism.10 In ad-
dition, the neonatal red cell population has a younger mean red cell age than the adult erythrocyte.3

Because of the physiologic peculiarities of neonatal erythrocytes, reticulocyte count and EC were measured in cord blood in an attempt to evaluate the usefulness of red cell creatine as an estimate of mean red cell age in neonates.

Materials and Methods

Blood was obtained from 44 normal subjects, 25 males and 19 females, ranging in age from 11 to 70 years. Samples were collected in ethylenediamine tetracetic acid (EDTA)* for both reticulocyte count and creatine determinations. All subjects had normal hemoglobins, red blood cell counts and indices determined by Coulter Model S.† Cord blood samples in EDTA were obtained from 43 healthy term neonates who had unremarkable perinatal histories (21 males, 22 females). For comparison, blood was obtained from 12 pediatric patients with hemolytic anemia, ages 2 to 11. Reticulocyte counts for all samples were done in duplicate on 1000 cells by two laboratory technicians according to the method of Miale.9

For creatine measurements, blood samples were stored at 4°C and analyzed within 14 days of collection. Immediately before analysis, red cells were concentrated by centrifugation twice at 2000 to 3000 RPM for 10 minutes followed each time by removal of the serum and buffy coat. Packed cells were mixed vigorously in a vortex mixer;‡ to ensure homogeneity and then lysed by diluting 0.1 ml of the packed cells into 0.9 ml of 0.1 percent saponin.§ The creatine content of the hemolysate was then determined by the automated diacetyl-1-naphthol procedure5 and expressed as milligrams of creatine per deciliter of packed red cells. The reagents required for the procedure were as described.6

Previous work in our laboratory has shown that glutathione, which is present in high concentrations in the erythrocyte, inhibits the diacetyl reaction for creatine, resulting in an underestimation of creatine content. To prevent this inhibition, the sulfhydryl inhibitor, p-chloromercuribenzoic acid (PCMB)‖ was added to the saline diluent at a concentration of 0.09 percent. Recoveries of added creatine from hemolysates in the presence of PCMB averaged 94.3 ± 1.1 percent. The sulfhydryl inhibition of the creatine reaction has recently been examined by Li et al.,8 and a more detailed discussion is presented there.

The analysis of separate variance estimate was used for statistical evaluation of the differences of reticulocyte counts and red cell creatine between the two sample groups. Regression equations were calculated by the least squares method for the following relationships: blood reticulocyte count expressed in percent of red cells and EC for normal adults, patients with hemolysis, and cord blood.

Results

The mean reticulocyte count for the 44 normal subjects was 0.9 ± 0.45 percent of red cells (mean ± S.D.) with a range of 0.2 ± 2.2 percent. The mean EC concentration was 7.2 ± 1.4 mg per dl (mean ± S.D.) with a range of 2.9 to 11.0 mg per dl. These results agree with the creatine values previously noted in normal adults.1,5,13

The mean reticulocyte count for the 43 cord blood samples was much higher, 4.3

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± 1.3 percent of red cells (mean ± S.D.) with a range of 1.3 to 7.2 percent. These results agree with values obtained by other investigators. The mean EC was 9.8 ± 3.2 mg per dl with a range of 4.9 to 20.2 mg per dl.

A highly significant difference (p < 0.001) was found between the mean reticulocyte count in normals and neonates. The difference between the mean EC values for these two populations was also significant (p < 0.001).

The mean reticulocyte count for the 12 patients with hemolysis was much higher than that seen in the first two groups, 12.3 ± 6.8 percent of red cells (mean ± S.D.) with a range of 1.8 to 23.9 percent. The mean EC concentration was 42.2 ± 18.1 mg per dl (mean ± S.D.) with a range of 15.6 to 70.5 mg per dl.

A significant correlation (r = 0.53, p < 0.001) was found between reticulocyte count and EC in normal adults (figure 1). This correlation was not improved by comparing absolute reticulocyte numbers (retics per mm³) rather than reticulocyte percent (r = 0.44, p < 0.01—data not shown). A good correlation between reticulocyte count and EC was also noted in patients with hemolysis (r = 0.67, p < 0.05) (figure 2). In contrast, the cord blood samples showed no significant correlation between the two parameters (r = 0.17, p > 0.1) (figure 1).

Linear regression analysis of the data gave the following regression lines for the three populations studied: normal adults, y = 1.75x + 5.7; patients with hemolysis, y = 1.78x + 20.2; and cord blood, y = 0.43x + 8.0.

Discussion

The significant elevation of reticulocyte counts observed in cord blood sam-
amples when compared with normal adult blood samples indicates the presence of a young mean red cell age in cord blood. Bratteby and co-workers\(^3\) have noted an increased production and an increased destruction of red cells during the last two months of gestation that results in a relative rate of red cell production that is three to five times that found in adults. Using equations derived from accumulated data on neonatal red cell life span, they calculated the mean life span of cells produced during the last 60 days of fetal life to be between 45 to 70 days, which is considerably less than the 120-day life span of a normal adult erythrocyte.

The significant correlation between reticulocyte count and EC concentration noted in normal subjects and in patients with hemolysis (see figures 1 and 2) confirms the usefulness of EC concentration as an estimate of mean red cell age in these populations. Although most of the hemolysis patients included in this study exhibited very high reticulocyte counts (with correspondingly high EC concentrations), studies of patients with mild hemolytic disease, in which the reticulocyte counts are lower, have also noted good correlations between the two parameters.\(^4\)\(^1\)\(^2\) That creatine has also been reported to correlate well with red cell survival times\(^4\) provides further evidence of its close relationship with red cell age.

No correlation between reticulocyte count and EC concentration was observed in the cord blood samples (see figure 1). Although the reticulocyte counts in these samples were significantly elevated, the creatine levels in most cases were not correspondingly high. This leads to the speculation that perhaps creatine is not well synthesized by neonatal erythrocytes. Creatine synthesis requires S-adenosyl methionine, which in turn requires adenosine triphosphate (ATP)—activated methionine. The hypoglycemia and hypoxia common in the neonate could result in a decrease of available ATP, thereby reducing the amount of creatine synthesized.

The inherent variability of reticulocyte counting suggests that other indicators of red cell age such as hexokinase and serum glutamic oxaloacetic transaminase (SGOT) (which have been shown to correlate well with EC in hemolytic and non-hemolytic anemias\(^1\)) might provide a more meaningful comparison with neonatal EC. In the present study, the small amount of cord blood obtained made additional sample analysis for these enzymes impractical.

In summary, red cell creatine concentration has been found to give an accurate assessment of mean red cell age in normal adults and in patients with hemolytic disease, as reflected by its correlation with reticulocyte count. The lack of correlation between these two measurements in cord blood may be due to decreased creatine synthesis in the neonatal red blood cell. Additional comparisons of creatine with other indicators of red cell age must be done before the role of creatine in the neonatal erythrocyte can be delineated, and its use as an index of neonatal red cell age established.

Acknowledgments

The authors wish to express their appreciation to Janet Sakell who performed the statistical analysis of the data.

References

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