CONCISE REPORT

An Objective Sign in Painful Crisis in Sickle Cell Anemia: The Concomitant Reduction of High Density Red Cells

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The etiopathologic basis of painful crisis in sickle cell anemia is largely unknown, and no objective criteria for its diagnosis and follow-up exist at present. We have studied 11 patients through 14 painful crises and observed a significant decrease of the densest fraction of red cells in 12 of the 14 crises as determined by isopycnic Percoll-Stractan continuous density gradients. If the first observation is normalized to 100%, the average decrease in dense cells was 77% with a range of 36% to 94%. The time needed for the percentage of dense cells to return to the steady-state level varied from seven to more than 30 days. These findings were in sharp contrast to the stability of the density pattern observed in another group of sickle cell patients, who were studied during crisis-free periods. The mechanism of the disappearance of dense cells could involve selective destruction by the reticuloendothelial (RE) system, selective sequestration in the areas of vascular occlusion, or a combination of both factors.

The etiopathologic mechanism of the painful crisis that punctuates the clinical course of sickle cell anemia is poorly understood. Little is known about the physiologic events that precede and accompany its initiation and development, and no objective signs have been associated with painful crisis. This lack of knowledge leads to problems ranging from uncertainty of diagnosis to the design and evaluation of rational therapy.

Our laboratory has previously reported striking differences in the distribution of red cell densities among sickle cell patients. Using a continuous density gradient system, we have described four classes of progressively denser red cells in the sickle cell anemia patient. These different density fractions have distinct rheologic and hemodynamic properties that may be presumed to play a role in the pathophysiology of the disease.

We report here that, while the density distribution pattern is stable during the steady state of the disease (and further data presented here), the pattern changes significantly during a painful sickle cell crisis: the proportion of densest fraction of cells (fraction IV) decreases significantly, and in some cases dramatically, in coincidence with the event.

MATERIALS AND METHODS

Patient Selection

All patients homozygous for hemoglobin S with the acute onset of moderate to severe painful crisis (six or more hours of unreolved pain as judged by physician and patient) who were admitted to Rockefeller University and Jacobi Hospitals during the months of April to July of 1983 were included in this study after informed consent was obtained. The bilirubin levels and white cell counts of the patients were not higher than usual and they were either afebrile or exhibiting a temperature no higher than 101°F. Only two classes of patients were excluded: (1) patients who were treated by transfusion during their crisis or during the preceding 3 months, and (2) patients with S-T-thalassemia.

Evaluation of Pain

Pain was evaluated daily, immediately before analgesic administration. It was characterized as mild (movement did not increase pain), moderate (movement did increase pain), or severe (pain prevented movement). A score was calculated taking into account the number of locations affected and pain intensity. These data were translated to a scale of 1 to 4. In some patients it was difficult to assess the exact starting date of the pain and its intensity before hospitalization. The scoring of pain presented here should be considered an approximation.

Treatment

Patients received hydration treatment (noted in parenthesis when the individual crisis is discussed). All patients received variable amounts of intramuscular meperidine. In four cases nasal oxygen was used.

Density Gradients and Their Evaluation

Percoll (colloidal silica coated with polyvinylpyrrolidone; Pharmacia Fine Chemicals, Inc, Piscataway, NJ) and Stractan (arabinogalactan polysaccharide; St Regis Paper Co, West Nyack, NY) (prepared as described by Corash) gradients were formed from a mixture of Percoll and Stractan (density 1.207, determined from refractive index), water, and ten-fold balanced salts in a ratio of 3.5:3.2.8:0.7. The pH and osmolarity were adjusted to 7.35 and 290 mosm, respectively. Batches large enough to make 200 to 300 determinations were prepared and frozen in 50-mL aliquots. A

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0.1-mL aliquot of a well-mixed sample of whole blood with the hematocrit adjusted to 50, preserved in citrate phosphate dextrose (CPD), was added to 5.9 mL of the gradient mix; the tubes were mixed by inversion, and finally they were spun in a Sorvall SS-34 rotor (Sorvall Instrument Co, Newton, Conn) for 40 minutes at 17,000 rpm (35,000 g) at 38 °C. The resulting gradient is continuous but nonlinear. Each group of eight tubes included a sample from an individual homozygous for hemoglobin A and a tube with Pharmacia density marker beads to ensure that conditions were constant from one centrifugation to the next. Scrupulous care in resuspending the density marker beads to ensure that conditions were constant from the densest cells selectively adhere to the bottom of the tube. The density gradients were photographed with Polaroid 46-L (Cambridge, Mass) continuous density transparency film to give a 5.2-cm image of the distance between the meniscus and the bottom of the tube. They were read in a Corning model 720 densitometer (Corning Medical, Medfield, Mass) to give ten densities. The bottom three readings were summed to give a value for fraction IV. The MCHC at the beginning of F4 is 40 g/dL; this corresponds to a density of about 1.10 g/mL. These values were obtained from calibration curves shown in Fabry and Nagel.’

RESULTS

Steady State

Observation of six patients in the steady state (no crisis within the month before the observation) for a period of 14 months demonstrated that fraction IV remained constant to within ±5% (Fig 1) for five individuals and ±7% for the remaining patient.

Sickle Cell Crisis

Figure 2 illustrates density gradients obtained during the first 11 days of painful crisis for the patient C.T. The densitometer determinations from these gradients are plotted in Fig 3B.

Figure 3A depicts observations on patient A.W. during 51 days following admission on the second day of crisis (treatment: IV isotonic saline for four days). The initial drop in the percentage of fraction IV (F4) was followed by four nearly constant values. The X marks the onset of an hepatic crisis, which was followed by a further decline in F4. A second crisis began on day 20 (treatment: oral hydration), when F4 represented less than 3% of the total. Following the second crisis, F4 gradually increased over a period of seven days to about 16%, which was maintained for another six days. On day 45, a third crisis began (treatment: oral hydration), accompanied by a dramatic decline in F4.

Patient J.P. (Fig 3A) (treatment: IV hypotonic saline [½ saline, ½ glucose] for six days) was followed for 92 days. Again, a dramatic decrease in the number of dense cells (F4) followed the onset of the crisis. The percentage of F4 plateaued at a value of about 8% on days 28 to 38, at which time he was hospitalized to treat his leg ulcers and then increased to a stable 15%.

The patients C.T. (treatment: IV hypotonic saline for five days) and A.C. (treatment: IV hypotonic saline for two days) (Fig 3B) had the highest initial concentration of F4 before the onset of crisis, 36% and 39%, respectively. The density gradients for the patient C.T. were shown in Fig 2. The decreases to 9% and 12%, respectively, were the largest absolute changes observed. Another patient followed through two crises was G.H. (Fig 3B). The onset of the first crisis was one day before admission (admission 1, IV hypotonic saline for four days; nasal O2 days 3 and 4). Following termination of the first crisis, F4 increased to 32% by day 52. On day 56 a second crisis began (admission 2, IV hypotonic saline for one day, nasal O2 prn, days 57 to 60), which again resulted in a dramatic decline in the number of dense cells.

Patient G.G. (Fig 3C) (treatment: IV hypotonic saline for four days) exhibited a sharp decline in the number of dense cells. Patient A.A. (Fig 3C) (treatment: IV hypotonic saline for three days, no hydration for two days, and IV hypotonic saline for two days) suffered an extended crisis with two exacerbations of pain that were followed by modest declines in the number of dense cells. Patient M.G. (Fig 3C) (treatment: oral hydration only) also exhibited a decrease in F4. Patient E.H. (not shown) (treatment: 5% dextrose in water for seven days, nasal O2 for seven days) was admitted after five days of severe pain and had a decrease in F4 from 12% to 5%. Patient M.C. (treatment: IV hypotonic saline for three days) was the only patient monitored who did not exhibit a decrease in F4. This patient had the least severe crisis (grade 1 pain maximum) of the eleven patients in this study. Patient V.W. (followed for 35 days, not shown) (treatment: IV hypotonic saline for six days, oral hydration for two days, nasal O2 on days 9 and 10) was admitted after two days of moderate pain (level 2) that subsequently increased to level 3. A small decline in F4 from 6% to 2% was observed on days 4 to 7. Following the crisis, F4 gradually increased to a five-day plateau at 9% on days 8 to 14 and then increased again to a nine-day plateau at 14%.

The patient sample consisted of six male and five female patients with an average age of 27 years and a
range from 21 to 34 years. During the steady state, their hemoglobin concentration had a mean of 8.8 ± 0.8 g/dL (±SD). The MCV (mean corpuscular volume) was 89.5 ± 8.5 fL. The reticulocyte count averaged 14.5 ± 5.5%. White blood cells were 11.9 × 10⁹ ± 2.8 × 10⁹. The percent fetal hemoglobin was 2.9 ± 2.7% SD. Bilirubin was 2.5 ± 1.1 mg/mL. Blood urea nitrogen averaged 6.2 ± 3 mg/dL and LDH had a mean of 463 ± 162 mU/mL. During crisis routine hematologic determinations using a Coulter Counter S+ (Coulter Electronics, Hialeah, Fla), manual reticulocyte counts, and blood chemistry determinations (SMAC) were performed on all patients at some time and on most of the patients daily. We found moderate to extreme (+1,000 mU/mL) elevation of lactate dehydrogenase (LDH) during the course of eight of 11 crises for which data was available, although specific red isoenzyme will have to be measured in the future for a precise understanding of this finding. In none of the patients did sodium levels fall outside of the normal range. In a separate series of ten patients in crisis, the osmolality averaged 288 mosm on admission.
second day, an average decrease of osmolarity of 10 mosm was observed. Subsequent variations were random. When dense cells are exposed to 278 mosm, no changes in density are detected in our gradient. No changes in bilirubin (except in the patient who developed hepatic crisis) or white cell count were observed.

DISCUSSION

We have followed the evolution of the percentage of densest red cells (F4), in 11 sickle cell anemia patients who had 14 painful crises. In 12 episodes, the event was accompanied by a significant reduction in the percentage of densest cells. When the first observation was normalized to 100%, the mean decrease was 77%, with a range between 36% and 94%, and a standard deviation of ±17%. There were two exceptions: In patient A.W. (Fig 3A), no significant decrease was observed during the second crisis but the percentage of F4 was already depressed as a consequence of the preceding crisis. Patient Mi.G. had no change during seven days of observation but had the mildest crisis (in both intensity and duration) in this sample. The time needed for the percentage of densest cells to return to their steady-state value after the end of the crisis ranged from as little as seven days to as long as 30 days.

Hemoglobin levels decreased slightly, 1 to 2 g/dL, at the onset of most crises but were probably compensated by increased reticulocyte production (Figs 3A to C). Although F4 decreased an average of 77% (from the initial value) during these patients’ crises, this fraction accounted for less than 30% of the total number of red cells in all but two patients (C.T. and A.C.). Therefore, their hemoglobin concentrations would not be expected to decrease significantly during crisis.

A sickle cell crisis was observed while the patient A.W. had a very low percentage of dense cells (Fig 3A) which is evidence that these cells are not essential nor causative for the onset and continuation of a sickle cell crisis. This observation is in accordance with the lack of apparent correlation between the incidence of sickle cell crisis and the percentage of F4 red cells (unpublished observations).

The fate of the densest cells during painful crisis in the sickle cell anemia patient requires further study. The trivial explanation that this disappearance is the consequence of treatment can be discounted for the following reasons: (a) Patient Mi.G. (not shown), who was rehydrated and received IM meperidene for three days did not exhibit a decrease in dense cells. (b) No correlation was observed between the type of hydration given (oral, IV hypotonic, or IV isotonic) and the disappearance of dense cells. (c) Based on our experience with the effect of hypotonicity on red cell density in vitro, as studied with density gradients, changes in hydration would, at the most, cause the densest cells (F4) to repopulate fraction III, but not disappear. This is not what we observed in these patients (Fig 2). (d) In at least two instances [the first crisis of G.H. (Fig 3B) and V.W. (not shown)], it seems probable that a decrease in fraction IV had occurred before the first observation and before treatment, since their percentage of dense cells subsequently increased to values greater than the first observation. (e) Nasal oxygen was administered to three of the eleven patients during four crises with no apparent effect on the percentage of F4, and in one instance (V.W.) was given while the dense cells were maintaining a constant value. This is in accordance with the observation of others. Finally, the reticulocyte response observed in most patients is evidence in favor of the actual disappearance of the dense cells from the circulation during crisis. Interestingly, we have observed a correlated phenomena that is readily accessible in a clinical setting. A Coulter Counter generated value, the red cell distribution width (RDW), falls in parallel to the decrease in F4. This result has been the subject of a preliminary report and will be discussed in detail in a separate publication.

The mechanisms that could be invoked to explain the disappearance of the densest cells during the painful crises of sickle cell anemia include: (1) selective destruction of these cells by the RE system, and (2) selective sequestration of these cells in one or more areas of vaso-occlusion. Further work is needed to explore these possibilities, but neither explanation requires, necessarily, that the consequences of extravascular hemolysis be observed in these patients.

With the exception of Diggs and Rieben et al, very little work on irreversibly sickled cells (ISCs) or dense cells during the course of crisis has been reported. The former reported no change in ISC (a feature that is difficult to quantitate because of the presence of deformed cells) in crisis, and the latter reported that the ISC count decreased in all of their patients during crisis. This and the increased filterability observed within 12 days of the onset of crisis can be entirely explained by the data presented here.

The remarkable decrease of the densest red cells described here is an objective sign of the presence of painful crisis. Its elucidation should increase our insight into the pathophysiologic events that follow its onset and further observations may prove helpful in the diagnosis of crisis and in the evaluation of therapy.

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