Detection of Prostaglandin Induction of Erythrocyte Sickling

Malcolm Johnson, Israel Rabinowitz, Anthony L. Willis, and Paul L. Wolf

Sickle cell crisis may be precipitated by pregnancy or infection, conditions in which prostaglandin concentrations can be elevated. We have investigated the effects of exogenous prostaglandins on whole blood and isolated, washed erythrocytes from patients with sickle cell anemia disease. Differential counting, light scattering, and optical absorption techniques indicate that prostaglandin E₂ can induce and potentiate sickling of these cells under conditions of reduced oxygen tension. Absorption characteristics of sickle cell hemoglobin indicate that prostaglandin E₂ affects hemoglobin only in an intact cell. These initial results suggest that prostaglandin E₂ may represent a cofactor initiating or enhancing sickle cell crisis. A new application of a light-scattering technique appears a valuable method for continuous monitoring of the sickling process.

Additional Keyphrases: sickle cell anemia • light-scattering technique • mechanism of pathology • prostaglandin E₂ • infection • pregnancy • hemoglobin absorption curve

Among the factors that can evoke a sickle cell crisis in susceptible individuals are infection, fever, and pregnancy (1,2). It is now known that concentrations of prostaglandins in maternal amniotic fluid and blood are elevated in late pregnancy (3,4), and there is mounting evidence for involvement of E-type prostaglandins in inflammatory processes and fever (5,6). Allen and Rasmussen (7) have shown that prostaglandin E₂ can decrease red cell deformability in concentrations approaching one molecule of prostaglandin per erythrocyte. These considerations, plus the growing awareness that biochemical characteristics of the pathological erythrocyte may be related to gross structural changes, have led us to investigate the effect of prostaglandins on the shape of both normal and sickle cell erythrocytes. To facilitate the rapid and continuous detection of sickling, we are developing new applications of a light-scattering technique, used to detect shape changes in a viable population of erythrocytes.

Materials and Methods

Normal or sickle cell whole blood was used on the day of collection and washed erythrocytes not later than four days after collection. Erythrocytes were washed four times in isotonic saline buffered with Tris [tris(hydroxymethyl)aminomethane], pH 7.3-7.5, and diluted in Tris–saline before use. Crystalline prostaglandins were taken up in ethanol–water (70:30, by vol), evaporated to dryness just before use, and redissolved in either Tris–saline for erythrocyte studies or phosphate buffer (40 mmol/liter, pH 7.5) for hemoglobin studies. Prostaglandin concentration was 250 ng/ml, except in hemoglobin studies, for which the concentration was 300 ng/ml (1 μmol/liter). Hemoglobin was prepared from normal and sickle cell blood by the method of Drabkin (8) and used in concentrations of 0.1 or 0.01 mmol/liter. Changes in cell shape were followed at 502 nm in the light-scattering attachment of an “Acta V Spectrophotometer” (Beckman Instruments, Inc., Fullerton, Calif. 92634) and oxygen tension was monitored with a Clark-type oxygen electrode (Beckman, Model No. 1008). Differential cell counts were performed by using wet mounts and Wright-Giemsa stained erythrocytes.

A block diagram of the spectrophotometric flow cell arrangement used to detect sickling of erythrocytes is shown in Figure 1.

Results

We have previously demonstrated that sickling can be induced in sickle cell anemia erythrocytes by addition of PGE₂² under conditions of reduced oxygen tension (9). Figure 2(a) confirms that lowered oxygen tension alone will induce some sickling, while addition of vehicle (evaporated ethanol in Tris–saline) has no effect. The sickling effect is markedly potentiated by adding PGE₂ (250 ng/ml) before lowering the oxygen tension (Figure 2b). Figure 3 illustrates that this sickling effect of prostaglandin can also be followed by monitoring the absorption spectrum of hemoglobin. Prostaglandin E₂ (300 ng/ml) has no effect on the absorption spectrum of Hb in normal erythrocytes under either increased or decreased oxygen tension. In sickle cell erythrocytes, on the other hand, there is a marked change in this spectrum, especially in the Soret band near 400 nm, under reduced oxygen tension. The Soret band maximum moves 4–5 nm toward shorter wavelength, with an increase in its extinction. The Soret peak shift is more apparent in whole blood, where it is also accompanied by changes in peak-to-peak distance of the 575 nm and 545 nm absorption bands. Figure 4

² Nonstandard abbreviations used: PG, prostaglandin, and Hb, hemoglobin.
shows that prostaglandin added to pure hemoglobin of either normal or sickle cell donors has no effect. The apparent difference in the Soret band spectrum of the sickle cell donor seen in Figure 4(d) is almost entirely the result of a dilution effect, as seen by the dotted line representing addition of vehicle. This hemoglobin was purified from the same blood sample of the individual whose washed erythrocytes responded to PGE$_2$ and N$_2$ as shown in Figure 2(b).

**Discussion**

Patients with sickle cell anemia may develop a crisis following an infection, and it has been demonstrated that they have a higher incidence of infection. One of the reasons for this association is that they do not possess a functional spleen and lack splenic lymphocytes. They undergo autosplenectomy after several years because of infarction and fibrosis of the spleen. Prostaglandins are produced in leukocytes, platelets, and from other sites in response to the inflammatory condition. Furthermore, sickle cell crises are more prominent during pregnancy, and prostaglandins are increased in amniotic fluid and blood; there is a greater incidence of abortion, maternal mortality, and fetal wastage in persons with sickle cell disease. We now believe that the increased frequency of crises accompanying infection and pregnancy may be ascribable to elevated endogenous prostaglandins acting on the sickle cell erythrocyte.

Our studies have shown that prostaglandins can cause sickling of sickle cells and that the erythrocyte must be intact for this prostaglandin effect to be observed, since no effect on hemoglobin was observed in a hemolysate. These specific changes are taken as presumptive evidence for the involvement of the erythrocyte membrane in the sickling event. In addition, this involvement implies a more active process than merely the event of mechanical collapse via tactoid hemoglobin formation. We are currently investigating erythrocyte membrane-bound enzyme responsiveness to prostaglandins, both in the normal and pathological state. The Soret peak shift in sickle cell erythrocytes, but not in pure hemoglobin, is of interest, as it is known that this absorption band is responsive to changes in the environment of the iron-heme coordination complex (cf., 10). It may, for example, be a consequence of the hemoglobin stacking phenomenon first noted by Murayama (11).

We should caution that we have no occasion observed nonresponsive sickle cell anemia erythrocytes, and conversely, “responsive” normal erythrocytes. These anomalies are in part the result of incompletely established experimental protocol and in part of physiological factors. For example, several of our sickle cell donors had been taking aspirin and other analgesics, which have been reported to inhibit prostaglandin synthesis (12). Other medication, including antibiotics, will interfere with normal enzyme action and ion flux in the erythrocyte. The responsiveness of normal blood may also be due to temporary medication or low-grade infection. In addition, we have yet to establish the critical reduction in oxygen tension and concentration of prostaglandin that will sickle anemic cells but not affect normal cells.

We have demonstrated that light scattering is a valuable technique for the detection and continuous monitoring of the sickling event. It is known that
Fig. 3. Effect of prostaglandin E₂ on the absorption spectrum of hemoglobin in intact erythrocytes of normal (left) and sickle cell anemia subjects (right), under increased (A,C) and diminished (B,D) oxygen tensions, respectively. In each part, the two upper curves represent spectra in the presence and absence of PGE₂ (10⁻⁶ mol/liter) and the lower curve the resulting difference between these curves. Optical density (absorbance) and wavelength scales are indicated.

Fig. 4. Absorption spectra of pure hemoglobin from normal persons (left) and sickle cell anemia patients (right) in the presence and absence of prostaglandin E₂ (10⁻⁶ mol/liter). Experimental conditions and curves as described in legend to Figure 3. The effect of dilution (by addition of vehicle) on the observed spectra is demonstrated by the dotted line.
very low oxygen tension can sickle normal blood, and as we have observed crenation of normal red blood cells after N₂ bubbling and addition of prostaglandin, we are now improving the technique to differentiate between sickling and crenation. A semi-automated, multiparameter, sensitive detection system such as the one outlined in the block diagram should prove invaluable in the continuous monitoring of the sickling process.

Thus, we believe that prostaglandins may represent a cofactor initiating or enhancing sickle cell crisis. The association of a plasma cofactor in the sickling process has been previously suggested by Murayama and Hasegawa (13). The symptoms of fever and arthralgias during a crisis may relate to increased blood prostaglandins. Our results have not ruled out the possible involvement of other blood components in crisis, and we hope further to explore prostaglandin-sickle cell interaction and attempt to inhibit prostaglandin induced sickling both in vitro and in vivo.

This work was in part sponsored by NIH contract No. NHL 1-72-2987-B. Prostaglandins were a generous gift from the Upjohn Co. The loan of the Acta V Spectrophotometer, Oxygen Analyzer, and Circulating Constant Temperature Bath from Beckman Instruments is gratefully acknowledged. We are grateful to Andrew W. White, Jr., M.D., for his clinical advice and cooperation, and to San Francisco-Oakland Sickle Cell Anemia Research and Education (S.C.A.R.E.) for cooperation and assistance. M.J. acknowledges receipt of a Wellcome Travel Grant.

References