Is There a Role for Selective Vasodilation in the Management of Sickle Cell Disease?

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To test the hypothesis that microvascular obstruction to blood flow at the level of the arteriole may be significant in individuals with sickle cell anemia, the ophthalmologic effects of orally administered nifedipine were monitored in 11 steady-state patients. Three patients with evidence of acute peripheral retinal arteriolar occlusion displayed a prompt reperfusion of the involved segment. Two other patients showed fading of retroequatorial red retinal lesions. Color vision performance was improved in six of the nine patients tested. The majority of patients also demonstrated a significant decrease in the amount of blanching of the conjunctiva which reflects improved blood flow to this frequently involved area. Such improvements were not observable in a control group of untreated stable sickle cell subjects. These findings support the hypothesis that inappropriate vasoconstriction or frank vasoospasm may be a significant factor in the pathogenesis of the microvascular lesions of sickle cell disease and, further, that selective microvascular entrapment inhibition may offer an additional strategy to the management of this disorder. We believe a larger, placebo-controlled study with nifedipine and similar agents is warranted. This is a US government work. There are no restrictions on its use.

VASCULAR INVOLVEMENT in sickle cell disease has long been assumed to result from microvessel occlusions due to in situ “sickling” of red cells. This has been thought to occur primarily in capillaries and venules where the oxygen tension is lowest, and hence obstruction to blood flow was probably initiated at these sites. Accordinly, therapeutic interventions have been targeted at the inhibition of hemoglobin S (HbS) polymerization through hemoglobin modification, erythrocyte alterations, or genetic engineering. The actual site of the initial obstruction to blood flow, however, has never been definitively demonstrated. It is now known that detectable amounts of polymerized HbS exist inside sickle erythrocytes even at oxygen saturation values of 90%, saturation levels that are commonly found in the arterial blood of sickle cell patients. Further, the amount of polymer increases in a hyperbolic fashion in the range of desaturation that occurs on the arteriolar side of the microvasculature. This polymer may sufficiently alter the rheological properties of the red cell to cause obstruction at the point of greatest resistance to blood flow, namely, the terminal arterioles. Indeed, sickled erythrocytes have been observed in animal preparations to cause frequent occlusion at the precapillary sphincter, and arteriolar obstruction has been seen ophthalmoscopically in the retinal microvasculature of sickle cell patients. These regions of the circulation are sensitive to vasodilators, and thus, it would appear that a trial of selective vasodilator therapy to diminish the microvascular entrapment of HbS-containing erythrocytes may be presently justified.

The purpose of the foregoing study was to investigate the effects of an arteriolar vasodilator, nifedipine, on the most directly observable microvasculature, the retinal and conjunctival vessels, in patients with sickle cell anemia.

PATIENTS AND METHODS

We studied a consecutive series of 11 patients with stable homozygous sickle cell disease who were admitted to the Clinical Hematology Branch of the National Institutes of Health for routine follow-up. The patients were considered stable if they were not in crisis for 1 month before or after the study date, had not received a blood transfusion in the prior 4 months, and were not receiving long-term medication other than folic acid. Their ages ranged from 20 to 39 years.

The diagnosis of sickle cell anemia was made on the basis of hemoglobin electrophoresis on alkaline cellulose acetate and on acid citrate agar, DNA analysis of bone marrow aspirates, and peripheral blood examination and confirmed by family studies when possible. After informed consent was obtained and after baseline hematology and ophthalmologic studies, a single, 10-mg oral dose of nifedipine (Procardia, Pfizer, Inc, New York) was administered to each patient after at least two hours without food. Once the response to the single dose was evaluated, nifedipine, 10 mg every eight hours, was given to the patients for the next five days. At this point, based on the individual responses to the drug, the dose was continued or increased to a maximum of 60 mg/d. Nine of the 11 patients were able to tolerate 20 mg and the other two, 10 mg every eight hours. These doses were continued for five days to achieve a steady state. Treatment with nifedipine was thereafter discontinued on the tenth day. There were no significant side effects in any patient.

The hematologic studies, performed on days 0 and 10, included a complete blood count with red cell indexes, reticulocyte count, and fraction of fetal hemoglobin (HbF). To rule out any direct effect of nifedipine on the polymerization tendency, red cell density profiles for each patient were determined by the calibrated phthalate ester density method, and the median density (Dm), middle 60% density range (D60), and the fraction of dense cells (specific gravity, >1.120; corpuscular hemoglobin concentration (CHC), >37 g/dL) were calculated.

After the patients had been accepted to participate in the study, ocular assessment (M.S.R.) was performed before institution of nifedipine therapy and was repeated at day 10. Thus, inclusion in the study was not dependent on the ocular findings. Assessment of the conjunctiva was done with the slit lamp biomicroscope and the degree of blanching, the presence of isolated “segments,” and the
severity of the “comma” sign (mild when less than 10 and severe when more than 10 commas in any quadrant) noted. Care was taken to avoid prolonged exposure of the conjunctiva to the heat of the slit lamp. The ocular examination also included biomicroscopy of the anterior segment and a detailed examination of the retina by direct and indirect ophthalmoscopy as well as Goldmann 3 mirror contact lens examination. Retinal findings were recorded on a fundus drawing. Fundus photography and fluorescein angiography were also performed. Color vision was assessed by the Farnsworth-Munsell 100-Hue test.4 In nine of the 11 subjects. This served not only to establish the stability of the ocular findings with time but also to examine the possibility of a “learning” effect associated with repeated color vision testing.4 Results of the 100-Hue test were considered normal if there was no axis present and if the score, calculated according to the instruction manual, was within the normal range for the age of the patient as reported by Verriest et al.14 If a blue-yellow, red-green, or mixed axis was present, the defect was noted as mild if the score was within the mean ± 2 SD for that age and as severe if greater than that.4 A separate series of ten consecutive stable sickle cell patients matched for age who were not given nifedipine were used as controls. These patients, who constituted the “nonsimultaneous” controls, were subjected to repeat hematologic and ophthalmologic assessments at intervals similar to the treatment group. Because of the design of this exploratory study, the investigators knew whether the patient was or was not being treated with nifedipine.

Statistical analysis of the results was performed by using the Wilcoxon signed rank test to determine the significance of differences between the pretreatment and treatment mean values of ratio variables.13 Fisher’s exact test was performed on pretreatment and treatment-paired categorical variables.13

RESULTS

The 11 patients consisted of six males and five females with a mean age of 29 ± 9 years, (± 1 SD), which was not significantly different from that of our ten controls (six males and four females), 30 ± 9 years. The clinical and hematologic data before and during nifedipine treatment are shown in Table 1. In our nifedipine-treated group we found the average indirect bilirubin content to be significantly higher pretreatment than during the treatment phase (2.6 ± 1.1 and 2.1 ± 0.2 mg/dL respectively, P < .03). In eight of 11 patients in whom it was measured sequentially, the plasma hemoglobin value was also significantly reduced during nifedipine administration (P < .02, Table 1). There was, however, no statistically significant difference observed between the pretreatment and treatment values of hemoglobin, reticulocytes, R60, or the fraction of dense cells (Table 1). The control group of patients displayed no significant changes in any hematologic parameter on repeated measurement (data not shown).

At the baseline examination, three study patients showed an acute and total occlusion of a peripheral retinal arteriolar segment, which was found to be reopened at day 10 of nifedipine treatment (Fig 1). In two other study patients, we noted localized retroequatorial red retinal lesions before treatment. We have previously suggested that these may be retinal areas where blood flow has been compromised.16 After nifedipine treatment there was a marked fading of all these red retinal lesions in two patients. The patients serving as controls had various degrees of stages 1 and 11 of sickle retinopathy.17 There were no noticeable changes in the sickle cell retinal lesions on repeated examinations.

In nine study patients who were tested with the 100-Hue color vision test,3,14 there was a significant decrease in the mean score (reflecting fewer errors) after ten days of nifedipine therapy for right eyes and for the mean score for both eyes (P < .03 and .04 respectively; Table 2). In six of 18 eyes,
the color vision defect changed from severe to mild or mild to normal. Furthermore, one patient who underwent repeat color vision testing 1 week after nifedipine treatment had been discontinued demonstrated in his right eye a return to his pretreatment severity score (patient 6, Table 2). No significant change in error score was found in the control patients (Table 2).

Conjunctival examination of the 11 patients initially showed that four patients had a mild comma sign and seven patients had severe commas. After nifedipine treatment one of the four in the mild category and three of the seven in the severe category showed a decrease in the number of commas, whereas the severity of the comma sign of the 20 eyes in the control group was unchanged. This finding was statistically significant (Fisher exact test, $P < .01$). In addition, after nifedipine treatment, most patients showed a decrease in the blanching of the conjunctiva, which reflected less vasoconstriction (Fig 2A and B), a finding again not observed among the patients serving as controls.

**DISCUSSION**

The present report suggests that conjunctival and retinal perfusion as well as the color vision performance of stable patients with sickle cell disease may be improved after the administration of a selective vasodilator, nifedipine. In addition, we noted that during the course of treatment, these patients demonstrated a significant decline in their indirect bilirubin and plasma hemoglobin levels (Table 1).

Whether the reperfusion of the retinal arterioles in our three patients or the fading of the retroequatorial red retinal lesions in another two patients could have happened sponta-
Fig 2. (a) Photographs of the bulbar conjunctiva shows blanching and isolated "segments" (arrows). (b) Same area after a ten-day course of oral nifedipine shows reperfusion of many more conjunctival vessels (arrowheads).

Table 2. Farnsworth-Munsell 100-Hue Score in Nine Patients With Sickle Cell Disease Before and During Nifedipine Treatment and Ten Controls

<table>
<thead>
<tr>
<th>Patient</th>
<th>OD Day 0</th>
<th>OD Day 10</th>
<th>OS Day 0</th>
<th>OS Day 10</th>
<th>Mean OU Day 0</th>
<th>Mean OU Day 10</th>
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<tbody>
<tr>
<td>1</td>
<td>197 (S)</td>
<td>207 (S)</td>
<td>204 (S)</td>
<td>180 (S)</td>
<td>200</td>
<td>193</td>
</tr>
<tr>
<td>2</td>
<td>104 (M)</td>
<td>86 (M)</td>
<td>79 (N)</td>
<td>25 (N)</td>
<td>92</td>
<td>55</td>
</tr>
<tr>
<td>3</td>
<td>98 (M)</td>
<td>61 (M)</td>
<td>54 (M)</td>
<td>56 (M)</td>
<td>76</td>
<td>58</td>
</tr>
<tr>
<td>4</td>
<td>63 (M)</td>
<td>36 (N)</td>
<td>189 (S)</td>
<td>48 (N)</td>
<td>126</td>
<td>42</td>
</tr>
<tr>
<td>5</td>
<td>176 (S)</td>
<td>118 (S)</td>
<td>180 (S)</td>
<td>159 (S)</td>
<td>178</td>
<td>137</td>
</tr>
<tr>
<td>6</td>
<td>193 (S)</td>
<td>40 (N)</td>
<td>77 (M)</td>
<td>76 (M)</td>
<td>135</td>
<td>58</td>
</tr>
<tr>
<td>7</td>
<td>122 (S)</td>
<td>105 (M)</td>
<td>189 (S)</td>
<td>102 (M)</td>
<td>178</td>
<td>103</td>
</tr>
<tr>
<td>8</td>
<td>164 (S)</td>
<td>192 (S)</td>
<td>100 (M)</td>
<td>166 (S)</td>
<td>132</td>
<td>179</td>
</tr>
<tr>
<td>9</td>
<td>48 (M)</td>
<td>46 (N)</td>
<td>34 (N)</td>
<td>44 (N)</td>
<td>41</td>
<td>45</td>
</tr>
</tbody>
</table>

Mean ± SEM: 134.4 ± 18.9 98.8 ± 21.2 122.9 ± 22.3 95.1 ± 19.7 128.7 ± 17.4 96.9 ± 19.9

P < .03  NS < .04

Abbreviations: OD, right eye; OS, left eye; OU, both eyes. 

Severity of abnormality: (S), severe, score is above 2 SD of expected mean for that age, and there is a blue-yellow or mixed confusion axis; (M), mild, score is within the mean for age + 2 SD, and an axis is present; (N), score is within ± 2 SD for age, and there is no axis.

†Measurement after nifedipine therapy had been discontinued.

‡Wilcoxon signed rank test.

Abnormalities in color vision have been reported as an early manifestation of ischemic retinal vascular diseases such as diabetes. To date, there are only a few case reports of color vision defects in sickle cell patients with occlusion of major retinal vessels, yet our patients exhibited no overt macular occlusive changes on clinical examination and fluorescein angiography. Although Verriest et al have shown that familiarity with the 100-Hue test may lead to an improved performance on the test (ie, by about ten points in our age group, a finding we also observed in our controls, Table 2), it is noteworthy that in four of nine treated patients the severity of the color vision defect decreased from severe to mild or mild to normal. This would not be expected from familiarity with the test alone.

The conjunctival sign in sickle cell anemia is also known to be labile; thus caution should be exercised in interpreting changes in the conjunctiva (Fig 2). In this pilot study it is the improvement in both the anatomic and the functional status (as assessed by color vision testing) of the conjunctiva and retina that leads us to suggest that nifedipine may have had a beneficial effect.

There are several reasons to suspect that treatment with
the calcium channel blocker nifedipine might be advantageous in patients with sickle cell disease. First, flow resistance is inversely proportional to the fourth power of the vessel radius, and hence minute changes in lumen diameter will have profound effects on blood flow. Because the site of maximal resistance to flow, the precapillary arteriole, is under neural and/or humoral control, any initial obstruction to flow may trigger reflex vasoconstriction, which may significantly impair local blood flow. We have previously suggested that amounts of polymerized HbS that normally exist inside sickle erythrocytes on the arteriolar side of the circulation may be sufficient to induce altered blood rheology in the narrowed precapillary region, even in a morphologically normal red cell. Accordingly, selective arteriolar vasodilation, by diminishing the entrapment of sickle erythrocytes, might be expected to decrease some of the hemolytic and vasoocclusive manifestations of sickle cell disease.

Second, it has been proposed that the early phases of sickle cell vasoocclusive events, including typical sickle cell crisis, involve an initial arteriolar vasospasm, which only later sets the stage for frank microvascular obstruction, a process that may be amenable to treatment with calcium channel blockers. Support for this hypothesis comes from pathological studies of autopsied patients with evidence of organ infarctions in the absence of vascular occlusions. “Reversible intense vasoconstriction” of conjunctival arterioles during crisis, or provocation with α-agonists in the steady state. There are also data to suggest that patients with other retinal vascular diseases treated for 6 to 36 months with calcium channel blockers show objective improvement of some retinal soft exudates presumed to be ischemic.

Calcium entry blockers, whose effect is on the final common pathway leading to vascular smooth muscle contraction, appear more specific in the arteriolar vasodilation of the capillary region of the microcirculation where fewer selective effects (ie, arteriolar, capillary, and/or venous vasodilation) are observed for other classes of vasoactive substances. Indeed, the lack of uniform positive responses when other vasodilators have been given to sickle patients may be explicable in part on this basis.

Finally, there is evidence that the terminal event resulting in cell death after prolonged ischemic injury or other noxious insults involves the intracellular accumulation of calcium. Thus, it has been proposed that inhibition of intracellular calcium entry may favorably modify a number of disease processes associated with ischemic injury.

Curiously, we also observed that our nifedipine-treated patients had a significant decline in their indirect bilirubin and plasma hemoglobin levels. Although there could be day-to-day individual or laboratory variability in these measurements, this would seem a less likely explanation for the statistically significant decrease in both of these parameters. Rather, these observations may be accounted for by a decrease in the rate of hemolysis, particularly of the small fraction of irreversibly membrane-damaged cells, which would be expected to accompany the diminished intravascular shear stress resulting from arteriolar vasodilation. We speculate that the salutary changes noted in the indirect bilirubin and plasma hemoglobin levels may proceed or be more sensitive indicators of intravascular hemolysis than either the hemoglobin or reticulocyte values over the short time course of this study.

Clearly, further studies in a larger series of patients, perhaps designed in a placebo-controlled, crossover fashion, are needed to confirm our preliminary observations. These proposed investigations should include objective assessments of other aspects of the microvascular status of the patients, possibly incorporating recently developed noninvasive methodologies as well as more traditional clinical evaluations.

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