ORIGINAL COMMUNICATIONS

STUDIES OF THE KALLIKREIN-KININ SYSTEM IN PATIENTS WITH SICKLE CELL ANEMIA

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Eight patients with sickle cell anemia (SS hemoglobin) were found to have decreased plasma levels of prekallikrein compared to normal control subjects or patients with other types of anemia. The prekallikrein levels in the patients with sickle cell anemia were found to decrease further during a sickle cell crisis. These results suggest that components of the kallikrein-kinin system are profoundly affected in patients with sickle cell anemia, and during crises may play a role in the clinical presentation of patients.

Sickle cell vaso-occlusive crises are characterized by pain, swelling, and loss of function.[1] The crises occur when large numbers of sickle cells cause mechanical obstruction of blood flow through small vessels. Later, activation of coagulation factors and thrombus formation may further compromise tissue-blood flow.[2,3] Pathologically, vaso-occlusive crises result in tissue hypoxia, ischemia, perivascular exudates and hemorrhages, infarction, and necrosis.[1]

Treatment of patients in crises is entirely symptomatic; it is assumed that pain, edema, and most other findings noted during a crisis are the result of tissue damage due to hypoxia. The sickle cell crisis is associated with many complex biochemical and physiologic changes that can free potential mediators from plasma or other tissues and cause extensive hemodynamic alterations. Indirect evidence already has been presented that local ischemia can alter the kinin system and may result in the local production of bradykinin.[4] The changes in coagulation factors and other components of the coagulation system, vasodilation, tachycardia, local edema, and pain—which are all usually present during crises—suggest that the study of certain components of the kinin system in patients with sickle cell anemia might be useful.

METHODS

Blood levels of bradykinin and plasma prekallikrein, prekallikrein activator (factor XII-like activity), and kininase activity were studied in each of eight patients with SS hemoglobin during vaso-occlusive crises and while in remission. Four patients with nonhemolytic anemia (hematocrits comparable to the SS patients), and ten normal

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volunteers also were studied. All patients were black.

Criteria for inclusion of patients with vaso-occlusive crisis were diagnosis of sickle cell anemia (SS hemoglobin), clinical evidence of vaso-occlusive crisis, onset of symptoms requiring medical therapy within 48 hours as a hospital inpatient, no recent transfusions (within 30 days), no clinical evidence of shock, and a minimum age of 18 years of all patients. The studies were repeated in patients with vaso-occlusive crises 72 hours after the symptoms had subsided as determined by the absence of analgesic therapy for 24 hours and general clinical evaluation.

Blood (3 mL) for bradykinin levels was drawn into a plastic syringe containing two volumes (6 mL) of cold 0.5 M perchloric acid. The bradykinin was separated from the deproteinized solution using a weak anion exchange column.[5] Samples were assayed in duplicate using a sensitive radioimmunoassay,[6] and values for bradykinin were corrected for 30 percent recovery.

Plasma for assay of prekallikrein levels, prekallikrein activator and kininase activity was obtained by drawing 5 mL of blood from an accessible arm vein without a tourniquet into a plastic syringe containing 0.1 mL of 20 percent sodium citrate pH 7.3. The plasma was separated by centrifugation at room temperature. Aliquots of 0.5 mL of plasma were stored at −20°C. Prekallikrein was assayed by a radiochemical esterolytic procedure.[7] This assay is based on the ability of kallikrein to hydrolyze tritiated *n*-alpha-tosyl-L-arginine methyl ester [(^{3}H) TAMe] that is labeled in the methyl group of the alcohol moiety. One TAMe unit (TU) is defined as the amount of kallikrein that hydrolyzes one micromole of TAMe per minute at pH 8.0 and 30°C.

Prekallikrein activator activity was measured as described by Webster, et al[5,8,9] with minor modifications. In brief, the assay is based on the ability of factor XII (Hageman factor) fragments derived from plasma samples to convert partially purified prekallikrein to kallikrein which can be measured by the TAMe assay. A 50-μL sample of plasma was mixed with 200 μL of a 50 percent solution of acetone in tris buffer (0.2 M, pH 8) and vortexed for one minute. A 50 μL aliquot of the mixture was added to 100 μL of a supercel suspension (100 mg/2 mL of tris buffer); 400 μL of tris was added so that the final volume was 500 μL. The mixture was stirred constantly for two minutes and the supernatant was separated by centrifugation. The absorbed supercel was washed twice with tris buffer and then resuspended in 200 μL of the buffer. A 10 μL aliquot was added to partially purified prekallikrein and assayed as described.[7,8] The values of Hageman factor activity are expressed as cpm of [^{3}H] methanol released from [^{3}H] TAMe. Although absolute values of Hageman factor activity can not be ascertained using only this assay, changes in Hageman factor activity in the same patient can be measured since the same batches of prekallikrein and TAMe were used for all plasma samples. Complete conversion of prekallikrein to kallikrein produced 14,000 cpm and complete hydrolysis of the (^{3}H) TAMe, 28,000 cpm. Prekallikrein activator activity is expressed as cpm (^{3}H) methanol released (rounded to the nearest hundred).

Plasma kininase activity was measured by adding 100 ng of synthetic bradykinin to 200 μL of plasma in 200 μL of 0.02 M tris HCl buffer pH 7.5 and incubating the mixture at 37°C for 10 minutes. Kininase activity is defined as the amount of bradykinin destroyed per minute per liter of plasma.

The average age of the sickle cell patients was 26 (range 18 to 44) and the male:female ratio was 1:1. While in crisis two of the patients received transfusions of packed cells after the study began. Blood samples were obtained for prekallikrein levels and prekallikrein activator activity from these two and from two of the other sickle cell patients at several different times while they were in remission and at least 30 days following the transfusion. Multiple blood samples also were obtained from five of the normal volunteers for prekallikrein levels.

RESULTS

The level of bradykinin in blood was normal (<2 ng/mL) during crises in seven of eight patients. In one patient the bradykinin level in blood was 10 ng/mL during a crisis. The plasma prekallikrein level in the sickle cell patients decreased significantly during crises compared to the prekallikrein level when the patients were not in crises (Table 1). Plasma prekallikrein levels in sickle cell

TABLE 1. COMPARISON OF PLASMA PREKALLIKREIN LEVELS DURING CRISIS AND REMISSION

Patient	Diagnosis	Bradykinin (ng/mL)	Prekallikrein (TU/mL)	Prekallikrein Activator [cpm (^{3}H)-methanol × 10^2]
1	Sickle cell crisis	10	0.52	31
	Remission	<2	0.79	38
	Crisis	<2	0.65	32
	Remission	<2	0.76	31
2	Sickle cell crisis	<2	0.28	34
	Remission	<2	0.53	35
	Remission		0.52	32
	Remission		0.48	36
3	Sickle cell crisis	<2	0.43	28
	Remission		0.60	26
4	Sickle cell crisis	<2	0.36	30
	Remission		0.58	27
	Remission		0.61	31
5	Sickle cell crisis	<2	0.44	24
	Remission		0.59	44
6	Sickle cell crisis	<2	0.32	33
	Remission		0.50	38
7	Crisis	<2	0.34	38
	Remission		0.56	37
8	Crisis	<2	0.68	42
	Remission		1.10	45

Note: All assays for bradykinin, prekallikrein, and prekallikrein activator activity were performed in quadruplicate. Prekallikrein activator activity is expressed as cpm (^{3}H) methanol × 10^2 released from TAMe. Mean prekallikrein level of patients: remission—0.64 TU/mL (±0.17); crisis—0.43 TU/mL (±0.12); the difference is statistically significant $P > .001$; mean prekallikrein activator activity: remission—3500 cpm (±600); crisis—3200 cpm (±500)

patients during remission were decreased when compared with normal volunteers and patients with other types of anemia (Table 2). In both the normal subjects and the patients with sickle cell anemia during remissions, the prekallikrein level in each individual remained relatively constant.

Plasma prekallikrein activator activity in patients with sickle cell anemia was not significantly different from that in the controls. Sickle cell crises did not consistently cause changes in the prekallikrein activator activity.

Normal plasma exhibited a linear rate of bradykinin inactivation for 20 minutes; more than 80 percent of the bradykinin was inactivated during this time. Plasma kininase activity of sickle cell patients did not change during crisis; the plasma kininase activity in these patients was the same as that found in normal volunteers. The mean plasma kininase activity of normal volunteers was 15.67 nmol (SD = 0.04) bradykinin destroyed per liter per minute; the mean plasma kininase activity of patients with sickle cell anemia was 15.63 nmol (SD ± 0.02) bradykinin destroyed per liter per minute.

TABLE 2. COMPARISON OF PREKALLIKREIN LEVELS FOR NORMAL VOLUNTEERS AND ANEMIC PATIENTS

Patient	Diagnosis	Prekallikrein (TU/mL)	Prekallikrein Activator [cpm (^{3}H)-methanol × 10^2]
Anemic Controls			
1	Myelofibrosis	1.2	
2	Myelophtasic anemia	0.97	
3	Iron Deficiency	1.1	
4	Iron Deficiency	0.84	
Normal Controls			
1		1.3	38
2		0.76	40
3		1.1	36
4		0.56	31
5		0.78,0.80,0.78	45
6		0.98,1.0,1.0	48
7		0.87	41
8		0.94,1.0, 1.0,0.99	39
9		1.1,1.0	42
10		0.70,0.78	36

Note: Mean prekallikrein level for anemic controls was 1.03 TU/mL (±0.16), and normal subjects had a mean prekallikrein level of 0.91 TU/mL (±0.19); this is significantly different from the prekallikrein level of patients with sickle cell anemia both during remission and crisis $P > .001$. The mean prekallikrein activator activity for normal subjects is 4000 cpm (±500) and it is not statistically different from that found in patients in sickle cell crisis.

DISCUSSION

In the present study, blood kinin and plasma prekallikrein levels, prekallikrein activator activity and kininase activity were investigated in patients with sickle cell anemia during crisis and remission. The level of circulating bradykinin was normal (<2 ng/mL) in seven of eight patients. An abnormally high level of circulating bradykinin (10 ng/mL) was found in one patient during crisis. Plasma prekallikrein was decreased in sickle cell patients compared with controls and there was further reduction in plasma prekallikrein during sickle cell crises. Plasma kininase activity in patients with sickle cell anemia was not different from that found in normal subjects, and there was no change observed during crises. Prekallikrein activator activity in patients with sickle cell anemia was not significantly different from the values found in controls. The combination of decreased prekallikrein synthesis during both remission and crises and increased local kinin production during crises is a likely explanation for the results found in patients with sickle cell anemia.

The level of plasma prekallikrein and the activity of kallikrein are determined by many different events and complex interactions with other substances (Figure 1). The low levels of prekallikrein observed in the patients with sickle cell anemia may result from increased kinin formation due to conversion of prekallikrein to kallikrein so that the plasma prekallikrein is consumed (activated) faster than it can be synthesized. Another explanation for the low plasma prekallikrein may be that the synthesis of this specific protein is diminished.

Plasma prekallikrein is a globulin that is synthesized in the liver.[10] Conditions that result in

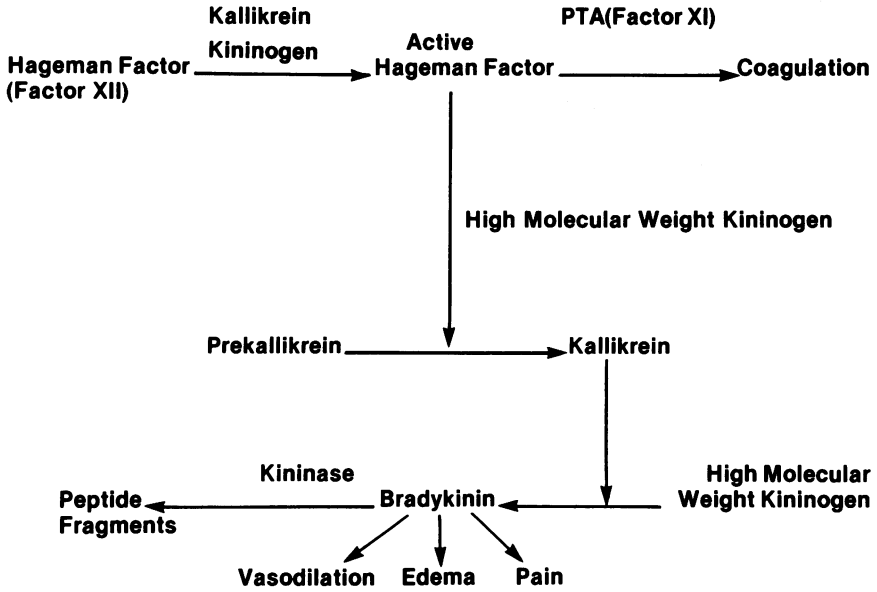

Figure 1. The kallikrein-kinin system interacts with components of the coagulation system. Activated Hageman factor initiates a series of reactions leading to kinin generation and coagulation. Prekallikrein may circulate as a complex containing high molecular weight kininogen. Kallikrein and high molecular weight kininogen can activate additional Hageman factor. The interdependence of the system suggests that conversion of even a small amount of prekallikrein to kallikrein can have a multiplication effect in the activation of additional Hageman factor and kallikrein

cellular damage, such as hepatitis or cirrhosis, can cause decreases in plasma prekallikrein levels.[11,12] Blood for liver function tests in the patients was not obtained at the time that these studies were carried out and no definite correlation can be made between the patient's hepatic function and the proteins involved in the kinin system. There is ample evidence, however, that abnormalities in liver morphology occur in patients with sickle cell disease.[13] Hepatic necrosis, portal fibrosis, regenerative nodules, and cirrhosis are frequently observed in these patients. The specific hepatic dysfunction that results from the altered morphology has not been documented as well. Hyperglobulinemia has been described in children with sickle cell disease, but this finding may not be directly attributable to liver dysfunction.[14] Although the total plasma globulin level can be increased in patients with sickle cell disease, synthesis of specific globulins such as prekallikrein may be diminished and could be reduced even further during crises because prekallikrein may be synthesized by hepatic cells that are more likely to be injured by reduced blood flow.

If the low levels of plasma prekallikrein are due to activation and consumption of the protein, ultimately the expected result would be generation of measurable levels of bradykinin. Using a sensitive specific method to measure blood levels of bradykinin, we did not observe consistently increases of kinin levels in the patients with sickle cell anemia while in remission or crisis. It is unlikely that increased bradykinin destruction explains these results because the kininase activity in plasma from patients with sickle cell anemia was not different from the kininase activity found in plasma from normal volunteers and did not change during crisis.

Local intravascular thrombosis and endothelial damage during sickle cell crisis have been described.[1,3,15] Damaged endothelium can activate factor XII[16] which initiates both intravascular coagulation and the conversion of prekallikrein to kallikrein (Figure 1). It is conceivable that

these events can result in the production of large amounts of kinins in certain tissues. The kinins act locally and are destroyed by tissue kininase; therefore, increased amounts of kinin are not found in the general circulation. These events may explain the diminished prekallikrein in the absence of increased levels of bradykinin in the circulation of patients during crises.

Factor XII (Hageman factor) is the most important known plasma prekallikrein activator. It is a β-globulin that is believed to be synthesized by the liver. The assay used in this study is based on factor XII conversion of prekallikrein and kallikrein; the assay is not sensitive enough to detect moderate changes in factor XII levels. It is estimated that a factor XII level of 25 percent of normal is adequate for complete conversion of prekallikrein to kallikrein.[17]

The generation of locally acting bradykinin may be an important determinant of some of the symptoms related to crises, eg, pain, swelling, and local vasodilation. In addition, locally generated bradykinin may have a role in important homeostatic mechanisms for maintaining tissue blood flow during the early phases of a crisis without causing major systemic hemodynamic changes. High local levels of kinin can cause arteriole dilation, venule constriction, and increased flow in capillary beds in normal man. In patients experiencing sickle cell crisis, greater sickling usually occurs in capillaries and venules; therefore, continued kinin induced venule constriction eventually could cause greater sickling, stasis, and tissue destruction than would otherwise occur.

The fact that prekallikrein is not diminished in other types of anemia may indicate the unique biochemical events, eg, coagulation and tissue destruction, that take place during the sickling process.

Although this study provides evidence for interactions of the kallikrein-kinin system in sickle cell disease, the precise mechanisms of these interactions and their importance to the clinical presentation of patients and the pathophysiology of sickle cell anemia will require further investigation. Many other potential mediators and vasoactive substances may also be generated during crisis. If any of the substances are important determinants in the production or amelioration of the clinical symptoms, specific antagonists or enhancers to these mediators might prove useful in treating the incapacitating symptoms in crises.

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