Glycated Hemoglobin in Uremic Patients as Measured by Affinity and Ion-Exchange Chromatography

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We estimated glycated ("glycosylated") hemoglobin in erythrocytes of hemodialyzed uremic patients by the aminophenylboronic acid affinity-chromatographic method and by an ion-exchange chromatographic method. As expected, apparent HbA1c concentrations were above normal in the uremic patients, owing to interference by the carbamylated hemoglobin. However, there was no significant difference between values for uremic and normal subjects when glycated hemoglobin was measured by the affinity method. Evidently it is unaffected by the presence of hemoglobin species modified by reactants not displaying a cis-1,2-diol group, such as carbamylated hemoglobin. For this reason it is preferable to the ion-exchange chromatographic method for accurate measurement of glycated hemoglobin.

Concentrations of hemoglobin A1c (HbA1c) reportedly are increased, both in diabetic patients and in patients with renal failure, when measured by ion-exchange chromatography (1, 2). However, when measured by the thiobarbituric acid method, a chemical method specific for the detection of glycation, patients with renal failure showed values for glycated hemoglobin similar to those observed in normal subjects, suggesting that the high values in these patients are a result of binding of something other than glucose to hemoglobin (2). Fluckiger et al. (3) later showed that hemoglobin carbamylation, resulting from a condensation of urea-derived cyanate with the N-terminal amino groups, was the cause of increased HbA1c in uremia; they also found that the values for HbA1c were proportional to the time-averaged concentration of blood urea.

Aminophenylboronic acid affinity chromatography, a simple method recently developed for measurement of glycated hemoglobin, is said to be more specific than ion-exchange chromatography and also more effective in distinguishing diabetic patients from normal subjects (4, 5). The method is based on the interaction of immobilized m-aminophenylboronic acid with the cis-1,2-diol group of the glucose residue attached to the hemoglobin molecule. Thus it should not be affected by hemoglobin that has been modified by reactants not displaying a cis-1,2-diol group, such as carbamylated hemoglobin. We sought to confirm that values for glycated hemoglobin in uremic patients with no history of diabetes are within the normal range when measured by affinity chromatography, thus further establishing that hemoglobin species modified by other reactants than glucose do not interfere.

Materials and Methods

Blood specimens: Whole blood, anticoagulated with EDTA, was obtained from two groups of subjects. The first group consisted of 28 normal subjects (13 women and 15 men, mean age 39 years, range 23–60 years) from the hospital staff. The second group consisted of 18 patients with renal failure, who were on intermittent hemodialysis (eight women and 10 men, mean age 44 years, range 22–60 years). Depending on the patient, dialysis was done two or three times a week for 3 or 4 h vs a fluid not containing any glucose. The mean pre-dialysis value for blood urea nitrogen for these patients was 36 (SD 5) mmol/L. There was no case of overt diabetes in either group.

I on-exchange column-chromatographic method: Hemoglobin A1c was measured at 22 °C with a commercially available minicolumn assay (Quik-Sep; Isolab Inc., Akron, OH 44321) according to the manufacturer's instruction, after the "labile" fraction was removed by incubating the erythrocytes at 37 °C for 30 min in 10 volumes of acetate buffer (0.2 mol/L, pH 5.5) (5).

Affinity chromatographic method: Glycated hemoglobin was also measured with a commercially available minicolumn assay (Glyco-Gel Test Kit; Pierce Chemical Co., Rockford, IL 61105) according to the supplier's procedure.

Results and Discussion

As expected, the mean percentage of apparent HbA1c in the uremic patients by ion-exchange chromatography (8.17%, SD 0.86%) significantly exceeded that in the normal subjects (6.31%, SD 0.55%; p < 0.001, Student's t-test) (Figure 1). It approximated the value found by Fluckiger et al. (3). On the other hand, results by affinity chromatography for the uremic patients (7.10%, SD 0.99%) did not differ significantly from those for the normal subjects (7.25%, SD 0.54%). Evidently carbamylated hemoglobin, which is present in higher concentration in the blood of uremic patients, does not interfere with measurement of glycated hemoglobin by the affinity method. The variance between these two groups was, however, significantly different (p < 0.01, F-test). When we established a normal range (mean ± 2 SD) by using the affinity data from the normal group (6.2–8.3), values for four of the 18 uremic patients fell outside this range (see Figure 1). It is not clear whether this higher variability in the uremic group as compared with the normal subjects reflects differences in glucose control or differences that are related to uremia, or both. As pointed out by Flückiger et al. (3), interpretation of data on concentrations of modified hemoglobin components in uremia is complicated by the anemia accompanying uremia and the need for frequent blood transfusions in hemodialyzed patients. The anemia of chronic renal failure involves a complex of factors that both suppress erythropoietin production and shorten erythrocyte life span, therefore decreasing any modified hemoglobin components. This accords with our finding of a positive correlation in the uremic group (r: 0.45; 0.1 < p < 0.2) between the hematocrit and the concentration of glycated hemoglobin as measured by affinity chromatography.

In keeping with our finding, the affinity method has also been shown to be free from many other interferences that affect the ion-exchange chromatographic method. The pres-
ence of hemoglobins F, S, C, and the "labile" fraction does not affect the determination of glycated Hb (4, 7, 8). Nor is it affected by the presence of up to 20% methemoglobin (9) or by the hemoglobin degradation products that appear during storage of whole blood (10). Moreover, the hemoglobin acetaldehyde adduct responsible for a falsely high value of HbA1 in alcoholics (11) and the acetylated hemoglobin present in the blood of patients on high doses of aspirin does not affect results by the affinity method (12, 13).

We conclude that all hemoglobin species modified by other reactants than glucose and not displaying a cis-1,2-diol group should not interfere with measurement of glycated hemoglobin by the phenylboronate affinity method.

Fig. 1. Proportion of glycated hemoglobin in erythrocytes of uremic and normal subjects as measured by affinity and ion-exchange chromatography

References