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Increased ^{125}I-labelled Concanavalin A Binding to Erythrocytes in Diabetes Mellitus

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Summary. Percentage binding of ^{125}I-labelled concanavalin A to erythrocytes in diabetic patients was significantly higher than that in normal subjects (12.2 ± 2.8 versus 8.1 ± 1.8%, mean ± SD, $p < 0.001$). Insulin-dependent diabetic patients showed significantly higher concanavalin A binding than non-insulin-dependent diabetic subjects (15.0 ± 1.4 versus 11.4 ± 2.5%, $p < 0.01$). There was a highly significant correlation between percentage binding of ^{125}I-labelled concanavalin A and glycosylated haemoglobin.

Key words: ^{125}I-labelled concanavalin A, human erythrocyte, diabetes mellitus, glycosylated haemoglobin.

In diabetes mellitus, enzymatic glycosylation of the glomerular basement membranes [1] and the vitreous glycoproteins [2], non-enzymatic glycosylation of haemoglobin [3], lens crystallins [4] and serum proteins [5] have been reported to be increased. Recently, Miller et al. [6] described an enhanced non-enzymatic glycosylation of the membrane proteins of erythrocytes in diabetic patients. These changes may be related to shortened erythrocyte survival [7] and reduced erythrocyte deformability [8] described in diabetic patients.

Concanavalin A is a plant lectin which has a specificity for D-mannose and D-glucose residues. Band 3 protein [10], one of the integral membrane glycoproteins of the human erythrocyte to which several membrane functions such as monosaccharide, anion and cation transport across the membrane have been attributed [11, 12], is the principal high affinity binding protein for concanavalin A [13].

The aim of the present study was to investigate ^{125}I-labelled concanavalin A binding to peripheral erythrocytes in diabetic patients.

Materials and Methods

Five Type 1 (insulin-dependent) diabetic patients (two females and three males, 29–44 years, mean 35 years) and 17 Type 2 (non-insulin-dependent) diabetic patients (ten females and seven males, 44–74 years, mean 54.8 years) were compared with 13 normal subjects (six females and seven males, 23–42 years, mean 33.6 years) in the binding of ^{125}I-labelled concanavalin A to erythrocytes. The Type 2 diabetics included seven diet-treated patients (four females and three males, 44–56 years, mean 43.6 years), five sulphonylurea-treated patients (four females and one male, 45–73 years, mean 61 years) and five insulin-treated patients (two females and three males, 46–61 years, mean 56.2 years). All participants gave their informed consent before entering the study.

Venous blood was collected into heparinized tubes after overnight fasting. After removing the buffy coat, the erythrocytes were purified by three successive washings of the erythrocyte pellet with incubation buffer.

Binding studies were performed as described previously [14]. ^{125}I-labelled concanavalin A with a specific activity of about 5 mCi/mg was obtained by the modified method of Hunter and Greenwood [14, 15]. About 4×10^7 erythrocytes were incubated with 50 ng radio-labelled concanavalin A in 0.5 ml of Tris-Hepes buffer (pH 7.0) containing 4-(2-hydroxyethyl)-1-piperazineethane sulphonic acid 75 mmol/l, Tris (hydroxymethyl)aminomethane 25 mmol/l, $MgCl_2$ 10 mmol/l, $CaCl_2$ 10 mmol/l, KCl 5 mmol/l, EDTA 2 mmol/l, NaCl 55 mmol/l and 0.5% bovine serum albumin [14] at 24° C for 3 h. Concanavalin A bound to erythrocytes was separated from free lectin by dibutyl phthalate gradient centrifugation at 4° C [14, 16]. Total and bound radioactivities were counted in a well-type gamma-counter. Non-specific binding determined in the presence of α-D-mannoside 0.1 mol/l was about 0.3 to 0.5% of the total radioactivities and was subtracted from the total bound radioactivities to obtain the specifically bound radioactivities. Results were the mean of duplicate determinations per patient and normalized to percentage specific binding of ^{125}I-labelled concanavalin A per 5×10^7 cells/ml.

Glycohaemoglobin concentration was determined by a micro-column chromatographic procedure using QUIK-SEP (Iso-Lab,

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Table 1. ^{125}I-labelled concanavalin A binding to erythrocytes and glycosylated haemoglobin in diabetic patients

	Number of subjects	^{125}I-labelled concanavalin A binding (%)	Glycosylated haemoglobin (%)
Controls	13	8.1 ± 1.8	6.8 ± 0.3
Diabetic patients	22	12.2 ± 2.8[a]	10.9 ± 2.8[a]
Type 1 diabetics	5	15.0 ± 1.4[a]	14.3 ± 3.9[a]
Type 2 diabetics	17	11.4 ± 2.5[a,c]	9.9 ± 1.2[a,c]
Treated with:			
Diet only	7	12.4 ± 2.3[a]	10.0 ± 1.4[a,d]
Sulphonylurea	5	10.7 ± 1.9[b,c]	9.9 ± 1.1[a]
Insulin	5	10.7 ± 3.2[b,d]	9.6 ± 1.1[a,d]

Results are expressed as mean ± SD.
[a] $p < 0.001$ compared with control subjects
[b] $p < 0.05$ compared with control subjects
[c] $p < 0.01$ compared with Type 1 diabetic patients
[d] $p < 0.05$ compared with Type 1 diabetic patients

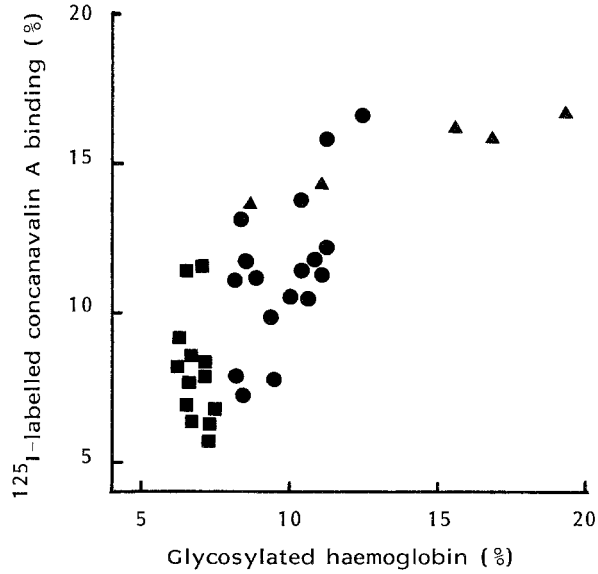

Fig. 1. The correlation between specific percentage binding of ^{125}I-labelled concanavalin A and percentage glycosylated haemoglobin. ■: normal subjects; ●: Type 2 diabetic patients; ▲: Type 1 diabetic patients. The equation of the linear regression is: $y = 1.62x + 6.16$ ($r = 0.77, p < 0.001$)

Akron, Ohio, USA) and corrected for temperature as indicated in the instructions.

Binding studies of radio-labelled insulin to erythrocytes were performed by a radio-receptor assay as previously described [17].

Results are expressed as mean ± SD and the significance of differences between means was calculated by Student's t test [18].

Results

Percentage specific binding of ^{125}I-labelled concanavalin A to erythrocytes was 12.2 ± 2.8% in diabetic patients and 8.1 ± 1.8% in normal subjects. This difference is statistically significant ($p < 0.001$). Among diabetic subjects, Type 1 diabetic patients showed significantly higher concanavalin A binding than Type 2 diabetic patients (15.0 ± 1.4 versus 11.4 ± 2.5%, $p < 0.01$) (Table 1).

Percentage glycohaemoglobin determined at the same time revealed a similar degree of differences as concanavalin A binding between diabetic patients and normal subjects (Table 1).

Figure 1 shows the correlation between percentage specific binding of ^{125}I-labelled concanavalin A to erythrocytes and percentage glycosylated haemoglobin ($r = 0.77, p < 0.001$). No overlap was observed between the Type 1 diabetic and normal subjects in percentage binding of ^{125}I-labelled concanavalin A, though many of the Type 2 diabetics had values in the normal range.

Percentage specific binding of ^{125}I-labelled insulin to erythrocytes (at 0.8 ng/ml radio-labelled insulin/ml) determined at the same time in 12 patients showed no significant correlation with percentage specific binding of ^{125}I-labelled concanavalin A ($r = -0.39$, $p > 0.05$).

Discussion

In the present study, ^{125}I-labelled concanavalin A binding to erythrocytes, which may mirror the degree of glycosylation of the erythrocyte membranes, was estimated in diabetic patients. It was shown that (a) erythrocytes from diabetic patients bind more ^{125}I-labelled concanavalin A than those from normal subjects and (b) percentage binding of ^{125}I-labelled concanavalin A to erythrocytes was directly proportional to the degree of glycosylated haemoglobin.

The pathophysiological changes responsible for enhanced binding of concanavalin A to erythrocytes in diabetics are unknown. Band 3 protein, which is a principal concanavalin A binding protein in the human erythrocyte membranes [13] and is thought to be responsible for monosaccharide transport [12] and/or other minor glycoproteins, may be increased in the diabetic state. Another possible explanation for increased concanavalin A binding is the enhanced non-enzymatic glycosylation of the membrane proteins.

Although not confirmed, valyno- and lysyno-1-deoxyfructose found in non-enzymatically glycosylated proteins [19] may interact with concanavalin A. Two experimental observations, that the neuraminidase treated erythrocytes bind more concanavalin A than the native erythrocytes [20] and that neuraminic acid contents of erythrocyte ghosts from diabetics are lower than those from normal subjects [21], suggest another possibility, namely incomplete glycosylation of membrane glycoproteins in diabetes mellitus. The lack of correlation between concanavalin A binding and insulin binding to erythrocytes indicates selective changes of cell surface components in diabetes.

A highly significant correlation between percentage binding of ^{125}I-labelled concanavalin A to erythrocytes and percentage glycosylated haemoglobin indicates that continuous hyperglycaemia induces the changes in both the erythrocyte membrane proteins and intracellular haemoglobin. Glycosylated haemoglobin is considered to reduce membrane elasticity and erythrocyte deformability because of its tight adherence to the inner surface of the erythrocyte membranes [22]. Over-glycosylation of the erythrocyte membranes may more directly affect the membrane properties and induce the functional abnormalities of erythrocytes found in diabetes [6, 7] and contribute to some of the long term complications of diabetes.

In conclusion, poorly controlled diabetic patients show an enhanced concanavalin A binding to erythrocytes, which may reflect over-glycosylation of the erythrocyte membrane proteins. There is considerable interest to know whether the enhanced concanavalin A binding also occurs in other tissues and to what extent these abnormalities correlate with the secondary complications of diabetes mellitus.

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