Acta Paediatrica Hungarica 30 (2) pp 241-250 (1990)

PEROXIDE HAEMOLYSIS OF ERYTHROCYTES IN CHILDREN WITH DIABETES MELLITUS

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Received 13 June 1988

Peroxide haemolysis of erythrocytes in children with diabetes mellitus was discussed. The peroxide haemolysis tests of erythrocytes of 32 healthy and 35 diabetic children were compared. The erythrocytes of diabetics were haemolyzed to a small extent when 2% H_2O_2 was used. If 5 and 10 mmol/l glucose were added, the peroxide haemolysis of the controls decreased but that of the diabetics did not change. The results of the examinations performed with pH 6.8 were compared to those of pH 7.4 and both in the diabetics and controls a slight haemolysis was observed.

INTRODUCTION

Our attention was drawn to the peroxide haemolysis test (PHT) in diabetes for several reasons. It is well known that the sulphydryl groups of the membrane take part in the glucose transport and in the development of the effect of insulin /22/. It is also accepted that the interactions between the sulphydryl groups and the double-bonds of the lipids may play a role in the permeability of the membrane, in the maintenance of the membrane structure and the cell deformability, and in the autooxidation of the cells /22, 26, 27/. In diabetes, where disorders of the sulphydryl-related functions are probable (disorders of the glucose transport, the deformability of the erythrocytes /5/, etc.), the question arises whether the interactions with sulphydryl reagents are also changed.

We have examined the PHT in children with diabetes. Via its

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effect on the membrane lipids and sulphydryl groups, H_2O_2 gives rise to haemolysis, the extent of which depends on the concentration applied and the antioxidant capacity of the erythrocytes /26/. It was assumed that in diabetes the result of the PHT may be changed.

PATIENTS AND METHODS

3-14-year-old children not suffering from any chronic disease, malabsorption or haemolytic disease were examined as controls.

Of the diabetes patients receiving care in our department, we examined those having neither ketoacidosis or other serious complication, nor accompanying disease.

Our diabetic patients did not receive intensified insulin treatment. Thus, with the exception of the remission phase, a normoglycaemic status could be achieved only in the minority of cases and for only a short period. A state of lability was characteristic for our patients. At the time of the examination, one hour after the meal following the morning insulin dose, the average glucohaemoglobin level was 8.09%, the blood sugar level was 16.69 mmol/l, while the 24-hour average urine sugar secretion was around 34.04 g.

The ages of the patients likewise varied between 3 and 14 years. The average daily insulin dose was 1.00 U/kg (0.44-1.66 U/kg). Actrapid MC and Monotard MC insulins were administered twice a day. The PHT described by György and Rose /8, 9, 21/ has been used by several authors /15, 16, 17/. We applied the PHT version reported by Lubin /12/.

The PHT examination was performed on the diabetic red blood cells, on normal red blood cells and in a system where the glucose concentration was 5 or 10 mmol/l. In this case the washed red blood cells were preincubated in glucose for 5-10 min at $37^{o}C$ before the reaction with H_2O_2. The investigations were also carried out after preincubation with insulin. The red blood cells were preincubated in buffer solution containing 100, 10 or 1 ng/ml insulin , for 2 hours, before the PHT. The PHT was also examined at pH 6.8.

The blood sugar levels in the diabetic patients were determined by the orto-toluidine method, and the glucohaemoglobin levels by the method of Davis and Nicol /4/. The micro-columns for the chromatographic measurements were prepared as described /4/. The normal examination results were $6.65 \pm 0.97\%$, expressed as a percentage of the total haemoglobin.

For statistical analysis of the results, the means and the standard deviations (SD) were calculated. Statistical significance of differences in the examined groups was ascertained with the two-tailed t-test.

RESULTS

1. The resistance of diabetic erythrocytes to H_2O_2, as examined with the PHT, proved to be significantly higher than that of the normal erythrocytes. This was revealed by the lower percentage values in the PHT, both at pH 7.4 and pH 6.8 (Fig. 1).

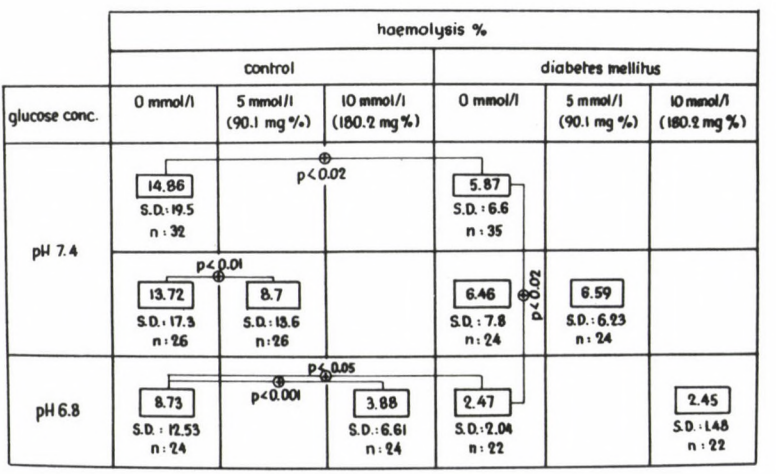

Fig.1. Extents of haemolysis induced by H_2O_2 in diabetic and control children at different glucose concentrations and pH values (indicates significant differences).

2. Incubation with glucose (5 mmol/l or 10 mmol/l) significantly increased the resistance of the normal erythrocytes to H_2O_2. The PHT results were lower in the presence of glucose, both at pH 7.4 and pH 6.8 (Fig. 1).

3. Neither 5 nor 10 mmol/l glucose in the blood increased the resistance of the diabetic erythrocytes to H_2O_2. The PHT result was not lower either at pH 7.4 or pH 6.8. (Fig. 1).

4. At pH 6.8, the resistance to H_2O_2 was higher in both normal and diabetic cases,i.e. the PHT results were lower (Fig.1).

5. In diabetic patients there was no significant correlation between the PHT result and the blood sugar level at pH 7.4, or between the PHT result and the glucohaemoglobin level (Fig. 2).

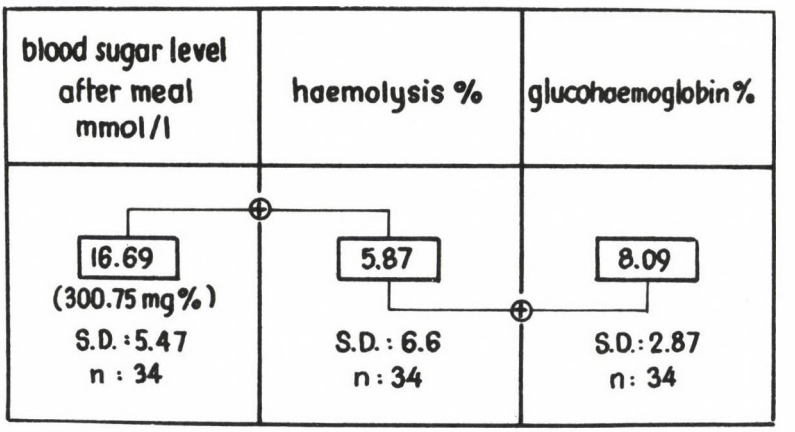

Fig.2. Blood sugar level (postprandial), extent of haemolysis induced by H_2O_2, and glucohaemeoglobin level in diabetic patients at pH 7.4 (indicates no significant correlation between the relevant values).

6. At the concentrations shown in Fig. 3, insulin added to the medium did not influence the result of the PHT in either diabetic or normal erythrocytes.

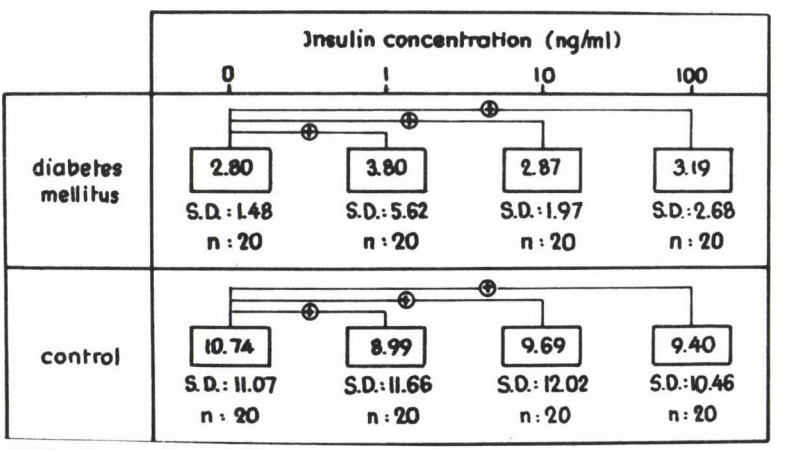

Fig.3. Effects of different insulin concentrations on PHT results of patients with diabetes mellitus and controls (indicates no significant correlation at the > 0.05 level).

DISCUSSION

The result of the PHT on normal erythrocytes may be either increased or decreased. Increased haemolysis (in malabsorption, or in premature infants) can be observed in the event of disturbances of the antioxidant system or a vitamin E deficiency. Initially we expected such an elevation. However, the present experiments revealed low results of the PHT in diabetes, when normal erythrocytes were incubated in the presence of glucose, and when normal and diabetic erythrocytes were incubated at pH 6.8. Evaluation of the results leads to a number of questions which can not be answered unambiguously.

The first question: what is the reason for the difference in the result of the PHT in diabetic and normal erythrocytes? It may be assumed that the membrane changes and other alterations /1, 5, 10, 19, 25/ characteristic of diabetic erythrocytes are connected with the increased H_2O_2 resistance observed.

It is possible that the smaller amount of unsaturated lipids in diabetic membranes is responsible for this /3/, because the unsaturated lipids can be peroxidized more easily /6/; however, other membrane changes may similarly be connected with the above deviation.

It is also possible that higher catalase activity /13, 14/ is responsible for the lower PHT results, but the presence of added H_2O_2 in an excess of 2% does not make this probable.

Theoretically, the fact that the cellular metabolic activity responsible for the antioxidative effect is different in normal and in diabetic subjects may likewise be an explanation. Nevertheless, an increase in the activity of these glucose-dependent metabolic processes in diabetes, where the glucose utilization is poorer than normal, is difficult to conceive.

The next question: what is the reason for the increase of the peroxide resistance of the erythrocytes by glucose in normal cases, while there is no such effect in diabetic patients? There are a number of well-known factors influencing the result of the PHT /26, 27/; one of them is glucose /26/, but its exact mechanism of action is not yet clear.

It may be presumed that a direct "antioxidative" membrane

effect plays a role in this process, when the interactions of glucose, sulphydryl groups, and lipids or other membrane elements would explain the phenomenon /3, 5/.

Perris /20/ found decreasing hypotonic haemolysis, i.e. the slow invasion of water into the cell, and this observation points to the interaction of glucose and other sugars with the normal red blood cell membrane. Sugars also interact with other membranes. In experimental animals Crowe described the dehydration of the polar main groups of the phospholipids in the microsomal membranes in response to trehalose, which was followed by a phase transformation in the membrane /2/.

Klose's studies suggest that glucose, effecting partial dehydration of the phase surface of the lipid double-layer, gives rise to a phase transformation, and this is followed by changes in the properties /11/.

It is also possible that this interaction has occurred earlier in the diabetic erythrocytes and hence can not be increased by the addition of glucose. The membrane changes and other alterations of the erythrocytes in diabetes have been discussed in numerous papers /1, 5, 10, 29/.

The difference in the glucose effect may also be connected with the consequences of the intracellular diabetic glucose metabolism. Glucose added to the medium during incubation may be a more effective intracellular substrate in red blood cells, which would have a more favourable influence on the antioxidative metabolism of the normal red blood cells than on that of the diabetic ones. Such a metabolic protective effect of glucose was postulated by Selwyn and Dacie /24/, who observed a decrease of the 24-hour autohaemolysis of normal erythrocytes in the presence of glucose. A protective effect was likewise suggested by Stocks and Dormandy /26/ in connection with the peroxide haemolysis of the erythrocytes, though it was not well expressed quantitatively.

An additional problem is the alteration induced in the oxidative haemolyis of the erythrocytes by the change of the pH. At low pH, a decreased autohaemolysis of the red blood cells was observed by Grimes and Young /7, 30/.

Stocks and Dormandy /26/ reported that at lower pH the PHT

effect can be avoided more easily than at higher pH. In connection with the phenomenon induced by the pH decrease, two points are worth mentioning: /a/ during acidosis the glucose utilization of the cells decreases, while it increases during alkalosis; /b/ on the other hand, the change of the pH, together with many other factors, may be involved in the alteration of the membrane fluidity /19, 29/.

Present experiments demonstrated that decrease of the pH from 7.4 to 6.8 increased the resistance of the erythrocytes to H_2O_2 in both normal and diabetic patients. At the same time, incubation in a medium containing a final glucose concentration of 5 or 10 mmol/l did not increase the resistance of diabetic erythrocytes to H_2O_2 at either pH 7.4 or 6.8, whereas it did increase the resistance of healthy red blood cells (Fig. 1).

This observation suggests that the change induced in the resistance to H_2O_2 by the presence of glucose, and the change induced in the resistance by decrease of the pH may take place by different mechanisms.

To decide whether the observed increased H_2O_2 resistance is connected with the enhanced glycosilation of the haemoglobin of diabetic red blood cells, we carried out a correlation analysis of the PHT and the Hb-A_1 results. The lack of any correlation is indirect evidence of the role of the membrane alteration in the increased H_2O_2 resistance in diabetic red blood cells.

Other authors have also stressed the possibility of membrane alterations, as concerns the deviations in the diabetic red blood cells /18/. Our experiments, too tend to implicate the membrane effect.

It may be presumed that in diabetic erythrocytes, in healthy red blood cells incubated in glucose, and in red blood cells incubated in acidic medium, a certain number of the functioning membrane groups (which, in fact, give rise to haemolysis in their reaction with H_2O_2) are inactivated or blocked; thus, they do not react with H_2O_2, and consequently the haemolysis is less extensive.

The observed effect (probably not specific) in diabetes is presumably due to glucose, and might be comparable with the effects of membrane stabilizers /23/.

In our in vitro experiments, the resulting elevated resistance can hardly be compared with the increased haemolysis in patients with severe diabetes, where the glucose concentration is higher, and ketone bodies and other factors may also be responsible for inducing haemolysis. From the aspect of diabetic complications, however, the possibility of similar interactions between glucose and the membrane in other cells may raise the question of a more general importance of the observed effect.

The ideas concerning the problems and questions raised need further consideration.

The authors thank Dr. Krisztina Boda of the Computing Centre, Szent-Györgyi Albert University, Szeged, for helpful assistance in the calculations.

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