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NORMALISATION OF RED BLOOD CELL PYRUVATE KINASE IN PYRUVATE KINASE DEFICIENCY BY RIBOFLAVIN TREATMENT

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Summary

A patient with an erythrocyte glutathione reductase activity of 50% of the normal value and an abnormal pyruvate kinase (PK) was given 36 mg riboflavin daily for 6 months. The glutathione reductase activity was restored and the abnormal pyruvate kinase was converted to normal. The clinical state of the patient improved. It can be concluded that the abnormality of PK, at least with this patient, is a secondary effect. Therefore, it is suggested that other abnormalities be searched for when an altered PK is detected. Treatment of this abnormality will help the patients more efficiently.

Introduction

Erythrocyte pyruvate kinase (ATP:pyruvate phosphotransferase, EC 2.7.1.40) deficiency is a well-known cause of haemolytic anaemia. Recently we suggested that the alteration in pyruvate kinase might be a secondary effect [1,2]. It was shown that incubation of pyruvate kinase with oxidized glutathione (GSSG), results in an enzyme with kinetic properties identical to that described in the literature for pyruvate kinase from most of the pyruvate kinase-deficient patients [3]. Also the thermostability of the "oxidized" enzyme was similar to the stability described for pyruvate kinase from the patients [4]. These data allow the conclusion that erythrocyte pyruvate kinase is sensitive to the redox state of the thiol groups in the red blood cell. Therefore, the primary enzyme defect responsible for an altered pyruvate kinase is likely to be related to enzymes which influence this redox state. This suggestion is supported by the existence of pyruvate kinase-deficient patients also showing a decreased glutathione reductase activity [5–8].

We were able to demonstrate [2] that the incubation of pyruvate kinase

of a patient (M.V.) with a decreased glutathione reductase activity with mercaptoethanol normalises the altered enzyme. It is known that administration of riboflavin to patients with a decreased glutathione reductase activity increases the activity of this enzyme [9]. For this reason riboflavin was administrated to the mentioned patient (M.V.). This report deals with the effect of this treatment on the properties of erythrocyte pyruvate kinase and the clinical state of the patient.

Materials and Methods

Pyruvate kinase from erythrocytes was purified up to stage 4, as described by Staal et al. [10], except that during the isolation procedure mercaptoethanol was omitted.

The final $(NH_4)_2SO_4$ precipitate was dissolved in 0.2 M Tris/maleate buffer, pH 8.2 and pyruvate kinase activity was assayed by following the decrease in absorbance at 340 nm in a coupled reaction with lactate dehydrogenase at 25° C, according to Valentine and Tanaka 11]. The reaction mixture contained 0.2 M Tris/maleate buffer (pH 8.2), 2.0 mM ADP, 0.09 mM NADH, 65 mM KCl, 20 mM $MgSO_4$ and 0.1 mg lactate dehydrogenase in a final volume of 3.0 ml.

Glutathione reductase activity was assayed according to Staal et al. [12] and glucose-6-phosphate dehydrogenase according to Kornberg and Horecker [13]. Thermostability experiments were done at 60° C for 1 min as previously described [10]. 36 mg riboflavin/day was administered orally for 6 months.

Results

Table I shows the effect of riboflavin administration on the total activities of some erythrocyte enzymes and some haematological parameters. The riboflavin treatment results (at least in this patient) in a decreased number of reticulocytes and an increased number of erythrocytes. The glutathione reductase activity which before treatment is about 50% of the normal value reaches its normal value. Fig. 1 (A and B) shows the v versus [phosphoenol pyruvate]

TABLE I

EFFECT OF RIBOFLAVIN ADMINISTRATION ON SOME ENZYMATIC AND HAEMOLYTIC PARAMETERS

	Before riboflavin administration	During riboflavin administration
Glucose-6-phosphate dehydrogenase (μmol/min per g per Hb)	11.0	8.1
Glutathione reductase (μmol/min per g per Hb)	1.8	3.3
Pyruvate kinase (μmol/min per g per Hb)	4.2	2.5
Hb (g%)	7.2	11.3
Ht	22	34
Erythrocytes ($\times 10^6$/mm^{3}blood)	1.8	2.6
Reticulocytes (%)	17.6	5.6

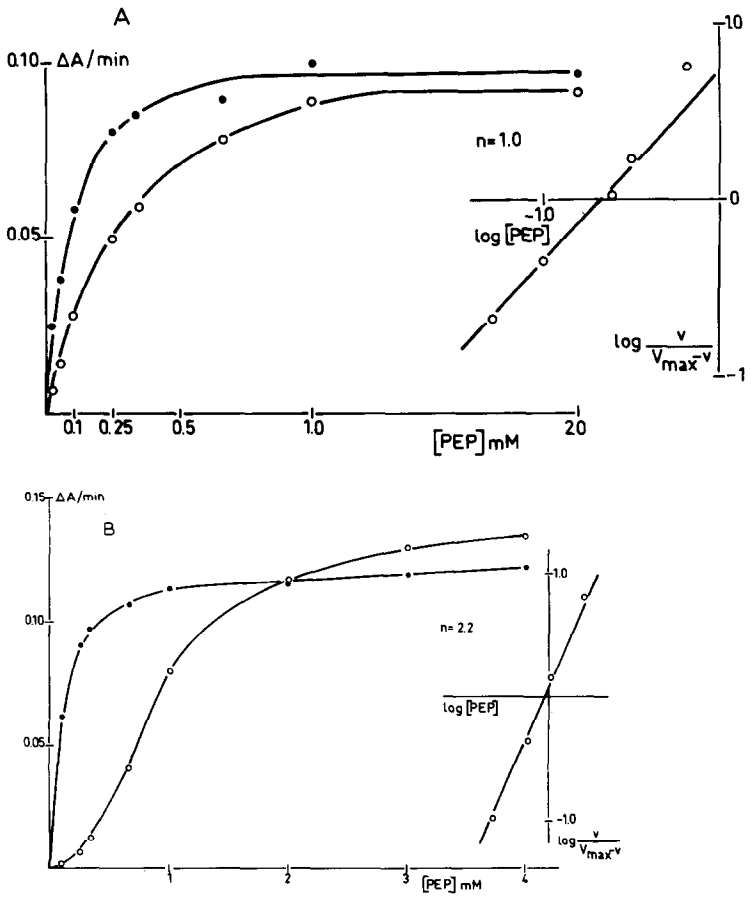

Fig. 1. The activity versus [phosphoenolpyruvate] plot of erythrocyte pyruvate kinase from patient M.V. measured in the presence and absence of Fru-1,6-P_2 (0.5 mM). [ADP] = 2 mM. The inserts are the Hill plots of the value obtained. The calculated Hill coefficients (n) are indicated. (A) The activity curve after incubation without mercaptoethanol, measured in the absence (○———○) and in the presence of Fru-1,6-P_2 (●———●) (from ref. 2). (B) The activity curve after incubation without mercaptoethanol (after 6 months of riboflavin treatment), measured in the absence (○———○) and in the presence of Fru-1,6-P_2 (●———●).

(PEP) at [ADP] = 2 mM in the presence and absence of 0.5 mM Fru-1,6-P_2 of the enzyme isolated from erythrocytes of patient M.V. before and after treatment with riboflavin. As reported earlier [2] the enzyme has lost its allosteric properties towards PEP (n value 1.0 s; insert Fig. 1A). Incubation of this enzyme with mercaptoethanol can restore the allosteric properties (see ref. 2). In contrast to the results before treatment, the enzyme isolated from erythrocytes of the patient at the end of a 6 month administration of riboflavin, exhibits allosteric properties (Fig. 1B) even before incubation with mercaptoethanol. The n value of 2.2 (see insert Fig. 1B) is equal to the value obtained with the enzyme from control persons [10]. Also the Fru-1,6-P_2 activation does not differ from that normally found. Incubation of the enzyme with mercaptoethanol does not change this pattern (not shown). The enzyme isolated at the end of the riboflavin treatment in contrast to the enzyme before treatment,

TABLE II

THERMOSTABILITY OF ERYTHROCYTE PYRUVATE KINASE AT 60°C

	Loss of enzyme activity (%)
Before riboflavin administration	70
During riboflavin administration	25

cannot be distinguished from the normal enzyme, at least on these kinetic parameters. It has been concluded earlier that the common property of pyruvate kinase from pyruvate kinase-deficient patients is the increased thermolability of the enzyme. Therefore, the thermostability was investigated, the results of which are summarized in Table II. It can be seen that the enzyme isolated at the end of the riboflavin treatment is more stable than before treatment. This result indicates that not only the kinetic properties are normalised but also the thermostability of the enzyme is restored.

Discussion

It has been demonstrated earlier that administration of riboflavin to glutathione reductase-deficient patients increases the glutathione reductase activity to its normal value. However, in contrast to the biochemical effect of riboflavin, there was no clinical improvement. In our patient to whom we administered riboflavin the increase of glutathione reductase activity was accompanied by a clinical improvement. Before, administration blood transfusions were necessary every month, whereas during the 6 months of riboflavin administration this was not necessary. It is suggested, therefore, to extend this type of treatment to other patients with this type of deficiency [6—8].

In a preceding paper [2] we showed that the abnormal pyruvate kinase determined in patient (M.V.) can be changed into normal by incubation of this enzyme with mercaptoethanol. This suggests that the abnormal pyruvate kinase was a secondary defect resulting from a modification of the thiol groups in the enzyme. The experiments here reported show that restoration of the glutathione reductase activity by administration of riboflavin is accompanied by a change of the abnormal pyruvate kinase into a normal enzyme. Also the thermostability of the enzyme is regained. These results confirm our earlier statement and prove that the enzyme modification in this patient is indeed a secondary defect. Of course the experiments reported here do not exclude the possibility of the occurrence of a real pyruvate kinase deficiency as a primary cause of a haematological disease [14—16]. However, the present and earlier [1,2] reported experiments clearly show that erythrocyte pyruvate kinase is an enzyme which is very sensitive to its cellular environment. This indicates that it is not necessary that when an altered pyruvate kinase is found, this abnormality is the primary lesion for an increased haemolysis [17]. By measurement of other enzymes, especially enzymes which play a role in the maintaince of the reduction state of thiol groups in the red blood cell, another abnormality may be detected and a therapy based upon this defect may help the patients.

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