Congenital Hemolytic Anemia in the Basenji Dog Due to Erythrocyte Pyruvate Kinase Deficiency

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SUMMARY

Congenital hemolytic anemia in the Basenji dog resembles pyruvate kinase (PK) deficiency in man as it is characterized by an abbreviated erythrocyte life span, an intense reticulocytosis, type II autohemolysis and splenomegaly. Glucose utilization and lactate production were inadequate with respect to the immature cell population. Analysis of enzymes involved in erythrocyte glycolysis revealed a deficiency of pyruvate kinase.

RÉSUMÉ

L'anémie hémolytique congénitale du chien Basenji ressemble à la déficience en kinase pyruvate (PK) chez l'homme. En effet, elle se manifeste par une diminution de la vie des érythrocytes, une réticulocytose intense, une autohémolyse de type II et de la splénomégalie. L'utilisation de glucose et la production de lactate s'avérèrent inadéquates, vis-à-vis l'ensemble des cellules immatures. Une analyse des enzymes impliquées dans la glycolyse des érythrocytes révéla une déficience en kinase pyruvate.

INTRODUCTION

Young Basenji dogs have recently been presented to veterinary hospitals in the United States suffering from a severe unremitting anemia (2, 13). Clinical findings of decreased activity, pale mucous membranes, and splenomegaly were usually detected within the first year of life. Unless repeatedly transfused, animals succumbed prior to the age of three years. Hematological findings in previously described affected Basenjis included lowered packed cell volumes, reticulocytosis, and bone marrow erythroid hyperplasia. The Coombs' test was consistently negative. Erythrocyte parasites could not be demonstrated and no sources of erythrocyte toxins were apparent. These observations plus the occurrence of the condition in littermates on several occasions suggest a congenital erythrocyte defect (2, 13). Hemoglobin electrophoretic studies failed to reveal an abnormal hemoglobin (13).

The purpose of this report is to describe a congenital hemolytic anemia in Basenji dogs associated with increased erythrocyte osmotic fragility and autohemolysis following incubation, impaired glycolysis, and pyruvate kinase deficiency.

MATERIALS AND METHODS

The packed cell volume was determined by the microhematocrit method (9). Reticulocytes were counted on films made from blood stained with new methylene blue (9). The number of reticulocytes was expressed in percent after counting 1000 erythrocytes.

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TABLE I. Hematologic Data

Subject	PCV	Reticulocytes (%)	^{51}Cr Survival Half-Life Days	Glucose Utiliz. (umoles/ml RBC)	Lactate Prod. (umoles ml/RBC)	Autohemolysis (%) Additive			
						Saline	Glucose (0.05%)	Inosine (0.02M)	Adenosine (0.02M)	ATP (0.02M)
Normal dogs	40—55	0—2	20	2.24±.49	3.92±.52	9.60	3.5	4.0	20.6	2.9
Reticulocyte control	25	8		3.63±.24	6.56±.78					
Patient 2170	23	50	3	3.58±.41	5.55±.97	83.3	68.8	66.7	28.5	16.9
Patient 2170c	22	50	2.5	3.47±.67	5.35±.93	78.9	66.3	58.1	31.1	19.7
Patient G-3	26	30		2.28	3.83	74.3	52.0	46.0	13.3	23.0
Patient G-6	21	45		3.23	4.99	72.7	68.4	50.2	17.0	21.7
Patient 7	27	40		2.54	3.72	72.0	75.1	54.1	16.1	15.9
Patient 8	28	35		1.96	3.79	68.0	60.6	57.8	10.0	21.4

Osmotic fragility of fresh blood and blood incubated at 37°C for 24 hours was determined using the method of Young *et al* (18). The autohemolysis test was carried out as modified by Young *et al* (18) and de Gruchy *et al* (1).

Autologous red cell survival in two affected dogs and a normal dog was determined by the ^{51}chromium technique (14). Fifty microcuries of ^{51}Cr[1] were added to 10 ml of patient's blood in 2.5 ml of acid-citrate-dextrose solution.

In vitro erythrocyte glucose utilization and lactate production were measured as previously described (3, 8, 10). Blood was defibrinated by means of glass beads.

Assay procedures as reported elsewhere were performed for glucose-6-phosphate dehydrogenase, phosphogluconic dehydrogenase, hexokinase, glucose phosphate isomerase, phosphofructokinase, aldolase, triose phosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, phosphoglyceromutase, enolase and lactate dehydrogenase (6).

The pyruvate kinase (PK) assay procedure was a modification of that reported by Tanaka *et al* (12). The final concentration of phosphoenolpyruvate in the cuvettes was 3 millimoles. Cuvettes were maintained at 37°C throughout the assay period.

Normal dogs of various breeds, including Basenjis, were used as controls in all procedures. A normal Basenji dog with reticulocytosis induced by phlebotomy (reticulocyte control) was included in order to assess the effects of red cell immaturity on glycolytic rates and enzyme activities.

RESULTS

Osmotic fragility was normal on fresh blood but markedly increased following incubation at 37°C for 24 hours. Routine hematological data, ^{51}Cr survival times, and autohemolysis results are presented in Table I.

Glucose utilization and lactate production in six affected dogs were similar to or slightly greater than normal dogs but generally lower than an 8% reticulocyte control dog. The mean lactate:glucose ratio in affected dogs was 1.62 and in the reticulocyte control Basenji 1.82.

[1]Mallinckrodt Chemical Works, St. Louis, Missouri.

With the exception of PK, all of the erythrocyte enzymes studied had greater activity than those of red cells from the normal Basenji with a reticulocytosis induced by phlebotomy.

Pyruvate kinase analysis revealed a range of values of 0.34 to 1.17 units for the anemic Basenji dogs and 1.11 to 1.59 units for normal dogs. A reticulocyte control dog (5%) had 2.01 units of PK activity (Table II). Erythrocyte PK activity was decreased therefore in Basenji dogs with congenital hemolytic anemia, especially with regard to cell age.

TABLE II. Erythrocyte Pyruvate Kinase Data

Subject	PK Activity Units[a]	Reticulocytes (%)
Normal dogs (n = 14)	1.36 ± .25	0-2
Reticulocyte Control	2.01	5
Patient 2170	.96	50
Patient 2170c	.88	50
Patient G-3	.50	30
Patient G-6	.34	45
Patient 7	.64	40
Patient 8	1.18	35
Patient A-1	.56	33

[a]one unit is expressed as one micromole of (NADH) oxidized to (NAD) per 10^{10} red cells per minute under conditions of this assay.

DISCUSSION

Congenital hemolytic anemia in the Basenji dog resembles the heterogeneous group of disorders in man referred to as the congenital nonspherocytic hemolytic anemias. The common features of this group of anemias in man include the absence of an abnormal hemoglobin, lack of spherocytosis, and normal osmotic fragility of fresh cells (1). An important differential feature within the group is the response to the *in vitro* autohemolysis test as described by Selwyn and Dacie (11). Type I autohemolysis is characterized by minimal hemolysis which is only partially prevented by glucose. Type II patients on the other hand exhibit marked autohemolysis after 48 hours incubation, with little or no prevention by glucose. De Gruchy and co-workers (1) studied type II patients and found an increase in osmotic fragility after incubation in three out of four cases. They also showed significant correction of autohemolysis by adenosine triphosphate (ATP). After demonstrating low glucose utilization, reduced erythrocyte ATP, and an accumulation of certain phosphorylated glycolytic intermediates, Robinson and coworkers (7) postulated a defect in glycolysis beyond the point of 2, 3-diphosphoglycerate formation in type II patients. In 1961, Valentine *et al* (15) found a specific deficiency of the erythrocyte enzyme pyruvate kinase (PK) in three patients with type II congenital hemolytic anemia.

The finding, in blood from anemic Basenji dogs, of an increase in osmotic fragility following incubation and the marked autohemolysis which was not prevented by glucose, but was reduced by ATP, resembles PK deficiency in man.

Erythrocyte metabolism involves the continual transport of glucose into the cell where 90% enters the anaerobic Embden-Meyerhof pathway. The energy requirements of the cell are met solely in this manner since mature erythrocytes have no citric acid cycle (19). Yunis (19) and Oski (4) have found that glucose utilization doubles when the reticulocyte count is between 10 and 20%. The reticulocyte control Basenji (8%) employed in this study, had glucose utilization and lactate production which was higher than the anemic dogs with reticulocyte numbers ranging from 35-50%. We may conclude, therefore, that the erythrocytes from the anemic Basenjis do not have a glycolytic rate commensurate with their immaturity. The lower lactate: glucose ratios in affected dogs is also in keeping with a glycolytic defect.

The demonstration of erythrocyte PK deficiency in anemic Basenji dogs serves to explain the impaired glycolysis. This enzyme normally catalyzes the conversion of phosphoenolpyruvate to pyruvate. When this reaction becomes significantly rate-limiting the conversion of glucose to lactate in the Embden-Meyerhof pathway is impaired. This would be expected to result in a deficiency of ATP and an increase in glycolytic intermediates in the anaerobic pathway proximal to the PK reaction.

It is now apparent that there is considerable heterogeneity of red cell PK deficiency in man. Pyruvate kinase mutants have been

described with low activity and normal Michaelis constants for the substrate phosphoenolpyruvate. Other patients have had normal or decreased PK activity with abnormal Michaelis constants for phosphoenolpyruvate (5). Similar kinetic studies are currently underway in which normal canine red cell PK is compared with that from erythrocytes of Basenji dogs with congenital hemolytic anemia.

Tasker and co-workers (13) were the first to describe this condition. However, they were unable to demonstrate any abnormalities in PK activity. This may have been the result of incomplete removal of leukocytes from blood containing many immature erythrocytes. White cells in man are reported to have 300 times the PK activity of red cells which necessitates their exclusion from the assay system (12). The failure to employ a reticulocyte control dog also offers an explanation for their assumption that PK activity was normal.

Congenital hemolytic anemia due to an erythrocyte enzyme deficiency has not been described previously in veterinary hematology. We believe, however, that the congenital hemolytic anemia occurring in some blood lines of Basenji dogs is due to impaired erythrocyte energy metabolism and premature cell senescence associated with PK deficiency. The discovery of this animal model of human disease should advance our understanding of the genetics and biochemistry of erythrocyte pyruvate kinase deficiency in both species.

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