

Effect of Splenectomy on Free Amino Acid Concentrations in Erythrocytes of Patients with Hereditary Spherocytosis

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ABSTRACT

Free amino acid concentrations in erythrocytes obtained from children with hereditary spherocytosis were measured before and after splenectomy. The concentrations of about half of the free amino acids were elevated before splenectomy, and this elevation may be due in part to increased numbers of reticulocytes. After splenectomy, however, the concentration of erythrocyte amino acids was not normalized and that of six to eight amino acids was still elevated. This elevation after splenectomy may be due to spherocytes themselves.

Hereditary spherocytosis is characterized by spherocytes in the peripheral blood, reduced erythrocyte survival time, varying degrees of anemia and splenomegaly, and full clinical remission after splenectomy. In this disease a congenital hemolytic process is associated with an intrinsic defect in the erythrocyte itself. A good deal of evidence indicates that the disorder is due to the altered physiology of the erythrocyte membrane^{5,6,11}. The biochemical status of erythrocytes in this disease is probably abnormal and is affected by splenectomy. In this study we measured the free amino acid concentrations in the erythrocytes of patients with hereditary spherocytosis before and after splenectomy.

MATERIALS AND METHODS

We examined four children (two boys and two girls) with hereditary spherocytosis who were treated by splenectomy. Their ages, at the time of splenectomy, ranged from one to ten years. The diagnosis was based on peripheral blood smears, increased osmotic fragility, negative Coombs test, and other findings of hemolytic anemia. Free amino acid concentrations were

measured in plasma, lymphocytes, and granulocytes, as well as in erythrocytes, before and after splenectomy. The methods for preparation of blood cell samples and for chromatographic analysis of amino acids were reported in previous communications^{3,4}.

RESULTS

Free amino acid concentrations in erythrocytes before and after splenectomy were recorded per 10^{10} cells (Table 1). The values of six amino acids were decreased after splenectomy. Before splenectomy the values of nine amino acids were higher than the control values, while after splenectomy the values of eight amino acids were higher than the control values. The concentrations were also recorded per gram of hemoglobin (Table 2). Values higher than the control values were recorded for eight amino acids before and six amino acids after splenectomy. The free amino acid concentrations in plasma, lymphocytes, and granulocytes were not significantly different from the control values, and were not changed after splenectomy.

Table 1. Concentrations of free amino acids in erythrocytes of patients with hereditary spherocytosis

Amino acid	Concentrations of free amino acids (nmol/10 ¹⁰ cells) ¹			
	Pre-splenectomy	<i>p</i> ²	Post-splenectomy	control ³
Taurine	40.04 ± 5.15 ⁷	0.05	27.72 ± 4.48	27.78 ± 3.97
Aspartic acid	496.65 ± 266.55		280.73 ± 40.24	220.09 ± 103.72
Threonine + glutamine	865.64 ± 182.90 ⁷	0.05	506.67 ± 131.22 ⁴	369.73 ± 100.03
Serine + asparagine	359.93 ± 43.36 ⁷	0.005	212.95 ± 28.59 ⁵	154.68 ± 36.00
Glutamic acid	316.27 ± 141.47		211.37 ± 35.05	181.25 ± 50.72
Glycine	1134.04 ± 166.19 ⁵	0.001	414.36 ± 72.29	333.28 ± 72.05
Alanine	490.89 ± 91.26 ⁷	0.005	257.02 ± 36.79	211.93 ± 59.90
Valine	39.01 ± 12.32 ⁵		37.99 ± 7.82 ⁵	20.32 ± 11.30
Methionine	8.60 ± 2.23		11.86 ± 3.41 ⁴	7.04 ± 4.01
Isoleucine	9.32 ± 4.97		9.78 ± 2.48 ⁷	4.06 ± 2.10
Leucine	28.17 ± 3.60		28.86 ± 5.58	24.30 ± 5.22
Tyrosine	50.11 ± 9.95 ⁴		60.40 ± 19.61 ⁶	32.83 ± 13.97
Phenylalanine	6.94 ± 1.11 ⁵		7.96 ± 1.43 ⁷	3.91 ± 1.81
Histidine	63.70 ± 13.10		69.85 ± 6.46 ⁴	57.23 ± 10.74
Ornithine	96.21 ± 10.74	0.05	70.93 ± 9.12 ⁴	97.44 ± 20.44
Lysine	121.46 ± 18.65 ⁴		94.26 ± 23.07	93.29 ± 21.08
Arginine	21.62 ± 2.91		20.74 ± 4.90	25.28 ± 9.37

¹Data expressed as mean ± SD.²*p*, probability by Student's *t*-test for comparing the means between Pre- and Post-splenectomy tests.³Age-matched control (*n* = 16).⁴Significantly different from control; *p*<0.05.⁵Significantly different from control; *p*<0.01.⁶Significantly different from control; *p*<0.005.⁷Significantly different from control; *p*<0.001.

Table 2. Concentrations of free amino acids in erythrocytes of patients with hereditary spherocytosis

Amino acid	Concentrations of free amino acids (nmol/g Hb) ¹			
	Pre-splenectomy	<i>p</i> ²	Post-splenectomy	Control ³
Taurine	126.16 ± 8.63 ⁷	0.001	86.22 ± 8.57	91.40 ± 14.41
Aspartic acid	1613.36 ± 934.93		872.32 ± 120.39	705.46 ± 366.07
Threonine + glutamine	2759.77 ± 690.41 ⁴	0.05	1594.75 ± 519.53	1212.18 ± 323.45
Serine + asparagine	1135.67 ± 98.83 ⁷	0.001	666.78 ± 69.18 ⁴	505.83 ± 109.73
Glutamic acid	988.96 ± 425.51		660.84 ± 138.65	595.66 ± 164.62
Glycine	3578.59 ± 445.93 ⁷	0.001	1282.74 ± 181.64	1094.71 ± 238.67
Alanine	1550.47 ± 278.12 ⁷	0.05	794.04 ± 62.54	693.63 ± 189.77
Valine	124.95 ± 46.94 ⁴		117.16 ± 18.32 ⁴	65.98 ± 37.13
Methionine	26.96 ± 5.56		36.43 ± 8.61	27.51 ± 15.06
Isoleucine	29.94 ± 17.63		30.11 ± 6.17 ⁷	13.23 ± 6.97
Leucine	89.28 ± 13.48		89.11 ± 12.89	78.71 ± 17.33
Tyrosine	159.11 ± 36.86		184.95 ± 50.31 ⁵	107.46 ± 46.46
Phenylalanine	22.16 ± 5.02 ⁴		24.69 ± 4.07 ⁶	12.34 ± 6.34
Histidine	210.27 ± 33.88		218.05 ± 6.97 ⁴	189.38 ± 31.99
Ornithine	305.15 ± 44.68	0.05	221.46 ± 21.94 ⁴	314.69 ± 69.28
Lysine	384.44 ± 62.41 ⁴		292.18 ± 59.14	298.93 ± 70.71
Arginine	68.88 ± 13.71		64.86 ± 14.83	75.80 ± 25.85

¹Data expressed as mean ± SD.²*p*, probability by Student's *t*-test for comparing the means between Pre- and Post-splenectomy tests.³Age-matched control (*n* = 16).⁴Significantly different from control; *p*<0.05.⁵Significantly different from control; *p*<0.001.⁶Significantly different from control; *p*<0.001.⁷Significantly different from control; *p*<0.001.

DISCUSSION

The concentrations of about half of the free amino acids measured in erythrocytes were elevated in patients with hereditary spherocytosis. Before splenectomy six amino acids were more concentrated in erythrocytes than after splenectomy. The reticulocytes of several species have been shown to accumulate amino acids and incorporate them into hemoglobin. An increased uptake of glycine by erythrocytes has been described in anemic patients with high reticulocyte counts²). It has also been found that an active alanine transport is present in reticulocytes and that this is greatly decreased or lost as cells mature in rats¹) and in sheeps⁹). The elevation of amino acids before splenectomy may be due in part to increased numbers of reticulocytes. Actually before splenectomy the reticulocyte count was high (6.2–24.4%) and decreased to 0.1–1.5% after splenectomy. After splenectomy, however, the concentration of erythrocyte amino acids was not normalized and that of six to eight amino acids was still elevated. This elevation may be due to spherocytes themselves rather than reticulocytes. Since the sodium ion is reported to influence the amino acid transport through the erythrocyte membrane^{1,10}), the elevation of amino acids after splenectomy may be associated with an increased spherocyte membrane permeability to sodium ion in patients with hereditary spherocytosis^{5,6}).

In patients with various types of anemia, including hemolytic anemia, plasma amino acids were reported to show little deviation from normal^{7,8}), and our patients with hereditary spherocytosis also showed no significant deviation. Free amino acid concentrations in lymphocytes and granulocytes of patients with hereditary spherocytosis have not yet been reported.

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