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TRADITIONAL MEDICINE

Alterations of erythrocyte ATPase activity and oxygen consumption in patients with liver-blood deficiency syndrome

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Abstract

AIM: To investigate the pathophysiology of erythrocyte energy metabolic changes of patients with the traditional Chinese Medicine (TCM) liver-blood deficiency syndrome (LBDS).

METHODS: Erythrocyte membrane ATPase activity and oxygen consumption rate (OCR) were determined in 66 patients with LBDS, including 35 patients with iron deficiency anemia and 31 patients with chronic aplastic anemia. Thirty healthy adults served as controls.

RESULTS: ATPase activity and OCR were decreased in patients with LBDS.

CONCLUSION: The decreased erythrocyte ATPase activity and OCR might cause the energy hypometabolism in LBDS patients.

Key words: Erythrocytes; Cell membrane; Oxygen consumption; Adenosine triphosphatase; Liver-blood deficiency syndrome; Iron-deficiency anemia; Aplastic anemia

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INTRODUCTION

Erythrocytes, the most common blood cell, exhibit metabolic characteristics. We have conducted hemorrheologic studies in patients with liver-blood deficiency syndrome (LBDS)[1], and observed that their hematocrit (Hct) was significantly decreased. This indicated a reduced erythrocyte count. There are few reports on erythrocyte metabolic alterations in patients with LBDS. In this study, the erythrocyte membrane ATPase activity and the erythrocyte oxygen consumption rate (OCR) were determined in patients with anemia, including iron deficiency anemia and chronic aplastic anemia.

MATERIALS AND METHODS

Diagnostic criteria

General clinical data are listed in Table 1. The patients enrolled in this study all had traditional Chinese Medicine (TCM) differentiated LBDS syndrome diagnosed by two clinicians according to our certified standard[2]. The symptoms included dizziness, decreased visual acuity and/or blurred vision, numbness of the extremities, face, lips, and nails appeared pale and malnourished, tongue appeared pale, and pulse taut and/or thready. Patients presenting with decreased visual acuity and/or blurred vision and numbness of the extremities along with an additional two symptoms (excluding those with Yinxu, Yangxu and Qixu) were diagnosed with LBDS. Iron deficiency anemia (IDA) was diagnosed according to the "Diagnostic Criteria and Curative Improvement Standard of Clinical Diseases" [3]. Chronic aplastic anemia (CAA) was diagnosed according to the June 1987 Baoji Conference revised standard[4]. Healthy adult blood donors served as controls.

Instruments

Equipment used included a portable automatic balanced recorder (XWT-104, Shanghai Dahua Instrument Factory), a Clark electrode and SP-2 dissolved oxygen assay controller (China Academy Vegetal Physiology Institute), a 2219-Ⅱ thermostat circulation water bath (LKB), and a 751 spectrometer (Shanghai 3rd Analytic Instrument Factory).

Preparation of the erythrocyte membrane

Five milliliters of heparin anticoagulated fasting venous blood was centrifuged at 3000 rpm for 10 min. The buffy coat was discarded. The remainder was washed with isotonic Tris-HCl (310 mOsm, pH

Table 1 General clinical data

	n	Male/Female	Age (yr)	Diseases
Control group	30	15/15	34.2 ± 10.4 (20-46)	
Erythrocyte membrane ATPase	31	11/20	36.6 ± 13.6 (19-56)	IDA 16, CAA 15
Erythrocyte OCR	35	13/22	35.8 ± 15.2 (21-48)	IDA 19, CAA 16

Table 2 Comparison of ATPase activities

Groups	*n*	Mg^{2+}-ATPase	Na^+-K^+-ATPase	Ca^{2+}-ATPase
		(μmol Pi·mg protein·h)		
Control	30	0.300 ± 0.160	0.250 ± 0.120	0.620 ± 0.170
LBDS	31	0.130 ± 0.072[b]	0.154 ± 0.081[b]	0.530 ± 0.159[a]
CAA	15	0.132 ± 0.044[b]	0.156 ± 0.067[b]	0.562 ± 0.130
IDA	16	0.132 ± 0.091[b]	0.152 ± 0.093[b]	0.468 ± 0.215[a]

[a]$P < 0.05$, [b]$P < 0.01$ *vs* control.

Table 3 Comparison of erythrocyte OCR (oxygen consumption rate)

Groups	*n*	Oxygen consumption rate (μL 100·h·mL compressed RBC)
Control	30	107.26 ± 18.46
LBDS	35	82.25 ± 36.39[b]
CAA	16	68.83 ± 24.83[b]
IDA	19	100.17 ± 13.86[a]

[a]$P < 0.05$ *vs* CAA, [b]$P < 0.01$ *vs* control.

7.4) twice at a ratio of 1:30 with cool hypotonic Tris-HCI (20 mOsm, 2 mmol/L EDTA, pH 7.4). After centrifugation at 12000 rpm for 35 min, a pink fluid precipitant could be seen adhering to the wall of the tube. This was washed with hypotonic solution twice, and a white cell membrane preparation was obtained. The whole procedure was performed at 0 ℃ to 4 ℃, and the membrane product was stored at -20 ℃. Twenty minutes before the ATPase activity assay, the membrane was dissolved with 2 g/100 L saponin. The membrane protein content was determined by the improved Lowry method[5].

Determination of erythrocyte membrane ATPase activity

The Mg^{2+}-ATPase, Na^+-K^+-ATPase, and Ca^{2+} ATPase activities were assessed according to Reinila *et al*[6].

Determination of erythrocyte oxygen consumption rate

The erythrocyte oxygen consumption rate (OCR) was determined using the film oxygen electrode Clark technique and formula[7].

Statistics

Results were expressed as mean ± standard deviation. A *t*-test and ANVOA were used for statistical analysis.

RESULTS

The Mg^{2+}-ATPase, Na^+-K^+-ATPase, and Ca^{2+} ATPase activities in patients with LBDS were significantly decreased as compared with the healthy controls, ($P < 0.01$, $P < 0.01$, and $P < 0.05$, respectively) (Table 2). The patients diagnosed with CAA did not have a significant difference in the Ca^{2+}-ATPase activity compared to normal controls.

The erythrocyte OCR was generally decreased in the LBDS patients compared with the healthy controls. The erythrocyte OCR of patients diagnosed with IDA was not significantly significant from the healthy controls ($P > 0.05$). The erythrocyte OCR of patients diagnosed with CAA was significantly decreased from the healthy controls ($P < 0.01$). The difference between the IDA and CAA patients was significant ($P < 0.01$) (Table 3).

DISCUSSION

Na^+-K^+-ATPase is responsible for the active transport of sodium and potassium across the membrane, which maintains a high intracellular concentration of potassium and a low intracellular concentration of sodium. ATP is required for the active transport of these molecules[8], If there is a decrease of Na^+-K^+-ATPase on the erythrocyte membrane, then there will be an increase of intracellular sodium concentrations, which could lead to a hypoenergetic status of the erythrocytes. Furthermore, if phosphorylation of membrane proteins is impaired in the ATPase deficient erythrocyte, then the formation of membrane protein polymers will be hindered. This affects cytoskeleton stability[9], resulting in abnormalities of the erythrocyte structure. Therefore, Na^+-K^+-ATPase is essential for the maintenance of the normal morphology, structure and function of the erythrocyte[10]. In mature erythrocytes, glucose catabolism is very active in order to provide the sodium pump with energy and to maintain the normal functioning of the erythrocytes (90% of the energy from glycolysis and 10% from the pentose phosphate pathway)[7].

We observed that the activities of the ATPases, including the Mg^{2+}-ATPase, the Na^+-K^+-ATPase and the Ca^{2+} ATPase, were significantly decreased compared to the normal controls. However, no differences were observed between the patients diagnosed with IDA and CAA. In addition, the oxygen consumption rate of the LBDS patients was decreased compared to the controls, especially the patients with CAA. Taken together, the results suggest that the erythrocyte ATPase activity and OCR are decreased in LBDS patients, which could lead to pathophysiological changes of decreased energy metabolism.

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