MÖSSBAUER STUDIES OF HEMOGLOBIN AND THE IMMUNE FUNCTION OF RBC OF THE PATIENTS WITH HEPATIC DISEASES*

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ABSTRACT

Red blood cells (RBC) of the patients with cirrhosis and with primary liver cancer were studied by using Mössbauer spectroscopy. The measurements of RBC from normal adults were made as control. Results showed that the subspectrum representing deoxy-Hb either disappeared or diminished, but no other subspectrum was detected in the samples from the patients. In addition, the immune function of RBC of these two types of patients was measured by using immune-adherence haemagglutination (IAHA), which indicated that its immune function was significantly lower than that of normal adults ($P<0.01$). These experimental results showed that the components of hemoglobin in RBC are correlated with the immune function of RBC.

Keywords: Mössbauer Spectroscopy Red blood cell Cirrhosis Primary liver cancer

1 INTRODUCTION

Cirrhosis and primary liver cancer result from a variety of causes. It is generally held that patients with heptitis can develop cirrhosis or liyer cancer which may be related to the deposit of immune complex in the liver of patients. One of the functions of RBC is to remove immune complex from the circulation system of the human body. Therefore, it is worth studying whether the denatured composition and structure of hemoglobin in RBC of these patients will affect its function of removing immune complex. RBC of 4 patients with cirrhosis and 7 patients with primary liver cancer were investigated by using Mössbauer spectroscopy. The measurements of RBC from 3 normal adults were made as control. Furthermore, the immune functions of RBC of 22

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patients with cirrhosis, 22 patients with primary liver cancer and 20 normal adults as a control group were investigated by using immune-adherence haemagglutination (IAHA).

2 MATERIAL AND METHODS

2.1 *Sample* Twenty two cases of patients with cirrhosis were provided by Naval Hospital 411, Shanghai, 22 cases of patients with primary liver cancer were from Ruijin Hospital, Shanghai. Definite diagnosis of the diseases was confirmed by clinical CT examination. Twenty subjects (normal adults) were staff members of our laboratory.

2.2 *Mössbauer spectroscopy* RBC were separated from venous blood of normal adults, and of the patients by using lymphocyte separatory liquid and were washed twice with physiological saline. Then, RBC extracted by centrifugation (speed 3 000 r/min, duration 15 min) were placed in a plexiglass container (height 1 cm and diametre 1.5 cm) and immediately frozen in liquid nitrogen. The Mössbauer spectra were recorded at 80 K with a constant acceleration spectrometer. A 1×10^9 Bq radioactive source of ^{57}Co/Pd and a proportional counter to detect γ -ray were used. The Doppler velocimeter was calibrated by using a highly pure α -Fe, 25 μ m thick. All the isomeric shift values (IS) were relative to that of α -Fe at room temperature. The spectra were fitted with a least-square program by using Lorentzian Shape.

2.3 *Measurement of the immune function of RBC* The immune function of RBC of these patients was measured by IAHA. Added respectively in each well of the plate were 25 μ l of aggregated human Gammaglobulin (AHG), complement, dithiothreital solution and erythrocyte suspension. Then the plate was incubated at 24°C for 60 min for further observation of haemagglutination pattern, and the immune-adherence activity of erythrocytes was expressed by the reciprocal of 2^N, the highest dilution of AHG that induced haemagglutination.

3 RESULTS

Table 1

Activity of RBC-CRI of the patients with hepatic diseases

Sample No.	IAHA titre (2^N)											Mean±SD
N	2^{11}	2^5	2^{10}	2^{11}	2^{11}	2^{11}	2^9	2^7	2^9	2^9		$2^{9.55\pm1.72}$
(20 cases)	2^{11}	2^{10}	2^{11}	2^{10}	2^{11}	2^{10}	2^{10}	2^7	2^9	2^7		
C	2^7	2^3	2^6	2^7	2^7	2^7	2^6	2^5	2^7	2^5	2^5	$2^{4.45\pm2.50}$
(22 cases)	2^6	2^5	2^6	2^5	2^5	2^0	2^0	2^1	2^0	2^5	2^0	
L	2^0	2^3	2^8	2^7	2^1	2^7	2^7	2^5	2^8	2^0	2^0	$2^{3.63\pm3.30}$
(22 cases)	2^2	2^0	2^6	2^0	2^5	2^0	2^7	2^9	2^0	2^5	2^0	

Note: N-Normal adults C-Patients with cirrhosis L-Patients with primary liver cancer

a. Changes in the immune function of RBC of the patients with hepatic diseases (see table 1).

Table 1 shows that the immune function of RBC of those patients decreased significantly when compared with that of normal adults ($P<0.01$).

b. Changes in the Mössbauer spectroscopy of RBC of the patients with hepatic diseases (see table 2).

Table 2 indicates that the number of deoxy-Hb of the patients decreases significantly when compared with that of normal adults.

Table 2

Mössbauer parameters of the patients with hepatic diseases

Sample No.		Oxy-Hb		Deoxy-Hb		Deoxy-Hb
		IS (mm/s)	QS (mm/s)	IS (mm/s)	QS (mm/s)	Oxy-Hb
	1	0.26±0.02	1.86±0.03	0.93±0.04	1.95±0.06	3.2
Normal	2	0.26±0.03	1.92±0.03	0.93±0.04	2.05±0.06	3.5
adults	3	0.23±0.02	1.89±0.02	0.91±0.04	2.08±0.04	3.9
	1	0.26±0.02	1.87±0.04			
Patient	2	0.24±0.02	1.90±0.04			
with	3	0.26±0.03	1.94±0.04			
cirrhosis	4	0.23±0.02	1.92±0.04	0.94±0.04	2.06±0.07	3.3
	1	0.27±0.02	1.92±0.02			
	2	0.24±0.02	1.89±0.02			
Patient	3	0.24±0.02	1.89±0.02			
with	4	0.23±0.02	1.80±0.03			
primary	5	0.26±0.03	1.69±0.05			
liver	6	0.22±0.02	1.77±0.03	0.88±0.06	1.73±0.12	4.6
cancer	7	0.26±0.03	1.88±0.04	0.92±0.03	2.09±0.05	4.0

4 DISCUSSION

The transport of O_2 and CO_2 is done by hemoglobin, which is a tetramer consisting of two α and two β subunits. The deoxy-Hb combines with oxygen to form oxy-Hb which carries O_2 to the tissues of the body. The oxy-Hb bears the function of oxygen transport in the body of mammals. In deoxy- Hb, the iron cation is Fe^{++} in high-spin state ($s=2$), and in oxy-Hb it is Fe^{++} in low-spin state ($s=0$). In our experiment, the subspectra for the ferrous $s=2$ were not detected in most of the samples taken from the patients. Even if some new branches between portal vein and hepatic artery could be formed, causing a mixed flow of arterial and venous blood, in the case of patients with liver cancer or cirrhosis, it wouldn't result in the disappearance of the subspectrum $s=2$. This change in oxy-Hb of RBC of the patients with cirrhosis and primary liver cancer certainly affect oxygen transport and immune function of the body. On the other hand, no ferritin, haemosiderin or other ferruginous components were detected in the samples from these two kinds of patients.

This implies that neither synthetic disturbance nor denaturation of hemoglobin exist in RBC.

To explore further the intimate relationship between the structure and the immune function of RBC in these patients, the immune function of RBC of these patients were determined by IAHA. Results showed that the immune function of RBC in these patients decreased significantly when compared with that of normal adults.

To sum up, the difference in the hemoglobin and immune function of RBC between patients and normal adults has shed new light on further research into the physical structure of RBC of patients with hepatic diseases. And further research is also required to reveal the intimate relations concerning pathological changes in RBC induced by these two kinds of hepatic diseases.

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