THE EFFECT OF SOME MEDICAL TREATMENTS ON THE RED BLOOD CELLS IN THE PATIENTS WITH THALASSEMIA*

Zhang Xiufang (张秀芳), Shen Linming (沈林明), Bao Hongxia (包红霞), Ding Xiaolan (丁晓岚), Wang Rongxin (王容新)* , Liu Yuanyuan (刘源源), Gao Naifei (高乃飞) and Huang Youwen (黄有文)

(Tsinghua University, Beijing 100084, China.

** 303 Hospital of PLA, Nanning 530021, China)*

(Received October 1991)

ABSTRACT

The Mössbauer spectroscopy and circular dichroism measurements have been used to investigate the effect of some medical treatments on the red blood cells (RBCs) of the patients with HbH disease and β -thalassemia major, respectively. The results indicate that both splenectomy and treatment with myleran are effective to alleviate the symptoms of anemia for some patients, but both of them are different in the effect on the RBCs of the patients. On the basis of the results, a hypothesis on the course of denaturation in hemoglobin of the patients is proposed.

Keywords: β -thalassemia major Mössbauer spectroscopy Circular dichroism HbH

1 INTRODUCTION

At present, common medical treatments for HbH disease and β -thalassemia (Thal.) major are blood transfusion, splenectomy, some medicines and so on. Blood transfusion is expensive, and it leads to deposition of iron in some organs of the patients and iron-overload disease. Splenectomy can increase life of RBCs and alleviate the symptoms for most patients. But there is danger that the patients get some complication, for example, postoperative infection. Medicines such as desferrioxamin usually have side effects[1,2,3]. In the last several years myleran (1,4 dimethyl sulfonyl butane) has been used for treating β -Thal.[4,5]

We have investigated whole RBCs and hemoglobin (Hb) of the patients by Mössbauer spectroscopy (MS) and circular dichroism (CD), respectively. In combination with clinical analysis, the effects of splenectomy and myleran on the RBCs in the patients have been discussed, and a hypothesis on the course

* This work was supported by the Science Foundation of Tsinghua University

of denaturation in the Hb of the patients is proposed.

2 EXPERIMENTS AND RESULTS

The blood samples of the patients with Thal. were collected in Nanning area of Guangxi Provice (see Table 1). The diagnosis of the disease was made by routine blood examination, Hb electrophoresis and gene analysis. The Table 2 shows the changes in the Hb and RBCs of some patients before and after treatments. A fresh blood sample of normal adult was got from the Beijing Red Cross Blood Bank. The above blood samples were preserved with ACD blood-preserving fluid (Anticoagulant-Citrate-Dextrose) at 4°C[6]. They were prepared as the samples for MS and CD measurements very soon (see the text below). The blood sample of umbilical cord of a newborn baby was collected in Tsinghua Hospital, and heparin was used to prevent coagulation.

Table 1

The blood samples from the patients

No.	1	2	3	4	5
Kind	HbH		β -thalassemia		major
Sex*	M	F	M	M	M
Age	46	29	11	14	4
Nationality* *	H	H	H	Z	Z
Treatment* * *	U	S	U	S	M

* M: Male F: Female

* * H: Han Z: Zhuang

* * * U: the untreated S: the splenectomized M: the treated with myleran

Table 2

The changes of Hb and RBCs in the patients due to treatments

No.	Treat-ment	Hb(g/l) before/after	RBCs ($\times 10^{12}$/l) before/after
2	S	75/95	3.61/4.17
4*	S	52/90	2.81/4.38
5* *	M	72/94	2.57/3.70

* The patient can go to school after splenectomy

* * The spleen of the patient reduced by 2 cm and the liver reduced by 1 cm

Table 3

The Mössbauer parameters at 80 K

No.	Compo-nent	IS(±0.02) (mm/s)	QS(±0.04) (mm/s)	W(±0.02) (mm/s)	I (%)
1	*a*	0.23	2.00	0.21	12.0
	b	0.89	2.22	0.38	70.0
	c	0.60	0.68	0.22	18.0
2	*a*	0.28	2.11	0.38	75.0
	b	0.92	2.39	0.28	9.0
	c	0.56	0.80	0.38	16.0
3	*a*	0.22	2.00	0.38	84.6
	c	0.58	0.69	0.20	15.4
4	*a*	0.26	2.09	0.38	85.0
	c	0.52	0.78	0.24	15.0
5	*a*	0.27	2.10	0.38	94.6
	c	0.53	0.76	0.38	5.4

IS is relative to α -Fe at RT

Table 4

The conformations of the Hb in solution

No.	N*	1	2	3	4	5	B* *
Kind of Hb		HbH			HbF		
α -Helix (%)	64	58	54	46	43	48	50
β Structure (%)	8	14	5	16	17	16	17
Random coil (%)	28	28	41	38	40	36	33

* N: The normal adult * * B: The baby

2.1 Mössbauer spectroscopy measurements

The RBCs were separated from the blood samples by centrifugation (3000 r/min, 10 min) and washed three times with physiological saline. The RBCs samples were encapsulated in perspex containers of about 1.6 cm in diameter and thickness of 0.6 cm, and immediately stored in liquid nitrogen until measurements. The above

operations were accomplished at 4 °C, MS measurements were carried out on a constant acceleration Mössbauer spectrometer with a 9.25×10^8 Bq ^{57}Co/Rh source. The samples were maintained at 80 K during measurements. All Mössbauer parameters were obtained by least-square fitting using Lorentzian lines and listed in Table 3. In addition to the components *"a"* corresponding to oxy-Hb and the *"b"* to deoxy-Hb, the third component *"c"* appeared in the spectra of RBCs in all patients.

2.2 Circular dichroism measurements

The washed RBCs were gradually mixed with equal volume of distilled water and kept at 4 °C for about 10 h so that hemolysis of RBCs fully took place. The hemolysate was centrifuged (15000 r/min, 15 min) to obtain Hb solution without RBCs ghosts. The different components of Hb were separated by polyacrylamide gel electrophoresis[7]. The bands corresponding to HbA and HbF were cut off and placed in physiological saline, then we obtained the pure solution of HbA and HbF for CD measurements. The CD measurements were carried out on a J-500C spectropolarimeter, a 10 mm path length cell was used, and the obtained spectra were analyzed by computer program according to the method of Chen *et al.*[8] The conformations of Hb in solution obtained from CD were listed in Table 4.

3 DISCUSSION

Our clinical analysis showed that splenectomy could increase the amount of Hb and RBCs per liter blood for the patients and alleviate the symptoms of the disease. Myleran is also effective for some patients with β -Thal. major (see Table 2). But according to the results form MS and CD, we consider that splenectomy is different from myleran in effects on RBCs in the patients.

Splenectomy is effective, this is only because it removes the destruction of spleen to some RBCs, thus increases the amount of Hb and RBCs in peripheral blood, and partly alleviates the symptoms of anemia[1,2], but it doesn't improve the structure and functions of RBCs in the patients. So the third components in Mössbauer spectra, which are generally considered as a character of this kind of disease, were at same level for the untreated and the splenectomized patients with HbH or β -Thal. major. Furthermore, CD results indicated that the percentage of α -helix in Hb, which is a kind of regular conformation in protein and closely relative to its biological activity[9], reduced from the normal adult, the untreated to the splenectomized patients, but the percentage of random coil, which is considered to be relative to denaturation of protein, increased gradually. The situation of the splenectomized patient is the worst (see Table 4). It is probably because the life-span of the RBCs rose due to splenectomy, then the changes in structure of the Hb continued for more time.

Myleran treatment is different from splenectomy. Table 3 shows that the third

components *"c"* in Mössbauer spectra of the RBCs of the patients treated by myleran obviously reduced in comparison with the untreated patients. CD results also showed that α -helix conformation in HbF gradually increased from the splenectomized, the untreated, the treated by myleran to normal baby, which indicated that myleran can improve structure of the RBCs in some patients with β -Thal. major. Maybe myleran could affect DNA and/or RNA in yong RBCs and alter some of them by inserting between the nucleotide base pairs, thus stimulate the expression of γ -globin gene and thus improve the production of γ -chains and the formation of HbF when γ -chains are united with α -chains, therefore the denatured α -chain globin decreased in amount (the excessive α -chains are unstable and easy to be denatured in β -Thal.).

According to all results above, we could say that splenectomy, though quite efficent for some patients, especially those with HbH disease, is not a good treatment that solves the problems thoroughly. However, myleran maybe is one that solves the problems more efficiently and more reasonably for some patients with β -Thal. major.

Bauminger *et al.* considered that the third components in Mössbauer spectra of the RBCs in Thal. might be a ferritin-like iron compound, and some of the ferritin-like iron was in an insoluble or membrane-bound form[10]. Because the denaturation of Hb in RBCs of the patients must be a complex course, so we prefer to consider the third component *"c"* is a superposition of denatured Hb and those ferritin-like compound. Some of them deposit on the membranes of RBCs, others are present in solution in RBCs. We don't find the bands corresponding to the component *"c"* in electrophoresis atlas. It may be due to that the ferritin-like compound in solution in RBCs has too high molecular weight and too large volume to migrate in electrophoresis, remaining at the start or nearby. Comparing MS with CD results, it is found that both are consistent with each other in changing tendancy, which indicate that each of them could give reliable information about structure and functions of RBCs alone. There are some differences in Mössbauer parameters of the third components between Bauminger's and our results. It might be due to the variation of patients in different localities.

According to our study, we propose a hypothesis on the course of denaturation in Hb of the patients: first, due to some reasons, such as unbalance of α - and β -chains in RBCs, some of normal Hb changes in conformation, for example, the percentage of the regular α -helix and β -structure reduces, that of the random coil increases. Then the Hb decomposes into apo-Hb chains and Fe^{2+}/Fe^{3+} ions. These ions quick form FeOOH molecules, further the molecules gather and form cores of about 7nm in diameter. Apo-Hb chains and denatured Hb precipitate on the cores and form a protein shell. The cores, with and without the protein shell, the denatured Hb and apo-Hb gradually deposit on the membranes of RBCs, which induce the changes in physical and chemical properties of RBCs' membranes such as mobility. Finally,

hemolysis takes place.

This work is preliminary. Further investigations will be carried out.

ACKNOWLEDGEMENTS

The authors would like to thank Prof. I.Ortalli and Dr. G. Pedrazzi, Institute of Physical Sciences, University of Parma, Italy, and Prof. E.Preoteasa, Institute of Atomic Physics, Romania, for their assistance and helpful discussion.

REFERENCES

[1] Qin Wenbin ed. Hemoglobin diseases. Beijing: The People's Health Publication Press, 1984.

[2] Ao Zhongfang, Liu Baojue, Chen Yueshu *et al.* ed. Modern treatments of hemopathy. Nanjing: Jiangsu Publishing House of Science and Technology, 1990.

[3] Xu Liang. *Chinese Journal of Hematology,* 1985, 6:504.

[4] Liu Depei, Liang Chihchuan, Ao Zaohui *et al. American Journal of Hematology,* 1990, 33:50.

[5] Liu Depei, Liang Zhiquan, Liu Lijuan *et al. Journal of Chinese Medicine,* 1988, 68:214.

[6] Bo Naiqing ed. Preservation of blood. Shanghai Publishing House of Science and Technology, 1984:124.

[7] Zhang Longxiang, Zhang Tingfang, Li Lingyuan *et al* ed. Biochemical experimental methods and techniques, Beijing: The Higher Education Publication Press, 1984: 94.

[8] Chen Y H, Yang J T, Chau K H *et al. Biochemistry,* 1974, 13:3350.

[9] Lu Zixian, Cui Tao ed. The applications of optical dispersion and circular dichroism in molecular biology. Beijing: Science Press, 1983.

[10] Bauminger E R, Ofer S. Proc int conf on the applications of the Mössbauer effect. Jaipur, 1981. New Delhi: National Science Academy, Indian, 1982: 61.