Scripta Scientifica Medica, vol. 30 (1998), pp. 83-87

STUDY OF HEAT SHOCK PROTEINS IN THALASSAEMIC PATIENTS

D. Kovachev, E. Kolettas*, S. Bonanou-Tzedaki*

*Department of Biology, Medical University of Varna, Varna, Bulgaria and *Laboratory of Biochemistry, School of Medicine, University of Thessaly, Larissa, Greece*

When cells are exposed to a variety of stimuli, there is increased expression of stress or heat shock proteins, a major representative of which is hsp70. The objective of the present work was to investigate the endogenous expression of hsp70 in peripheral blood mononuclear cells and erythrocytes of patients suffering from b-thalassaemia and to correlate hsp70 levels with patients' antioxidant status. Blood samples were obtained from thalassaemia major patients aged 16-24 years. Hsp70 was identified with a mouse monoclonal anti-human hsp70 antibodies using the Western blot procedure. The total antioxidant status was determined by means of a commercial kit of RANDOX. It was established that: i) Hsp70 levels were low in mononuclear cells, ii) Hsp70 was not appreciably induced by incubation at 43°C, iii) in erythrocytes, however, there was a marked endogenous expression of hsp70 - thalassaemics express more hsp70 than control subjects, iv) the antioxidant status of thalassaemics was by about 20 % less than the control one. The increased endogenous hsp70 in thalassaemic erythrocytes was consistent with the hypothesis that the elevated levels of denaturated globins induced the expression of stress proteins during erythopoiesis. We are currently investigating whether there is a correlation between the severity of the clinical symptoms and hsp70 levels.

Key-words: Beta-thalassaemia, heat shock proteins, hsp70, antioxidant status, haemoglobin, erythrocytes

Homozygous b-thalassaemia is characterized by absent or reduced production of haemoglobin b-chains. The haemoglobin a-chains which remain in excess aggregate in insoluble homotetramers which precipitate intracellularly leading to premature red cell destruction (haemolytic anaemia). Regular red blood cell transfusions combat the anaemia but result in accumulation of tissue iron which if untreated is fatal in the second decade of life. The development of iron chelation therapy has led to dramatic improvements in survival and quality of life. Iron overload is, however, still a major concern. Under physiological conditions ferric ions are bound to proteins (transferrin and ferritin) and are not available to catalyse the conversion of molecular oxygen to

Address for correspondence:
D. Kovachev, Dept. of Biology, Medical University, 55 Marin Drinov St, BG-9002 Varna, BULGARIA
E-mail: biologie@asclep.muvar.acad.bg

reactive oxygen species (ROS), e. g., hydroxyl ions, superoxide and hydrogen peroxide. Under conditions of iron overload such as thalassaemia, however, there is an increase of low-molecular weight iron which promotes peroxidative damage and cell injury (4,5) and causes a decreased total antioxidant status of the patient (10).

When cells are exposed to a variety of stimuli (physiopathologic conditions and environmental assaults, including oxidative stress), there is an increased expression of certain classes of proteins, the so-called stress or heat shock proteins, a major representative of which is hsp70, which protect cellular proteins from denaturation (8). The human hsp70 family includes 11 genes expressed either constitutively (hsp70c), or after induction by various agents (hsp70i). Although the expression of hsp70 has been studied in various haemopoietic cells, there is no information about its expression in the white and red blood cells of patients suffering from iron overload. We reasoned that the increased iron levels found in b-thalassaemia would result in increased ROS formation and expression of hsp70. This stress response might confer considerable protection to red blood cells because it could decrease the effective concentration of free haemoglobin a-chains through association with hsp70, and to white blood cells which may become more able to withstand the damaging effects of ROS.

The objective of the present study was to investigate the endogenous expression of hsp70 in peripheral blood mononuclear cells and erythrocytes of patients suffering from b-thalassaemia and to correlate hsp70 levels with patients' antioxidant status.

MATERIAL AND METHODS

Blood samples were obtained from patients aged 16-24 years and suffering from *thalassaemia major* who were on Desferrioxamine treatment just before blood transfusion. Samples from age-matched blood donors were used as normal controls. Peripheral blood mononuclear cells and erythrocytes were obtained by density centrifugation of whole blood-EDTA samples through Ficoll. The cells were extensively washed with HANKS and then resuspended in RPMI 1640 containing 10 % fetal calf serum, 1 % glutamine, and penicillin-streptomycin. The cells were preincubated at 37°C for 2 hours, heat-shocked at 43°C for 2 hours, and allowed to recover at 37°C for 1 hour. After pelleting and washing, they were lysed in triple detergent buffer, boiled in x2 Laemmli sample buffer (6) and the proteins were separated by SDS-PAGE and transferred to nitrocellulose filters. Hsp70 was identified by probing with a mouse monoclonal anti-human hsp70 antibody (Sigma H5147) which detects both constitutive and inducible forms. Colour was developed after the addi-

tion of a reporter anti-mouse IgG-HPR antibody using a horseradish peroxidase chromogen solution. Human hsp70 (Sigma) was used as positive control. Patients' total antioxidant status was determined in serum samples by following the absorption of the radical cation ABTS (10) using a kit by RANDOX. Other clinical chemical analyses were performed at the Larissa Hospital Diagnostic Laboratories, Larissa.

RESULTS AND DISCUSSION

Fig. 1 shows the expression of hsp70 in the white and red blood cells of eight thalassaemic and six control subjects. They are representative of the Western blots obtained with several different samples.

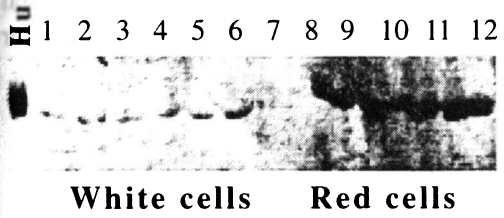

Fig. 1. *Expression of hsp70 in white and red blood cells of normal and thalassaemic subjects*

We find out that in peripheral blood mononuclear cells: i) endogenous hsp70 levels, ii) hsp70 is not appreciably induced by incubation at 43°C, iii) thalassaemic cells express even less hsp70 than control ones when equal amounts of proteins are loaded, iv) RNA extracted from mononuclear cells of normal and thalassaemic subjects also give a very faint signal for hsp70m RNA on Northern blots (results not shown). In erythrocytes, however, there is a marked endogenous expression of hsp70. Thalassaemics express more hsp70 than control subjects.

The total antioxidant status of the serum of thalassaemic subjects is by approximately 20 % less than that of normal subjects (Table 1).

Table 1

Total antioxidant status of control and thalassaemic subjects
(Each value represents the mean ± SD)

Subjects		mM / L
Control	(n=8)	1,49 ± 0,17
Thalassaemic	(n=8)	1,21 ± 0,16

($p < 0,1$ by Student's *t*-test).

The mean values of selected haematologic indices for the thalassaemic and control individuals are the following (Table 2):

Table 2

Haematologic parameters of control and thalassaemic subjects

Parameters	T	C
g % haemoglobin	10,2	14,3
g/ml iron	260,0	110,0
ng/ml ferritin	1,890	82

In haemopoietic cells the expression of hsp70 has been studied in chicken, rabbit, murine and human cells and in various leukaemic cell lines (2,3). It has been found that monocytes and granulocytes, but not lymphocytes, constitutively express hsp70 (2). Activation of either lymphocytes or monocytes by cytokines or exposure to oxidative stress leads to induction or increased expression of hsp70 (9). Its expression is induced during erythroid cell differentiation (1) and increases further by heat shock.

Our results with the mononuclear cells (which consist of approximately 85 % lymphocytes and 15 % monocytes) agree with the findings in the literature available about the low levels of endogenous hsp70 expression. The fact that we could not find any increase of hsp70 levels in the thalassaemic white cells was unexpected, given increased patients' iron and ferritin levels and the decreased serum antioxidant capacity. It suggests that the presumed oxidative stress either does not affect mononuclear cells, or is compensated by other mechanisms. The increased endogenous hsp70 in thalassaemic erythrocytess, however, is consistent with the hypothesis that the elevated levels of denaturated globins induce the expression of stress proteins during erythropoiesis. Finally, this 20 % decrease of the antioxidant status of the thalassaemic patients is comparable to the 14 % one reported in another study (7).

We are currently investigating whether there is a correlation between the severity of the clinical symptoms and hsp70 levels.

Acknowledgements:

We thank the University of Thessaly Research Committee for financial assistance and Drs Halkias and Vagenas for providing the blood samples.

REFERENCES

1. Bei-Paraskevopoulou, T.A., S. A.Bonanou-Tzedaki, H. V. Arnstein. *Biochem. Soc. Trans.*, **14**, 1986, 973-977.- 2. Clerget, M., B. S. Polla. *Proc. Natl. Acad. Sci. USA*, **87**, 1990, 1081-1090.-3. Fincato, G., et al. *Blood*, **77**, 1991, 579-588.- 4. Halliwell, B., J. M. C. Gutteridge. *Meth. Enzymol.*, **186**, 1990, 1-8.- 5. Hershko, C., G. Graham, G. W. Bates, E. A. Rachmilewitz. *Brit. J. Haematol.*, **40**, 1978, 255-261.- 6. Laemmli, U. K. *Nature*, **277**, 1990, 680-674.- 7. Livrea, M. A., et al. *Blood*, **88**, 1996, 3608-3614.- 8. Morimoto, R., A. Tissieres, C. Georgopoulos, Eds. The Biology of Heat Shock Proteins, New York, Cold Spring Harbor Laboratory, 1990.- 9. O'Farrell, F. J., B. M. Hannigan, Y. A. Barnett. *Biochem. Soc. Trans.*, **24**, 1996, 718-722.- 10. Rice-Evans, C. N. J. Miller. *Meth. Enzymol.*, **234**, 1994, 279-290.

Изследване на топлинно-шокови протеини при болни с таласемия

Д. Ковачев, Е. Колетас*, С. Бонану-Цедаки*

*Катедра по биология, Медицински Университет – Варна, България и *Катедра по биохимия, Медицински факултет, Тесалийски Университет - Лариса, Гърция*

Резюме: Когато клетката се подложи на редица външни и вътрешни въздействия рязко се увеличава експресията на т.н. стресови или топлинно-шокови протеини (ТШП). Счита се, че главна роля тук играят ТШП-70. Цел на настоящото изследване бе да се докаже ендогенната поява на ТШП-70 в еритроцити и мононуклеарни клетки на болни с b-таласемия. Проби от кръвта на болни на възраст от 16 до 24 г. бяха получени от градската болница в Лариса, Гърция. ТШП-70 бяха идентифицирани чрез моноклонални антитела. Антиоксидантният статус на болните бе определен с помощта на кит от фирмата RANDOX. Получените резултати показват, че: 1) нивото на ТШП-70 в мононуклеарните клетки е много ниско; 2) допълнителният топлинен шок на клетките при 43°C не повишава значимо изходното ниво; 3) при еритроцитите се наблюдава значителна експресия на ТШП-70 в сравнение с контролата, 4) антиоксидантният статус на болните е с около 20 % по-нисък в сравнение с контролата. Повишеното ниво на ТШП-70 в таласемични червени кръвни клетки е в съзвучие с хипотезата, че големите количества денатуриран глобин може да индуцира експресията на стресови протеини по време на еритропоезата. В ход са сравнителни изследвания между тежестта на клиничната картина и нивата на ТШП-70 при болни с таласемия.