VOLUME 2, 2011

PROTECTIVE FUNCTION OF CERULOPLASMIN AND ITS ACTION ON ERYTHROCYTES IN MEN WITH PROSTATE TUMORS

N. KOTRIKADZE, M. ZIBZIBADZE, L. RAMISHVILI, M. ALIBEGASHVILI., T. CHIGOGIDZE, L. MANAGADZE

Javakhishvili State University, Tbilisi, Georgia

ABSTRACT

Purpose: To investigate the protective action of Ceruloplasmin (Cp) on the lysis of erythrocytes in men with prostate tumors.

Material and Methods: The blood erythrocytes of the patients with benign hyperplasia of prostate (BHP) and prostate adenocarcinoma (CaP) were studied. Patients at the age 60-75 with early stages of a cancer have been investigated. The control group was consisted of apparently healthy males with the compatible age. n=15 for each group. The clinical stage of the disease was diagnosed by means of rectal, histological and echographic examination of the prostate gland. Photometric methods were applied to register lysis dynamics in order to test out the protective action of Cp.

Results: The nonspecific protective function of Cp preparation on BHP and CaP erythrocytes as well as on the control group erythrocytes was revealed. CaP erythrocytes have shown more sensitivity to the lysis provocative factor than BHP and the control group erythrocytes, that was presumably attributed to the structural and functional changes of the erythrocytes developed in the presence of malignant tumor.

UDC CODE & KEYWORDS

■ UDC: 616-006 ■ Ceruloplasmin ■ Erythrocytes ■ Prostate Tumor ■ Protective Action ■ Lysis Dynamics.

INTRODUCTION

Ceruloplasmin (Cp) is the antioxidant of blood plasma (Sato T, Orikaza M and Hasegawa H. 2004). It is known, that the specific receptors for Cp are localised on the surface of many kinds of non-hepatic cells (Hellman, N.E and Gitlin, J.D, 2002; Sasina L.K, at all. 2000). Such cells are monocytes, lymphocytes, erythrocytes, endothelial cells of aorta and heart, etc. It means that Cp action does not apply only blood plasma but is directed to target-cells as well. One of the functions of this enzyme is assisting in copper transport and expediting its delivery to tissues (Cabrera A, at all, 2008). Presumably, when Cp binds to the target cell it may also reveal the antioxidant activity. When this supposition is considered, the protective action of Cp on erythrocytes as one of the important links of organism defence system (Zhang Lan, at all, 2007) is of significant interest.

As the antioxidant Cp is an inhibitor of free-radical reactions in blood plasma (Hellman, N.E and Gitlin, J.D, 2002). It regulates some characteristics of hemopoiesis and works as a stimulating agent on the functioning of immune system in vitro (Banha J. at all 2008; Vashenko V. I and Vashenko T. N. 2006). At the same time Cp also protects erythrocytes from the lysis induced by the transition metal ions (Parfenov E. A., Zaikov G. E., 2000). It's considered that not only antioxidant and ferroxidase properties of Cp play a decisive role in protection of erythrocytes, the interaction of the enzyme with it's receptor also is of great importance for its defensive action.

The goal of the study was to investigate the influence of exogenous Cp on the oxidative stress promoted by the Fe^{2+} and accordingly, on the lysis of erythrocytes induced by the transition metal.

Materials and methods

The erythrocytes of patients with BHP and CaP, served as the material for the studies. The patients in the age of 60-75 were studied at primary revealing of a tumor. The control group was consisted of apparently healthy males with the appropriate age. n=15 for each group. The research data were supplied and the clinical stage of disease was diagnosed by the Georgian National Institute of Urology, by means of rectal, histological, and echographic examination of the prostate gland.

Erythrocytes were isolated from 10 ml of vein blood, added with 1 ml of heparin (5000ME). The blood was centrifuged at 3000 rpm, for 10 min. Erythrocytes ($4 \cdot 10^9$ cell) were washed three times with isotonic buffer containing 10mM Tris-HCl and 155 mM NaCl, pH=7,4.

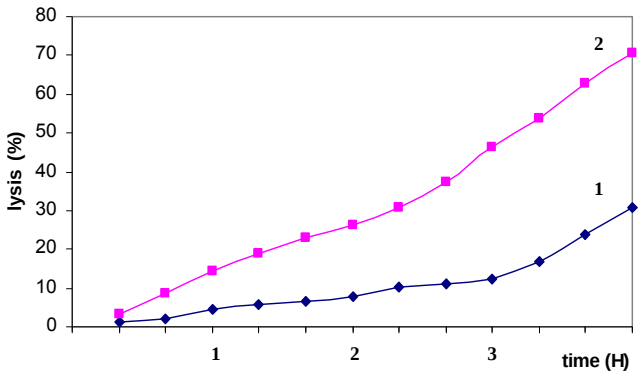
Erythrocytes were incubated with Cp preparation (Biopharma, A_{610}/A_{280}=0,041) or without it. Incubation was carried out at 37ºC, 1 h, on the water bath, in the presence of Ca^{2+}. Lysis was induced by Fe^{+2} (in form of Mohr's salt), erythrocytes were diluted in ratio 1:1000 and the dependence of lysis intensity on lysis duration was studied.

The percentage of lysed erythrocytes was calculated by the indices of optical density registered during the lysis. Registration of lysis dinamics was performed photometrically at λ=670 nm in definite intervals of time (Goryunov A.S, at all, 2000).

Results

The studies have demonstrated that in all studied groups, the erythrocytes, incubated in the presence of Cp before lysis, were less affected by the lysis inducing factor then the erythrocytes incubated in the absence of Cp, as it was expressed by lower percentage indices (Fig.1.1; 2.1; 3.1).

Figure 1: Lysis of control group erythrocytes incubated in the presence of exogenous Cp (1) or without it (2).

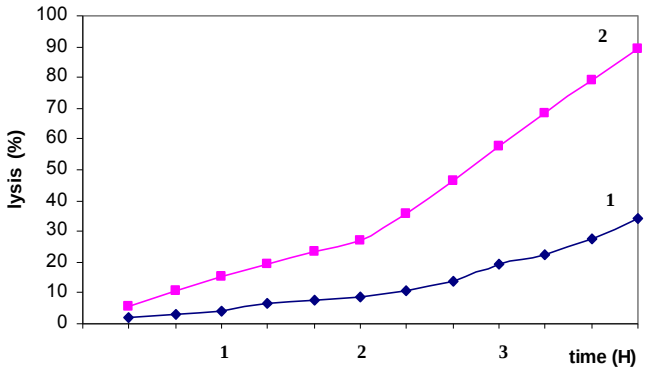


(1) Cp = 0,03μM, Ca^{2+} = 1mM, Fe^{2+} = 0,5mM.

(2) Ca^{2+} = 1mM, Fe^{2+} = 0,5mM.

Source: Author

Figure 2: Lysis of BHP erythrocytes incubated in the presence of exogenous Cp (1) or without it (2).

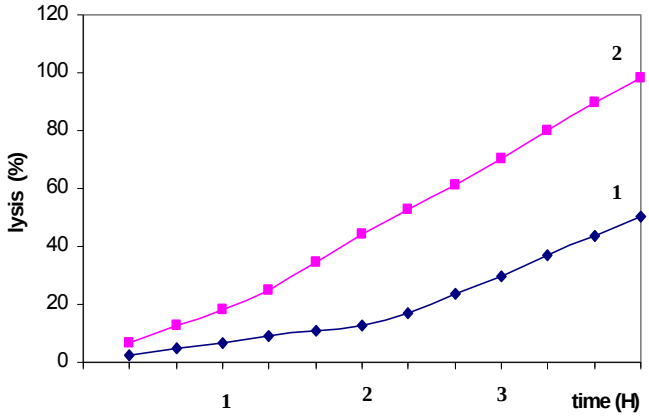


(1) Cp = 0,03μM, Ca^{2+} = 1mM, Fe^{2+} = 0,5mM.

(2) Ca^{2+} = 1mM, Fe^{2+} = 0,5mM.

Source: Author

Figure 3: Lysis of CaP erythrocytes incubated in the presence of exogenous Cp (1) or without it (2).



(1) Cp = 0,03μM, Ca^{2+} = 1mM, Fe^{2+} = 0,5mM.

(2) Ca^{2+} = 1mM, Fe^{2+} = 0,5mM.

Source: Author

After incubation with exogenous Cp the cell lysis of both apparently healthy men (Fig.1.1) and those with prostate tumors (Fig. 2.1; 3.1) was going similarly in the first two hours. After this period the lysis dynamics remained similar within the control group (Fig. 1.1) and in BPH (Fig 2.1), while the lysis intensity of CaP erythrocytes was prominently increasing (Fig. 3.1). As the erythrocytes, which were not incubated with Cp before the lysis, they were showing more sensitivity against the lysis initiating factor in all studied groups and have been lysed more intensively during the same period (Fig.1.2; 2.2; 3.2). In the absence of exogenous Cp the lysis intensity of CaP erythrocytes proved to be especially high (Fig 3.2), already after the first hour of the lysis.

The studies have also demonstrated that after incubation with Cp, the duration of the first phase of the lysis was increased in all studied groups. In the case of control group and BPH erythrocytes, the first phase duration was ~2,5-3h in the presence of Cp (Fig.1.1; 2.1) and only 2h when erythrocytes were incubated in the absence of Cp (Fig.1.2; 2.2). In terms of CaP erythrocytes, the first phase of the lysis was less expressed, compared with control group and BPH erythrocytes especially without Cp action, indicated once more that, these erythrocytes have demonstrated a lesser degree of resistance against lysis process (Fig.3.2).

Discussion

Current literature reveals that lysis of erythrocytes occurs in two phases. The first is a latent period, when the large amount of cells reveals certain resistance to the lysis inducing factor. This phase is followed by more intense phase, when the remaining erythrocytes lyse (Burak Çimen M.Y. 2008). It is established that the first phase of lysis is prolonged until erythrocyte's intracellular antioxidant system carries out its function properly and the intact structure of the erythrocyte's membrane is retained.

It was suggested that the sharply elevated degree of the lysis in CaP erythrocytes may be due to their structural and functional changes provoked by the profound physiological changes that take place in the presence of malignant tumor (Stepovaya E.A. at all, 2003]. Furthermore, it is evident, that in the case of control group and BPH the intensity of lysis is greatly reduced in the presence of Cp. While if the CaP erythrocytes are incubated with Cp, irreversible lysis begins after two hours. The process can be somehow suppressed by Cp, but this slowing down action is not carried out effectively.

In all studied groups erythrocytes incubated in the presence of Cp before the initiation of the lysis, were less affected by the lysis than when they were incubated without Cp. It means that after Cp binds to the specific receptor on the membrane of erythrocyte it also protects the cell membrane from the influence of the lysis inducing factor. The direct "provoking factor" of the lysis under the given conditions is generation of reactive oxygen radicals. They in turn interact with erythrocyte membrane inducing lipid peroxidation with following destruction of the cell membrane (Burak Çimen M.Y. 2008).

As to the Cp protective action, it must be attributed to antioxidant and ferroxidase activities of the enzyme and these synergies may be realised through the binding of Cp with its receptor. It is possible that the binding of Cp with its membrane receptor facilitates the interaction between Cp and Fe2+, or superoxide. There also may be another mechanism that can explain this process: the electrons, which are necessary to oxides Fe^{2+} to Fe^{3+} or to make superoxide harmless by redoxenzymatic reaction, Cp can accept directly from the definite structural component of erythrocyte membrane. In other words, from any reducing factor that is located in close proximity to the specific receptor. In this case, however, the tight binding with the membrane is apparently critical in Cp protective action.

It should be mentioned that there is enough number of studies that have described Cp as potent catalyst of low-density lipoprotein (LDL) oxidation *in vitro* (Fox P. L, at all, 2000; Makedou K. G, at all, 2009). The prooxidant activity of Cp requires an intact structure, and a single ,,prooxidant" copper atom at the surface of the protein. There is a suggestion, that oxidative stress changes ceruloplasmin from a protective to a vasculopathic factor [Shukla N, at all, 2006]. Althoug under the conditions we had used protective action of Cp was appreciable.

Conclusion

In conclusion, Cp protects the erythrocytes of patients with prostate tumors against the oxidative stress promoted by Fe^{2+} and accordingly from the lysis that is induced by metal ions. Furthermore, the results indicate that as antioxidant and ferroxidase Cp is capable to reduce the oxidative stress that develops during the tumor growth, and thus, it is capable of diminishing the toxic influence of tumor either in BPH or CaP.

REFERENCES

1. Banha J., Marques L., Oliveira R., Martins Mde F., Paixão E., Pereira D., Malhó R., Penque D., Costa L.Ceruloplasmin expression by human peripheral blood lymphocytes: a new link between immunity and iron metabolism.Free Radic Biol Med. 2008. v. 44. 483-492.
2. Burak Çimen M.Y.Free radical metabolism in human erythrocytes.Clinica Chimica Acta. 2008. v. 390. N. 1-2. 1-11.
3. Cabrera A., Alonzo, E., Sauble E., Chu Y-L., Linder M. C., Sato D. S., Mason A. Z.Copper binding components of plasma and cytoplasm, copper turnover and excretion, as determined in the mouse with large doses of 65Cu. Biometals. 2008. v. 21. 525–543.
4. Fox P. L., Mazumder B., Ehrenwald E., Mukhopadhyay C. K.Ceruloplasmin and cardiovascular disease. Free Radical Biology and Medicine. 2000.V.28. N.12. 1735-1744.
5. Goryunov A.S., Sukhanova G.A., Borisova A.G. Rozhkov S.P. Trout Erythrocytes Membranes. Thermoresistivity and Thermally induced structural transitions. Jour.Therm.Anal. Calor. 2000. v. 62. 29-37.
6. Hellman, N.E., Gitlin, J.D.Ceruloplasmin metabolism and function. *Annu. Rev. Nutr.* 2002.N. **22.** 439–458.
7. Makedou K. G., Mikhailidis D. P., Makedou A., Iliadis S., Kourtis A., Vavatsi-Christaki N., Papageorgiou G. E. Lipid Profile, Low-Density Lipoprotein Oxidation and Ceruloplasmin in the Progeny of Families With a Positive History of Cardiovascular Diseases and/or Hyperlipidemia. Angiology. 2009. N.60. 455 - 461.
8. Parfenov E. A., Zaikov G. E. in "Biotic Type Antioxidants: The Prospective Search Area for Novel Chemical Drugs."VSP, 2000. 560p.
9. N.E., Gaitskhoki V.S.Isolation and partial characterization of cDNA clone of human ceruloplasmin receptor.Bull Exp Biol Med. 2000.v.129.491-495.
10. Sato T., Orikaza M., Hasegawa H. Ceruloplasmin as an antioxidant in the serum: ability to protect glutamine synthetase under hydroxyradical generating conditions. Seikagaku. 2004.v.76. N.8. P.798.
11. Shukla N., Maher J., Masters J., Angelini G., Jeremy J. Does oxidative stress change ceruloplasmin from a protective to a vasculopathic factor? Atherosclerosis. 2006.v.187. Is. 2. 238-250.
12. Stepovaya E.A., Novitskii1V.V., Ryazantseva N.V., Gol'dberg V.E., Tkachenko S.B., Kolosova M.V.Structure and Properties of Lipid Bilayer of Erythrocyte Membranes in Patients with Malignant Tumors.Bull.Exp.Biol.Med. 2003. v.136. N.5.490-493.
13. Vashenko V. I.,Vashenko T. N. Ceruloplasmin — from Metalloprotein to Drug.Psychopharmacology and Biological Narcology. 2006. v. 6. N. 3. 1254- 1269.
14. Zhang Lan., Zhang Bai-gen., Zhang Ji-wei., Zhang Hao. Immune function of erythrocytes in patients with chronic venous insufficiency of the lower extremities.Chin. Med. Jour. 2007. v.120. 2224-2228.