

Some components of oxidative-antioxidative system in human blood plasma and serum

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Abstract

Comparison of the concentrations and activities of components in the oxidative-antioxidative system between blood plasma and serum. Blood plasma and serum samples were obtained from 38 healthy adults to evaluate malondialdehyde concentration, the total antioxidative capacity, superoxide dismutase activity, protein and non-protein sulphhydryl groups, ascorbate, haemoglobin, methaemoglobin and protein. Blood plasma shows higher activity of superoxide dismutase, as well as higher concentrations of low-molecular sulphhydryl groups and ascorbate, when compared to those in blood serum. The total plasma antioxidative capacity is also higher than that assessed in blood serum. Processes of blood coagulation and blood clot retraction lead to antioxidant consumption. The evaluation of oxidative-antioxidative system for diagnostic purposes should be performed in blood plasma.

Key words: plasma, serum, oxidative - antioxidative system.

Introduction

Plasma is a natural environment for blood morphological components. Serum is formed *in vitro* as a result of generation and retraction of either arterial or venous blood clot, creating its environment. Serum differs from plasma by the lack of I, II, V, VIII and XIII coagulation factors, as well as in higher concentrations of components, released from blood platelets, such as β -

thrombomodulin and platelet factor 4 [1, 2]. Blood platelets and erythrocytes in particular, contain numerous chemical compounds and enzymes, which are involved in oxidative - antioxidative processes [3, 4].

The aim of the study was to compare the concentration and activity of some components of the oxidative - antioxidative system in plasma and serum, obtained *in vitro* from blood of healthy volunteers.

Material and methods

Blood samples for the studies were collected from ulnar veins in 38 healthy adults, including 34 men and 4 women, aged from 25 to 30 years. The blood was collected into 0,1 mol/l sodium citrate (9:1 v/v) to polypropylene tubes and to glass ones without anticoagulant. Blood, collected into sodium citrate, was centrifuged (2700 x g; 30 minutes; 20C) to obtain plasma. Blood, collected without anticoagulant, was incubated for 1 hour at 37°C and then centrifuged in the same conditions as described above, to obtain serum. The following were evaluated in blood plasma and serum: malondialdehyde concentration [5], the total antioxidative capacity [6], superoxide dismutase activity [7], protein and non-protein sulphhydryl groups [8], ascorbate [9], haemoglobin [10], methaemoglobin [11] and protein [12]. The results obtained in plasma were multiplied by 1.25, due to dilution of the collected blood in citrate [13]. The obtained results were submitted to statistical analysis with Student's 't' test, accepting $p < 0.05$ as significant.

Results

Malondialdehyde concentration is higher in plasma than in blood serum (Table 1). The total plasma antioxidative capacity, superoxide dismutase activity, as well as concentrations of most antioxidative system components are also higher in plasma, than those in blood serum. Only the concentration of protein sul-

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Table 1. Some components of the oxidative - antioxidative system in blood plasma and serum.

Component	Plasma	Serum	p
	(1)	(2)	1 vs. 2
Malondialdehyde, $\mu\text{mol/dl}$	59.8 \pm 18.2	47.6 \pm 13.6	0.0014
Total antioxidative capacity, $\mu\text{mol/dl}$	175.3 \pm 34.3	128.2 \pm 33.4	0.0001
Superoxide dismutase, j/dl	378.1 \pm 118.0	276.3 \pm 113.2	0.0003
Protein SH-groups, $\mu\text{mol/dl}$	225.6 \pm 21.2	219.6 \pm 14.5	0.1541
Non-protein SH-groups, $\mu\text{mol/dl}$	34.2 \pm 3.9	28.4 \pm 2.8	0.0001
Ascorbate, $\mu\text{mol/dl}$	2.04 \pm 0.71	1.6 \pm 0.57	0.0039

Table 2. Haemoglobin, methaemoglobin and protein concentrations in blood plasma and serum.

Component	Plasma	Serum	p
	(1)	(2)	1 vs. 2
Haemoglobin, mg/dl	2.8 \pm 0.3	27.1 \pm 3.6	0.0001
Methaemoglobin, $\mu\text{g/dl}$	42.3 \pm 5.8	384.6 \pm 52.8	0.0001
Protein, g/dl	7.5 \pm 0.6	7.1 \pm 0.5	0.0023

phidryl groups in plasma is comparable to that, assessed in blood serum. Concentrations of haemoglobin and methaemoglobin are markedly lower, whereas protein concentrations is higher in plasma than those in blood serum (Table 2)

Discussion

The total antioxidative capacity, and some of its components, such as superoxide dismutase activity, as well as the concentrations of non-protein sulphhydryl groups and ascorbate, is higher in plasma, when compared to respective values in blood serum. It indicates their consumption in the processes, accompanying clot formation. Lower serum concentration of malondialdehyde can probably be the result of its non-covalent binding to low-molecular sulphhydryl compounds. Plasma haemoglobin concentration, lower than its concentration in serum, gives evidence of increased permeability of erythrocyte cell membranes after blood extravasation. Bivalent iron in haemoglobin takes part in reductive processes that leads to haemoglobin conversion to methaemoglobin. Hypoxia and acidosis are developed with the time, following thrombus formation in arteries or veins, which results in increased permeability of cell membranes in entrapped erythrocytes and other morphological elements. Enzymes, which are present in erythrocytes in big quantities, such as acidic phosphatase, cathepsin D, aminotransferase, some enzymes of glycolytic cycle and many antioxidative enzymes, as well as reduced glutathione, are released into serum [14, 15, 16, 17, 18]. Due to extensive differences between blood plasma and serum, the evaluation of oxidative - antioxidative

system for diagnostic purposes should be performed in blood plasma.

References

1. Triplett D.A.: Haemostasis. Igaku-Shoin Ltd., New York, Tokyo, 1985, 6-9.
2. Zieliński T, Wachowicz B. Secretory process in blood platelets. *Post Bioch*, 2003; 49: 175-93.
3. Brüne B, Von Appen F, Ullrich V. Oxidative stress in platelets. In: Sies H, editor. *Oxidative stress. Oxidants and antioxidants*. San Diego: Acad Press, 1991; 421-43.
4. Stocker R, Frei B. Endogenous antioxidant defence in human blood plasma. In: Sies H, editor. *Oxidative stress. Oxidants and antioxidants*. San Diego: Acad Press, 1991; 213-43.
5. Esterbauer H, Lang J, Zdravec S, Slater TF. Detection of malonaldehyde by high-performance liquid chromatography. *Meth Enzymol*, 1984; 105: 319-28.
6. Miller NJ, Rice-Evans C, Davies MJ, Gopinathan V, Milner A. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clin Sci*, 1993; 34: 407-12.
7. Sykes JA, McCormac FX, O'Brien TJ. Preliminary study of the superoxide dismutase content of some human tumours. *Canc Res*, 1978; 38: 2759-62.
8. Ellman GL. Tissue sulphhydryl groups. *Arch Biochem Biophys*, 1959; 82: 70-7.
9. Kyaw A. A simple colorimetric method for ascorbic acid determination in blood plasma. *Clin Chim Acta*, 1978; 86: 153-7.
10. Beau A. Method for hemoglobin determination in serum and urine. *J Clin Pathol*, 1962; 32: 111-2.
11. Elvelyn KA, Malloy HT. Microdetermination of oxy-hemoglobin, methemoglobin, and sulfhemoglobin in a single sample of blood. *J Biol Chem*, 1938; 126: 655-62.
12. Gornall AC, Bardawill CJ, David HM. Determination of plasma proteins by means of the biuret reaction. *J Biol Chem*, 1949; 177: 751-66.
13. Shinowara GY. Spectrophotometric studies on blood serum and plasma. The physical determination of hemoglobin and bilirubin. *Am J Clin Pathol*, 1954; 24: 696-707.
14. Caraway WT. Chemical and diagnostic specificity of laboratory tests. *Am J Clin Pathol*, 1962; 37: 445-64.
15. Greczaniuk A, Roszkowska-Jakimiec W, Gacko M, Worowska A. Determination of cathepsin D activity in blood plasma using hydrochloric acid denatured haemoglobin. *Diagn Lab*, 2000; 36: 97-101.
16. Loegering DJ, Carr FK, Saba TM. Cathepsin clearance from the circulation and reticuloendothelial function. *Exp Molec Pathol*, 1977; 27: 277-83.
17. Richterich R, Colombo JP, Weber H. Ultrametoden in klein klinische laboratorium. VII. Bestimmung der sauren prostata-phosphatase. *Schweiz Med Wschr*, 1962; 92: 1496-1500.
18. Solbach HG, Englhardt A, Merten R. Unterschiede der enzymativitäten in serum und plasma und ihre bedeutung für die klinische enzymdiagnostik. *Klin Wschr*, 1962; 40: 1136-39.