Biomed. Papers 145(2), 81–83 (2001)

DETERMINATION OF TOTAL ANTIOXIDANT CAPACITY IN PLASMA BY CYCLIC VOLTAMMETRY. TWO CASE REPORTS

Jitka Psotová[1*], Jana Zahálková[2], Jan Hrbáč[3], Vilím Šimánek[1], Josef Bartek[1]

[1] *Institute of Medical Chemistry and Biochemistry, Palacký University, 775 15 Olomouc, psotova@tunw.upol.cz*
[2] *3rd Clinic of Internal Medicine, University Hospital Olomouc*
[3] *Institute of Inorganic and Physical Chemistry, Faculty of Science, Palacký University, Olomouc, Czech Republic*

Received: October 12, 2001

Key words: Cyclic voltammetry / Plasma / Total antioxidant capacity / Silymarine / Dialysis

The cyclic voltammetry (CV) was used for the measurement of the plasma total antioxidant capacity from two types of patients. The first one consisted of 29 volunteers (men aged 18–21 years) who were administered placebo or silymarin at a dose of 858 mg/day. After two months of silymarine administration, CV revealed a statistically significant increase in total antioxidant capacity compared to placebo. No statistically significant changes in TBARS, SH-groups, creatininin, urea, and uric acid concentrations were found. The second group under study comprised 49 patients with chronic renal disease during dialysis therapy. After dialysis, CV revealed a decrease of total antioxidant capacity in the plasma, which was equivalent to a decrease in creatinine, urea and uric acid. CV was performed using a system consisting of a working glassy carbon electrode, an auxiliary platinum electrode, and a reference saturated calomel electrode; a linear change of voltage of 200 mV/s was applied. CV is a simple and relatively reliable method for assessment of body antioxidant status. It is also time and cost effective.

INTRODUCTION

Free radicals are highly reactive molecules generated by biochemical redox reactions that occur as a part of normal cell metabolism and in the course of free radical-mediated diseases such as diabetes mellitus, cancer, rheumatoid arthritis, renal, cardiovascular, inflammatory, infectious and neurological diseases[1]. Free radicals are eliminated from the body by their interaction with antioxidants. Two classes of antioxidants are known: (i) the low-molecular weight (LMW) compounds, e. g. vitamins E and C, ß-carotene, glutathione, uric acid, bilirubin, etc. and (ii) the proteins albumin, transferrin, caeruloplasmin, ferritin, superoxide dismutase, glutathione peroxidase, catalase, etc. Total antioxidant capacity (TAC) parameter summarizes overal activity of antioxidants and antioxidant enzymes. The depletion of TAC induced by oxidative stress is eliminated by release of stock organ antioxidants, mainly from liver and adipose tissue and the induction or activation of antioxidant enzymes. At a later phase of oxidative stress, the TAC falls due to depletion of antioxidants. Low molecular weight antioxidants penetrate specific locations in the cell where oxidative stress may occur and protect against reactive oxygen species (ROS). The clinical importance of determining TAC consists in identifying patients with increased risk of the above diseases and, deficient nutrition[2, 3, 4]. A significant increase of total antioxidant capacity really occurs after supplementation with vitamins C, E, and ß-carotene[5] and phenolics of green and black tea[6]. TAC can be also applied for monitoring and optimization antioxidant therapy.

At present, assessment of the antioxidant status of the body, as a clinical marker of oxidative stress, is established using three approaches: (i) determination of the concentration of total or individual low-molecular antioxidants in serum or plasma (ii) determination of activity of selected enzymes (iii) monitoring markers of oxidative stress, e. g. 8-hydroxyguanosine or products of LDL lipoperoxidation damage.

Determination of TAC is based on the evaluation of a total reduction effect of individual LMW antioxidants, of either a hydrophilic or a hydrophobic character. It provides information about antioxidant types and their concentration without exact qualitative differentiation. These methods have been predominantly based on monitoring color changes, which are recalculated as a quantity of antioxidants to the standard Trolox (analog of vitamin E) (ref.[7]). The following methods are frequently used. One, ferric reducing/antioxidant power (FRAP) directly analyses total LMW antioxidants[8]. At low pH values, reduction of Fe(III)-tripyridyltriazine complex to Fe(II)-form displays an intense blue color. Measurement is based on the ability of antioxidants in the sample to inhibit the oxidative effects of reactive species purposefully generated in the reaction mixture. This method is simple, speedy, inexpensive and robust. The oxygen radical absorbance capacity (ORAC) is a simple, sensitive, but time-consuming method (about 70 min) requiring a fluorescence detector. In this system the

2,2'-azobis(2-amidinopropane) dihydrochloride is used as peroxyl radical generator[9]. Similar method used for estimation of TAC is Trolox-equivalent antioxidant capacity (TEAC). TEAC is based on the formation of blue-green cation radical of 2,2'-azino-di-[3-ethylbenzthiazoline sulphonate] cation ($ABTS^+$) in the presence of metmyoglobine and H_2O_2 (ref.[10]). The LMW antioxidants inhibit color production proportionally to their concentration. The method is quick but the most expensive one of all the methods mentioned above. The CV determines TAC in plasma or other biological fluids, tissue homogenates and skin[11, 12]. The peak potential (anodic potential, E_p) or inflection point of current wave (half wave potential, $E_{1/2}$) of the anodic peak current wave (I_p) characterizes the reducing power of antioxidants and provides information on the type and ability of antioxidants to donate electrons (Fig. 1.). The anodic current corresponds to the concentration of antioxidants. The potential at the maximum of anodic wave reflects the reducing ability of antioxidants present in the plasma.

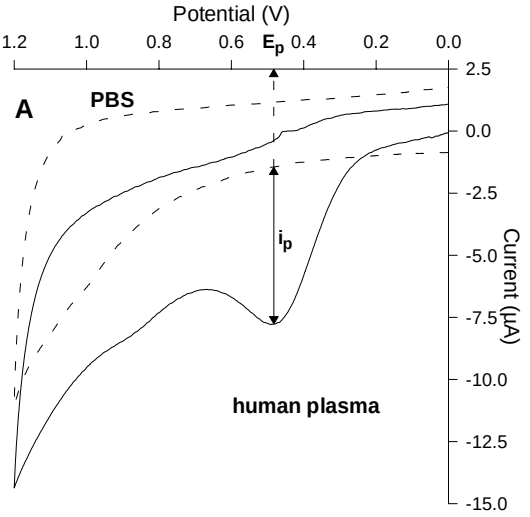

Fig. 1 Cyclic voltammogram of human plasma and phosphate buffer salina.

In this paper the plasma TACs for two types of patients were determined by CV. In the first case change of TAC was evaluated between silymarin and placebo-treated volunteers. The second case were dialyzed patients with renal diseases.

METHODS

Type 1. The study comprised 29 volunteers (men aged 18–32 years, BMI 22.61 ± 0.91) who were administered silymarine (858 mg/day) or placebo. Blood samples were collected at the beginning of the study and on 30th and 60th day. Uric acid, bilirubin, total SH-groups, products of lipid peroxidation that react with thiobarbituric acid (TBARS), TAC (anodic current I_p and redox potential E_a using CV) were determined in the EDTA plasma.

Type 2. A group of 49 patients with chronic renal disease was examined at the Center of Dialysis, 3rd Clinic, University Hospital in Olomouc. Within the dialysis program, the patients were subjected to dialysis during the period of April to October 1999. Patients with higher proteinuria (over 300 mg/l) were excluded from the study. The mean duration of dialysis therapy of the whole group of patients was 14.9 months. Patients were examined before and after standard four-hour hemodialysis using a dialyzer with polysulfon membrane (Fresenius) with an area of 1.6–1.8 m^2. Blood was collected into vacuum tubes (10 ml, Greiner) before and after dialysis. Blood samples were immediately centrifuged and the obtained EDTA plasma was separated for the measurement of uric acid, urea, creatinine, total SH-groups, TBARS and TAC.

Cyclic voltammetry.

The plasma antioxidant capacity was measured using Potentiostat Galvanostat Model 273, EG & G (Princeton Apllied Research, USA). A three-electrode system was used throughout the study. The working electrode: glassy carbon disk (Laboratory Instruments, Czech Republic) 2 mm in diameter, polished before each measurement; platinum wire served as an auxiliary electrode and calomel saturated electrode as the reference electrode. Plasma measurements (0.3 ml) were carried out in phosphate buffered saline, pH 7.4 (1.5 ml) at a scan rate of 200 mV/s. All cyclic voltammograms measurements were performed in the range (–0.4) – (0.8) V. Each sample was analysed twice.

Lipid peroxidation. Lipid peroxidation products in plasma were determined by reaction with thiobarbituric acid as thiobarbituric acid reactive substances (TBARS) and expressed as concentration of malondialdehyde[13].

Protein SH-groups and glutathione. Determination of the total thiol level in the plasma was estimated according to Hu (1994) using 2,2'-dithiobisnitrobenzoic acid (DTNB) (ref.[14]).

Plasma LMW components. Creatinine, urea and uric acid were measured on automated analyzer BM/HITACHI 917.

RESULTS

Type 1. Total antioxidant capacity, SH-groups, and TBARS were estimated at the beginning and end of experiment. After two months silymarin administration a significant increase in plasma total antioxidant capacity (I_p) was found. The plasma lipid peroxidation (TBARS) fell in silymarin group, but not significantly (Table 1). In our study the limits of total antioxidant capacity for health men was 123–178 nA.

Type 2. The selected plasma parameters (TAC, TBARS, SH-group, creatinine, urea and uric acid) were estimated before and after dialysis. It was found that decreasing TAC after dialysis mainly corresponded to diminution of the LMW substances, creatinine, urea and uric acid (Table 2).

Table 1. Effect of silymarin on lipid and oxidation plasma parameters in young healthy men

Parameters	Group	Mean ± SD	1.
Total SH-groups	Silymarin	0.51 ± 0.04	0
(mM)	Placebo	0.51 ± 0.05	0
TBARS	Silymarin	4.62 ± 0.62	4
(mM)	Placebo	4.88 ± 0.50	5
E_p	Silymarin	0.53 ± 0.04	0
(V)	Placebo	0.54 ± 0.02	0
I_p	Silymarin	14.3 ± 1.60	15
($A.10^{-8}$)	Placebo	14.96 ± 2.71	14

Table 2. Effect of dialysis on antioxidant capacity parameters

Parameter	%*
TAC	45.8 ± 0.3**
TBARS	90.4 ± 33.5**
Total SH group	117.1 ± 28.3**
Uric acid	34.0 ± 7.4**
Urea	35.0 ± 7.9**
Creatinine	44.0 ± 7.2**

*% Percentage of the initial value (100%) after dialysis.

**$p < 0.01$

CONCLUSION

The results obtained from TAC determination by CV in both types of patients reliably show changes and thus provide satisfactory information about the body antioxidant status. The method is suitable for monitoring of LMW antioxidant composition in nutrition. In the case of renal patients, CV is capable of monitoring a decrease in TAC and the effectiveness of dialysis. The method of CV is a simple and relatively reliable method for determination of TAC in plasma or serum.

ACKNOWLEDGEMENT

This work was supported by The Ministry of Education, Youth and Sports of The Czech Republic (MSM: 151100003).

REFERENCES

1. Kohen, R., Chevion, S., Schartz, R., Berry, E. M. (1996) Evaluation of the total low molecular weight antioxidant activity of plasma in health and diseases: a new approach. Cell Pharmacol. *3*, 355–359.
2. Free Radicals and Oxidative Stress: Environment, Drugs and Food Additives. Biochemical society symposia: No. 61. (Rice-Evans, C., Halliwell, B., Lunt, G. G., eds), Portland Press, London 1995.
3. Berry, E. M., Kohen, R. (1995) Is the biological antioxidant system integrated and regulated? Med. Hypotheses *53*, 397–401.
4. Zima, T., Štípek, S., Tesař, V., Plátenník, J., Crkovská, J. (1996) Free radicals – reactive oxygen species, antioxidants and antioxidant therapy. Remedia *6*, 35–58.
5. Ghiselli, A., Serafini, M., Natella, F., Scaccini, C. (2000) Total antioxidant capacity as a tool to asses redox status: critical view and experimental data. Free Rad. Biol. Med. *29*, 1106–1114.
6. Serafini, M., Ghiselli, A., Ferro-Luzzi, A. (1996) In vivo antioxidant effect of green and black tea in man. Eur. J. Clin. Nutr. *50,* 28–32.
7. Cao, G., Prior, R. L. (1998) Comparison of different analytical methods for assessing total antioxidant capacity of human serum. Clin. Chem. *44,* 1309–1315.
8. Benzie, I. F., Strain, J. J. (1996) The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal. Biochem. *239*, 70–76.
9. Cao, G., Alessio, H. M., Cutler, R. G. (1994) Oxygen-radical absorbance capacity assay for antioxidants. Free Rad. Biol. Med. *14*, 135–137.
10. Miller, N. J., Rice-Evans, C., Davies, M. J., Gopinathan, V., Milner, A. (1993) A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clin. Sci*. *84:* 407–412.
11. Kohen, R., Vellaichamy, E., Hrbac, J., Gati, I., Tirosh, O. (2000) Quatification of the overall reactive oxygen species scavenging capacity of biological fluids and tissues. Free Rad. Biol. Med. *28*, 871–879.
12. Kohen, R., Beit-Yannai, E., Berry, E. M., Tirosh, O. (1999) Overall low molecular weight antioxidant activity of biological fluids and tissues by cyclic voltammetry in: Methods in Enzymology *300,* 285–296.
13. Buege, J. A., Aust, S. D. (1978) Microsomal lipid peroxidation in: Methods in Enzymology, *52*, 302–310.
14. Hu, M.-L. (1994) Measurement of protein thiol groups and glutathione in plasma in: Methods in Enzymology, *233*, 380–385.