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Determination of Antioxidant Activity of Blood Serum in Health and Disease by Voltammetric Method

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ABSTRACT: The article presents the results of a study to determine the antioxidant activity of blood serum in health and disease in patients with renal failure. As a research method, we used cyclic voltammetry with the use of a film mercury-graphite electrode as an indicator. The p-benzoquinone-hydroquinone pair served as the mediator system. The zone of reference values of the level of antioxidant activity of blood serum in the range of 1.378 ± 0.214 mmol / L was revealed. The correctness of the method is confirmed by comparison with the TAS method (Randox). A high correlation was shown between the level of antioxidant activity and the values of the redox potential of the blood serum of patients with diseases of the kidneys and other organs was shown.

KEYWORDS: free radical, antioxidants, voltammetric determination, blood serum, reference zone, oxidation-reduction potential, kidney disease, norm and pathology

I. INTRODUCTION

Due to the development of ideas about the role of free radical processes in the body and their relationship with the work of the antioxidant system, more and more attention is paid to assessing the effectiveness of its work [14,15]. In a normally functioning human body, there is a balance between prooxidants, those, active oxygen forms (AOF) and free radicals, and components of the antioxidant defense system. Violations of this balance in diseases of various etiologies can lead to oxidative stress or slow down free radical processes, i.e. to violations of natural metabolic processes [5,7].

Currently, it is known that in the human body there are several barriers of the antioxidant system that provide a protective function at various levels [12,14]:

1. Primary antioxidants enzymes (glutathione peroxidase, superoxide dismutase), metal-binding proteins (ferritin and ceruloplasmin), which prevent the formation of free radicals.
2. Secondary - vitamins E and C, beta-carotene, uric acid, bilirubin and albumin - which help to remove the formed radicals.
3. Tertiary - enzymes that repair damaged cellular structures (for example, at the level of DNA repair).

In this regard, the problem of developing methods for the analysis of prooxidants and antioxidants in biological fluids is quite acute [6].

The most commonly used spectrophotometric methods for determining antioxidant activity require expensive and scarce reagents and depend on the color intensity of the biological material itself [10].

Within the framework of this problem, special attention should be paid to electrochemical methods for the determination of antioxidants due to their simplicity, selectivity, and rapidity [9]. At the same time, not all biologically active substances are electrochemically active, which does not allow their direct determination.

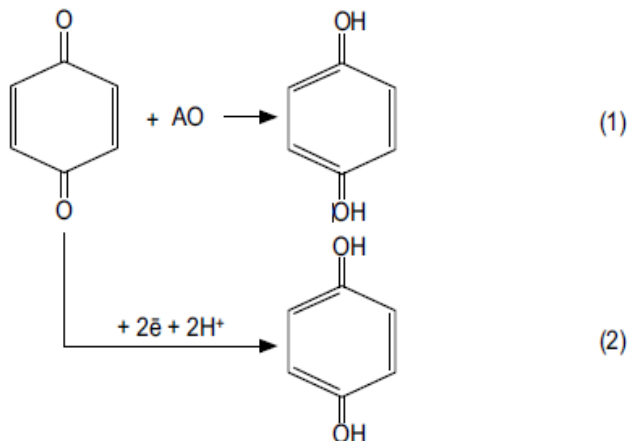
In this case, the most acceptable is the use of mediator reactions using redox pairs that change their ratio in the presence of antioxidants. It should be noted that the choice of the mediator system must meet a number of requirements: the thermodynamic possibility of the chemical reaction of the reagent system with the main antioxidants, the reversibility of the electrode reaction of the mediator system, the rapid establishment of an equilibrium potential, the Nernst dependence of the potential on changes in the concentration of system components and a fairly high reaction rate with the main antioxidants [8]. As such mediator systems, both inorganic ions and organic substances with electrochemical activity can be used, for example, iodine-iodide, ferro-ferricyanide, p-quinone-hydroquinone, and others. A change in the value of the measured electrical parameter in the presence of an antioxidant substance can become a measure of the antioxidant activity of this substance [1,3].

The aim of this work is to develop an electrochemical method for determining the level of antioxidant activity (AOA) of biological media using a mediator electrochemically reversible pair of quinone-hydroquinone and its application in clinical practice.

II. MATERIALS AND METHODS

We used p-benzoquinone (99.5%, Diaem, Russia), ascorbic acid (99%, Sigma Aldrich), trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid 97%, SigmaAldrich), quercetin dihydrate (97%, Sigma Aldrich). Voltammetric measurements were carried out using a computerized complex based on the modernized PU-1 polarograph [4]. A three-electrode cell with a working film mercury-graphite electrode made according to [2,13] was used. A platinum wire 0.5 mm in diameter served as an auxiliary electrode. All potential measurements were carried out relative to a silver chloride electrode. The pH of the solutions was determined using an I-130M ion meter with an ESL-63G glass electrode and an EVL-1M3 silver chloride reference electrode; the redox potential was measured using an ORPTESTER 169B ORPTESTER ORP meter (Malaysia).

The essence of the method lies in the interaction of the oxidizing agent p-benzoquinone with the biological environment (equation 1), after which the residual concentration of p-benzoquinone is determined voltammetrically (equation 2).



III. RESULTS AND DISCUSSION

First of all, we studied model solutions of antioxidants (ascorbic acid, trolox, quercetin dihydrate) in the concentration range of 10^{-5} - 10^{-3} M. For analysis, to 3 ml of phosphate buffer solution (pH 7.4, 0.15 M for NaCl) 1 ml of 10^{-2} M aqueous solution of p-benzoquinone and 1 ml of the analyzed antioxidant solution were added. The mixture was kept for 5 minutes at room temperature, after which the electrochemical determination of the residual concentration of p-benzoquinone was carried out. We used a three-electrode cell with a film-type mercury-graphite working electrode, a silver chloride reference electrode, and a carbon-graphite auxiliary electrode. The potential was cyclically scanned for 5 cycles in the range from -650 to +900 mV with a scan rate of $55 \text{ mV}\cdot\text{s}^{-1}$. The concentration of p-benzoquinone was determined by the decrease in the cathodic peak at potentials of $-160 \pm 20 \text{ mV}$ (Fig.1).

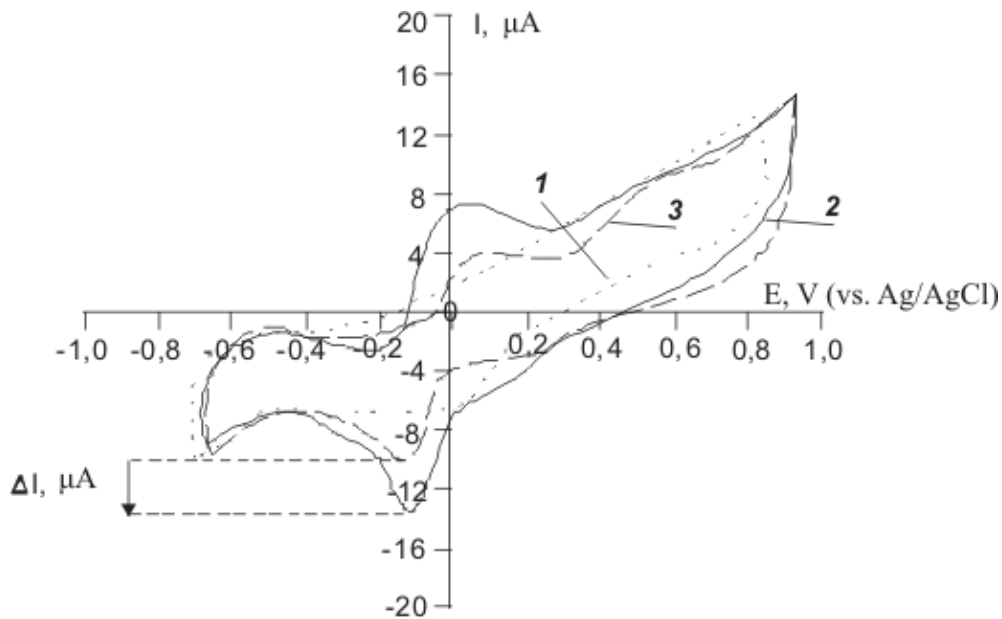


Fig. 1. Current-voltage curve of the p-benzoquinone / hydroquinone mixture:

- 1 - background solution (phosphate buffer pH 7.4 0.15 MNaCl);
- 2 - background solution + 0.01 M p-benzoquinone;
- 3 - background solution + 0.01 M p-benzoquinone with the addition of 1 ml of blood serum from a healthy donor.

Calibration curves were constructed based on the data on the analysis of model solutions of antioxidants. It has been experimentally established that all the solutions of antioxidants used show a linear dependence in the concentration range from 10^{-5} - 10^{-3} M. However, the use of a dihydroquercetin solution as the AOA standard showed the best correlation coefficient. Therefore, for further research, we chose dihydroquercetin as the standard for the level of antioxidant activity.

To establish the average values of total AOA in the blood serum and to determine the zone of construction of reference values [11] of the AOA level, a study of the blood serum of 23 practically healthy donors was carried out. It was shown that the level of AOA lies in the range $0.82 \div 1.88$ mmol / l, calculated on dihydroquercetin.

Statistical processing of the data set from 23 apparently healthy donors yielded a zone of reference values of 1.378 ± 0.214 mmol / l (Fig. 2).

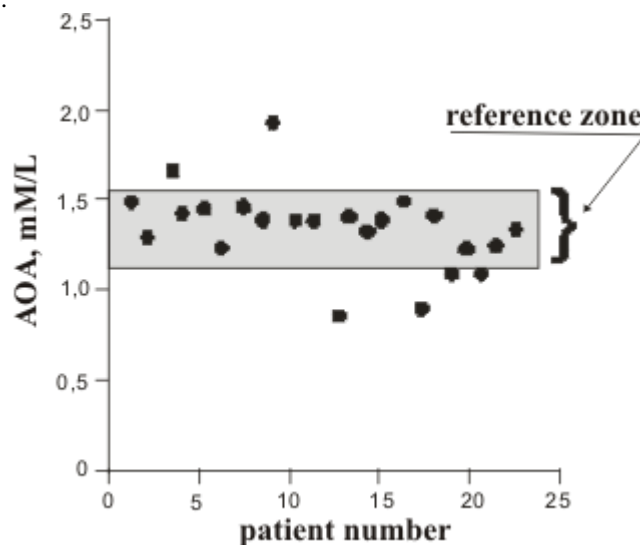


Figure: 2. The antioxidant activity of blood serum of 23 is the healthy donors.

When comparing the level of AOA in the blood serum of patients with a diagnosis of sepsis (6 people), measured by the proposed method, a high correlation was found with the spectrophotometric method (TAS, "Randox") (Fig..3)

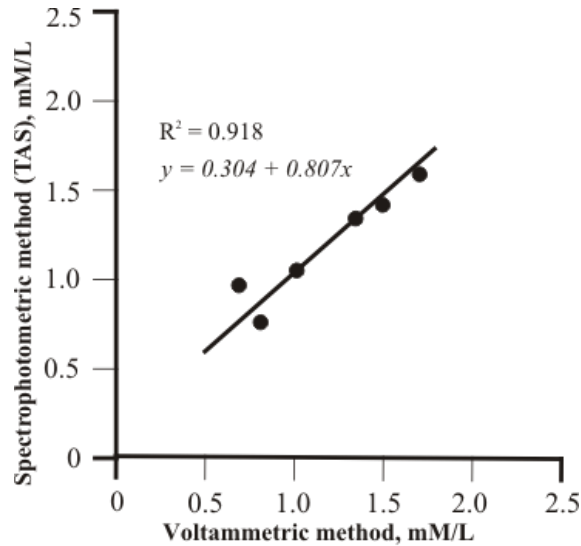


Figure: 3. Comparison of voltammetric and spectrophotometric (TAS, Randox) methods for the determination of AOA in septic patients.

In the study of patients of the nephrology department (11 people), a correlation was found between the level of AOA and the values of the redox potential (Fig.4).

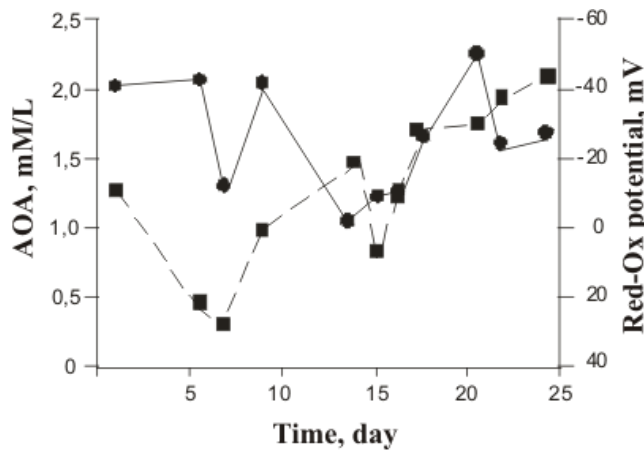


Fig.4. Dynamics of changes in AOA and ORP in blood serum of patients with kidney disease.

As follows from the diagram, in the course of treatment, there is a similar dynamics of changes in the AOA and ORP of blood serum. The reference zone for AOA can be attributed to the periods starting from 7 to 20 days of treatment.

Thus, the proposed method can serve as an additional criterion for assessing the condition of patients with diseases of the kidneys and other organs.

IV. CONCLUSION

As a result of the research, a method was developed for the voltammetric determination of the antioxidant activity of blood in health and disease. The reference values of the antioxidant activity of the blood serum of healthy donors were

established, lying in the range of 1.378 ± 0.214 mmol / L. When analyzing the blood serum of patients with kidney disease, the developed method shows adequate results. The correlation between the antioxidant activity of blood serum and its redox potential has been established. Conducting clinical trials of the method showed that in order to achieve the values of the reference zone for AOA during treatment, a period of one to three weeks can be attributed. Thus, the proposed method can serve as an additional criterion for assessing the condition of patients with diseases of the kidneys and other organs.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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