

Determination of Heavy Metals in Biological Objects by Method of Differential Impulse Polarography

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Abstract

The new microanalytical methods have been worked out for determination of copper, lead, cadmium and zinc in the samples of biological materials by method of differential impulse polarography.

Key words: *blood serum, hairs, polarography copper, lead, cadmium, zinc*

Introduction

Nowadays the polarographical methods of analysis are the most wide-spread in analytical practice. Among them the method of differential impulse polarography is especially perspective. This method is widely used for determination of admixtures in pure metals and reactive, at analysis of natural waters and mine rocks and in the other fields of science and technique. Estimation of ability of this method, the comparison with other high-precise methods used for determination of heavy metals in biological objects have been considered in the work [1]. The present work is devoted to the study of possibility of simultaneous determination of copper(II), lead(II), cadmium(II), zinc(II) ions in a little amounts of biological materials (blood, blood serum, hairs).

Materials and Methods

This work was carried out on the registered polarograph PU -2. As electrolyser was used the polarographic cell

of closed type [2]. As working electrode served the drop mercury electrode. As comparison electrode was used the saturated calomel electrode. The preventive removal of oxygen was carried out by means of blowing of argon through electrolyser. The used reactivities were of high purity or purified before usage at analysis. Biomaterials undergo the wet ashing. polarography in interval 0,0-0,1V. At the same time and the same condition was carried out the control experiment and on this ground the corrections were made.

The obtained polarogram contain 4 picks corresponding to copper, lead, cadmium and zinc. By the high of pick using the method of addition can be determined amount of metal. For this, after obtaining of the first polarogram the known amount of standard solution containing Cu, Pb, Cd and Zn was added into electrolyser. Then, the second polarogram is taken (fig.1). The high of pick is measured graphically, and the content of determined metal is calculated by formula:

$$X\% = CH_2 / (H_2 - H_1) \cdot g \cdot 10$$

Where H_1 - the value of pick of metal (in mm)

H₂ - the value of pick after adding of standard solution (in mm)

C - the amount of added metal (in mg)

g - the amount of biomaterial (in g).

Results

The results of present study revealed decreased antioxidant properties of blood (*Tab.1*) on the 21st day after implantation of Sarcoma C-45 which is manifested by increased intensity of EPR signals of oxidized ceruloplasmin (g=2,056) by 50% in comparison with norm.

At this time EPR signal of Fe³⁺ transferrin (g=4,2) is not changed. On the background of decreased antioxidant protection, lipid peroxidation is activated contributing to destruction of membrane structures and erythrocytes in particular, leading to hemolysis and production of methemoglobin. This last is confirmed by appearance of Met-Hb intensive signal (g=6,0) in the EPR spectrum. Furthermore, presence of signals of Mn²⁺ (g=2,14) and Mo⁵⁺ containing complexes in blood is the good evidence of membrane structures' destruction. Signal of Mn²⁺ complexes in turn indicates inactivation of mitochondrial superoxid-dismutase (SOD), while signal of Mo⁵⁺ complexes reflects exaggerated production of xantinioxidase and developed ischemia.

In the presence of activated POL, as a result of membrane structures' destruction, inactivation of adrenoreceptors (g=2,01) is obvious. Besides, high concentration of Mn²⁺ ions presented in blood of experimental animals, results in disconnection of adrenoreceptors from system of adenylatcyclase. At last, Mn²⁺ ions are promoters of process of peroxidation. It must be mentioned, that EPR spectrum of blood reveals intensive signal of FeS-NO complexes (g=2,03), indicating activation of NO synthesis.

Inactivation of adrenoreceptors and oppression of protective and compensatory processes of organism causes subsequent aggravation of pathological process.

Manifestation of ferritin bound Fe²⁺ ions which is not detected in norm, indicates destruction of tissue cells.

On the 21st day after tumor implantation EPR spectrum of liver (*Tab.2.*) demonstrates disorder of hepatocytes' mitochondrial respiration at NAD.H: ubiquinon oxidoreductase locus manifested by the increase intensity of signal of free radicals (g=2,0) and decreased half width of it (ΔH).

Sharply increased intensity of signal of Mn²⁺ containing complexes reveals membrane structures' destruction in liver and inactivation of mitochondrial superoxid-dismutase. Signal of Mo⁵⁺ containing xantinioxidase is increased as well, indicating ischemia of hepatocytes.

Intensified signal of FeS-NO complexes (g=2,03) reflects activation of NO production. In liver, signal of Met-Hb is detected as well.

In the EPR spectrum of spleen (*Tab.3.*), on the 21st day after tumor implantation, signals of Ribonucleotid reductase (RR) and Fe³⁺ transferrin are not changed, while in tumor tissue signal of RR is intensified, which is characteristic for activated proliferative processes.

On the 30th day after tumor implantation, signal of oxidized ceruloplasmin of blood (g=2,056) is slightly increased in comparison with data obtained on the 21st day. Signal of Fe³⁺ transferrin decreases, which might be the result of decreased peroxidative activity of ceruloplasmin diminishing antioxidant ability of apotransferrin and leading to malfunction of erythro- and hemopoiesis.

At the same time, intensity of signal of Mn²⁺ containing complexes increases much more, indicating tissue destruction and disorder of mitochondrial electron transport. In the presence of activated POL, signals, characteristic for adrenoreceptors inactivation are increased as well.

In hepatocytes, on the background of disordered mitochondrial electron transport at NAD.H: ubiquinon oxidoreductase locus, deficit of substrate of mitochondrial respiration takes place, which is revealed by the presence of decreased signal of free radicals in comparison with data of 21st day.

Decreased signal of Mn²⁺ ions could be determined by the efflux of above-mentioned ions in the blood. Signal of FeS-NO (g=2,03) increases extremely, indicating exaggerated production of NO.

Decreased intensity of Fe³⁺ signal probably is the compensatory reaction of organism. On the background of decreased signal of Fe³⁺ transferrin in blood, signal of cytochrom P-450 increases, which is characteristic for intoxication and inactivation of processes of detoxication.

Noteworthy, that after treatment with Plaferon, in the EPR spectrum of liver and blood, signal of FeS-NO (g=2,03) is revealed indicating NO modulator ability of Plaferon.

| | INACTIVE RECEPTOR S g=2,01 | Met-Hb g=6,0 | Fe ³⁺ TRANSFER Rg=4,2 | CERULO PLASMI N g=2,056 | Mn ²⁺ g=2,14 | Mo ⁵⁺ | Fe ²⁺ | FeS-NO g=2,03 |
|---|-------------------------------|-----------------|-------------------------------------|------------------------------------|-----------------------------------|-----------------------------------|------------------|------------------|
| Norm o | 0,9±0,1 | — | 33,0±2,3 | 20,0±1,2 | 2,0±0,8 | — | — | — |
| 21 st day after C-45 implantation x | 1,9±0,07 | 20,0±0,3 | 32,7±0,3 P _{0-x} >0,1 | 30,6±0,4 | 12,3±0,5 | 13,1±0,4 | 22,7±0,7 | 16,8±0,4 |
| 30 th day after C-45 implantation 1 | 2,8±0,009 | 24,5±0,4 | 28,3±0,8 | 27,6±0,7 | 16,0±0,4 | 14,1±0,6 | 31,4±0,7 | 15,3±0,2 |
| 30 th day after C-45 implantation +Plaferon 2 | 2,1±0,08 | 17,9±0,7 | 28,2±0,7 P ₁₋₂ >0,1 | 23,2±0,8 P ₀₋₂ >0,05 | 15,3±0,3 P ₁₋₂ >0,1 | 13,3±0,4 P ₁₋₂ >0,1 | 19,9±0,5 | 16,7±0,5 |
| 40 th day after C-45 implantation 3 | 2,5±0,06 | 22,0±0,5 | 21,4±0,8 | 37,6±0,8 | 12,8±0,3 | 13,6±0,4 | 29,7±0,9 | 11,5±0,6 |
| 40 th day after C-45 implantation +Plaferon 4 | 2,0±0,09 | 10,4±0,5 | 31,5±0,7 P ₀₋₁ <0,1 | 25,2±0,4 | 10,7±0,5 | 10,9±0,3 | 23,2±0,5 | 17,8±0,4 |

Tab.1 EPR spectrum of blood in rats during Sarcoma C-45 growth before and after treatment with Plaferon.

On the background of hypoxia, activated POL and inactivated SOD, proliferative processes of organism decrease, which is revealed by the decreased signal of RR in spleen.

On the 40th day after tumor implantation, EPR spectrum of blood reveals further decrease of antioxidant ability of organism (oxidized ceruloplasmin is increased and Fe³⁺ transferrin is decreased). POL is exaggerated, which is revealed by the presence of disordered chain of mitochondrial respiration at NAD.H: ubiquinon oxidoreductase locus, developed ischemia and increased production of generators of oxygen free radicals - ubisemiquinons and xanthinoxidase in liver.

It should be mentioned, that excess amount of NO, revealed by the intensive signal of FeS-NO (g=2,03), in the presence of superoxidradicals and inactivated SOD, converts into peroxinitrit, which is known as high active free radical, thereby makes favorable conditions for further activation of POL. Developed condition in turn decreases RR signal in spleen, indicating decreased proliferative processes.

Biochemical investigation of blood, on the 21st, 30th and 40th day after tumor implantation, in comparison with norm, revealed sharply reduced activity of antioxidant enzyme catalase by 40,5%, 56,2% and 60,3% correspondingly. Common activity of ceruloplasmin is decreased by 49,6%, 37,6% and 55,1% (p< 0,001) correspondingly.

Oxidized ceruloplasmin concentration is increased by 53,0%, 38,0% and 88,1% correspondingly. Ratio of oxidized ceruloplasmin to entire the ceruloplasmin concentration is increased, which points on elevation of quota of inactive ceruloplasmin, reduction of antioxidant ability of organism and activation of lipid peroxidation.

Restored antioxidant ability of blood in turn decreases POL, which is manifested by decreased amount of Met-Hb and reduced signal of cytochrom C oxidase.

| | NORM | 21 st DAY AFTER C-45 IMPLANT. | 30 th DAY AFTER C-45 IMPLANT. | 30 th DAY AFTER C-45 IMPLANT. +PLAFERON | 40 th DAY AFTER C-45 IMPLANT. | 40 th DAY AFTER C-45 IMPLANT. +PLAFERON |
|---------------------------------------|----------|---|---|--|---|--|
| | 0 | X | 1 | 2 | 3 | 4 |
| I intensity g=2,00 | 25,0±0,9 | 35,1±0,8 | 26,1±0,7 | 25,0±0,3 P ₁₋₂ >0,1 | 22,8±0,8 | 24,6±0,5 |
| ΔH half-width | 12,0±0,5 | 7,2±0,4 | 7,6±0,3 | 10,5±0,2 | 10,2±0,1 | 12,7±0, P ₀₋₄ >0,01 |
| Met-Hb g=6,0 | — | 28,1±0,5 | 21,9±0,8 | — | 15,7±0,5 | 2,8±0,5 |
| Fe ³⁺ transferrin g=4,2 | 33,0±2,3 | — | 28,3±0,8 | 28,2±0,7 P ₁₋₂ >0,1 | 21,4±0,8 | 31,5±0,7 |
| Cytochrom P-450 g=2,25 | 12,0±0,8 | 17,7±0,3 | 25,1±0,4 | 13,6±0,5 P ₀₋₂ >0,1 | 15,8±0,3 | 14,3±0,8 P ₀₋₄ >0,05 |
| FeS g=1,94 | 25,0±1,2 | 26,9±0, P _{0-X} >0,1 | 27,3±0,6 P ₀₋₁ >0,1 | 27,2±0,5 P ₀₋₂ >0,1 | 21,5±0,6 P ₀₋₃ >0,05 | 34,9±0,7 |
| Mn ²⁺ g=2,14 | 10,0±1,3 | 18,0±0,3 | 12,8±0,7 P ₀₋₁ >0,1 | 9,8±0,5 P ₀₋₂ >0,1 | 14,1±0,4 | 12,2±0, P ₀₋₃ <0,05 |
| Mo ⁵⁺ | 8,1±0,3 | 14,7±0,4 | 14,5±0,2 | 9,1±0, P ₀₋₂ >0,1 | 13,2±0,5 | 10,5±0,4 |
| FeS-NO g=2,03 | 10,0±1,4 | 14,8±0,5 | 22,1±0,6 | 32,2±1,0 | 19,3±0,6 | 24,1±0,8 |

Tab.2 EPR spectrum of liver in rats during Sarcoma C-45 growth before and after treatment with Plaferon

It is remarkable, that despite treatment, EPR signals characteristic for inactive forms of adrenoreceptors are still increased. It is probably the result of exaggerated production of catecholamines, desensitization of adrenoreceptors and disconnection of adrenoreceptors from system of adenylatcyclase after influence of Mn²⁺ ions presented in blood in excess amount.

On the background of Plaferon-LB, mitochondrial respiratory chain at NAD.H: ubiquinon oxidoreductase locus is restored, which is manifested by the normalization of free radical signals.

Increased signal of NAD.H: dehydrogenase (g=1,94) on the 40th day after tumor implantation probably is the result of Plaferon-LB, which is capable somehow increase mitochondrial membrane permeability.

Signal of Met-Hb is decreased in both - blood and hepatocytes' EPR spectrum reflecting decreased hemolysis.

In case of Plaferon background, signals of Mn²⁺ and Mo⁵⁺ in the spectrum of blood and liver are lower than in case of untreated animals, indicating decreased production of xanthinoxidase and restored activity of SOD.

Noteworthy, that after treatment with Plaferon, in the EPR spectrum of liver and blood, signal of FeS-NO (g=2,03) is revealed indicating NO modulator ability of Plaferon.

In the spleen tissue, signal of RR and proliferative processes are activated, while in cancer cells mentioned signal and processes are sharply reduced.

| | RIBONUCLEOTID REDUCTASE RR | | Fe ³⁺ TRANSFERRIN g=4,2 IN SPLEEN |
|--|-----------------------------------|------------------|--|
| | SPLEEN | CANCER TISSUE | |
| Norm 0 | 30,0±1,2 | — | 33,5±1,5 |
| 30 th day after C-45 implantation 1 | 21,2±1,0 | 27,4±0,4 | 20,4±1,4 |
| 30 th day after C-45 implantation+Plaferon 2 | 25,2±0,7 | 18,5±0,2 | 23,5±0,6 P ₁₋₂ >0,05 |
| 40 th day after C-45 implantation 3 | 17,7±0,6 | 32,7±1,0 | 21,2±1,0 |
| 40 th day after C-45 implantation +Plaferon 4 | 21,9±1,1 | 18,7±0,7 | 32,1±0,7 P ₀₋₄ >0,1 |
| 21 st day after C-45 implantation 5 | 33,1±0,9 P ₀₋₅ >0,1 | 31,1±0,4 | 32,8±0,8 P ₀₋₅ >0,1 |

Tab.3. EPR signals of spleen and cancer tissue (Sarcoma C-45) in rats before and after treatment with Plaferon.

In treated animals there is a tendency of catalase activation, however it is still decreased in comparison with norm. On the 30th day after tumor implantation common activity of ceruloplasmin in comparison with untreated animals (control) is increased by 63,2%. Moreover, it reaches the normal level .

On the 30th and 40th day of Sarcoma C-45 growth, Plaferon decreases concentration of oxidized ceruloplasmin by 15,9% and 33,0% correspondingly in comparison with control.

The ratio of oxidized ceruloplasmin to common rate of ceruloplasmin is decreased as well, that points on restored activity of enzyme.

Conclusions

1. During Sarcoma C-45 growth in the organism of experimental rats antioxidant properties of blood are decreased and mitochondrial SOD is inactivated;

2. Production of oxygen active form generators – Mn²⁺, NO, Xanthinoxidase, Cytochrom P-450 and POL are activated;

3. Protective and compensatory reactions of organism are reduced;

4. Membrane structures and electron transport of mitochondrial respiratory chain are disordered;

5. Hemolysis and hypoxia are developed;

6. After treatment with Plaferon-LB antioxidant protection of blood and activity of hepatocytes' mitochondrial SOD are improved;

7. Intensity of production of oxygen free radical generators - Ubisemiquinon, Xanthinoxidase, oxidized cytochrom P-450 and POL are decreased;

8. Destruction of membrane structures, especially erythrocytes and mitochondrial membranes are decreased contributing to reduction of Hypoxia;

9. Intensity of RR signal in cancer tissue is decreased indicating delayed growth of cancer tissue;

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Определение тяжелых металлов в биологических объектах методом дифференциальной импульсной полярографии

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Р Е З Ю М Е

Разработана микроаналитическая методика определения меди, свинца, кадмия и цинка в одном и том же образце биологического материала методом дифференциальной импульсной полярографии. На основании сравнения нижних пределов определения металлов методами дифференциальной импульсной полярографии и атомной адсорбции, предложенный метод оценивается нами как на порядок превосходящий по точности .

Ключевые слова: *кровь, сыворотка, волосы, полярография, медь, свинец, кадмий, цинк*