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Voltammetry Assay for Assessment of Oxidative Stress linked Pathologies in Brain Tumor suffered Childhood Patients

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Oxidative stress plays an important role in cancers due to several reasons including metabolism disorder and hypoxia in tissue near the cancer. Numerous researchers have reported participation of oxidative stress in the cancer pathology. However, the mechanism is not well understood. In this study, we investigated oxidative stress development and depletion of antioxidants in blood samples from medulloblastoma, neuroblastoma, glioblastoma and central nervous system cancer suffered childhood patients. The blood samples were analyzed using square wave voltammetry (SWV), dimethyl-4-phenylenediamine method (DMPD), 2´-azinobis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS), free radical method (FR), ferric reducing antioxidant power (FRAP), reduced and oxidized glutathione, and metallothionein. We proved significant correlation between the markers. The findings demonstrate crucial role of oxidative stress and antioxidants levels regulation in the cancer suffered patients.

Keywords: biosensor; electrochemistry; stress; cancer; medulloblastoma; neuroblastoma; glioblastoma; brain cancer; redox status

1. INTRODUCTION

It is a common knowledge that oxidative stress relates with imbalance between concentration of reactive forms and the antioxidant. Owing to some reported papers, it appears that these changes are very important in the pathophysiology of critically ill patients [1-4]. Currently, direct measurement of reactive oxygen species and oxidative stress markers is still difficult task in clinical medicine due to their instability and interferences of a lot of other compounds. Based on these facts, degradation products of biomolecules or antioxidants are assayed for estimation of oxidative stress appearance [5,6]. Recently, there is a growing interest about oxidative stress link to tumor progression as the pathology is tightly connected to the antioxidants imbalance [7,8]. Application of exogenous low molecular weight antioxidants (LMWA) then can serve as a therapeutic intervention [9].

Voltammetry is an elementary electrochemical method implemented in all of electrochemical analyzers. For this reason it is very accessible and easy-to-use for various users. In several published papers it has been shown its potential for monitoring oxidative stress and its relation to grave diseases as acute pancreatitis [10] and/or hemorrhagic shock [11]. The voltammetry techniques are reliable enough to assay a broad spectrum of antioxidants without any pretreatment of samples or use of specific reagents [12]. The fast assessment of LMWA presented in sample can serve for examination of pathological processes or prove of their depletion [13]. In an example, square wave voltammetry on platinum working electrodes was used for assessment of LMWA in blood plasma and the achieved results well correlated to ferric reducing antioxidant power (FRAP) as a standard method [14]. In another experiment, Bordonaba and Terry successfully used screen printed carbon electrodes and square wave voltammetry to assay polyphenols and other antioxidants in fruit juices [15]. For the here reported experiment, we chose square wave voltammetry as a low cost method sawing amount of sample and providing reliable results. Plasma samples from patients can be simply measured resulting in achieving of peaks responding to basic groups of antioxidants [14].

The main aim of this contribution is to study antioxidant activity by using of simple electrochemical method (square wave voltammetry) at carbon working electrodes. For this purpose, plasma samples from childhood patients with diagnosed medulloblastoma, neuroblastoma, glioblastoma and other brain tumors were chosen in order to demonstrate the method suitability for use in clinical practice.

2. EXPERIMENTAL PART

2.1. Chemicals, material and pH measurements

All chemicals of ACS purity used were purchased from Sigma Aldrich Chemical Corp. (Sigma-Aldrich, USA), unless noted otherwise. Water underwent demineralization by reverse osmosis using an Aqua Osmotic 02 instrument (Aqua Osmotic, Tisnov, Czech Republic) and then was purified using Millipore RG (Millipore Corp., USA, 18 MΩ) – MiliQ water. Value of pH was measured using a WTW inoLab pH meter (Weilheim, Germany).

2.2. Human blood serum

Blood samples were obtained from 48 children hospitalized at the Department of Paediatric Haematology and Oncology of Faculty Hospital Motol with newly diagnosed solid tumors. 48 samples, medulloblastoma (n = 17), neuroblastoma (n = 31), glioblastoma (n = 2), and other brain tumors (n = 6) were used for the experiment purposes. Average age of patients was 7.3 years. The blood samples were collected before chemo- and radiotherapy.

Preparation of serum samples. The samples were kept at 99 °C in a thermomixer (Eppendorf 5430, Germany) for 15 min with shaking in order to remove ballast proteins and peptides which could influence the electrochemical response. The denatured homogenates were centrifuged at 4 °C, 15 000 × g for 30 min. (Eppendorf 5402, Germany).

2.3. Square wave voltammetry

Square wave voltammetry was performed on screen printed sensor with graphite working, graphite auxiliary and Ag/AgCl reference electrodes (Metrohm, Switzerland). The polymer based was dot shaped with diameter 4 mm. The auxiliary and reference electrodes were ring shaped and polymer based as well. For the assay control, an electrochemical device EmStat (PalmSens, The Netherlands) was used. The sensor was placed into holder and set in horizontal position. The assay of low molecular weight antioxidants using square wave voltammetry was done in a slight modification of previously optimized protocol [14]. The voltage was applied within the range from 0 to 1.1 V with potential step as well as voltage amplitude 5 mV. Frequency of the waves was 1 Hz. In a total, 20 µl of the plasma samples was spread over the electrodes and voltammetry was run immediately. The sensors were used as disposable analytical devices. The achieved voltammograms were processed in software PSLite (PalmSens, Houten, The Netherlands) and area under peak in µA/V was calculated by integration using the software.

2.4. Differential pulse voltammetry - Brdicka reaction

Differential pulse voltammetric Brdicka reaction measurements were performed with a 747 VA Stand instrument connected to a 746 VA Trace Analyzer and 695 Autosampler (Metrohm, Switzerland), using a standard cell with three electrodes and cooled sample holder (4 °C). A hanging mercury drop electrode (HMDE) with a drop area of 0.4 mm^2 was the working electrode. An Ag/AgCl/3M KCl electrode was the reference and glassy carbon electrode was auxiliary. For data processing, GPES 4.9 supplied by EcoChemie was employed. The analyzed samples were deoxygenated prior to measurements by purging with argon (99.999 %) saturated with water for 120 s. For measurement the Brdicka supporting electrolyte containing 1 mM $Co(NH_3)_6Cl_3$ and 1 M ammonia buffer ($NH_3(aq)$ + NH_4Cl, pH = 9.6) was used. The supporting electrolyte was exchanged after each analysis. The parameters of the measurement were as follows: initial potential of –0.7 V, end potential of –1.75 V, modulation time 0.057 s, time interval 0.2 s, step potential 2 mV, modulation amplitude - 250 mV, Eads = 0 V, volume of injected sample: 20 µl (100 × diluted sample with 0.1 M phosphate

buffer pH 7.0). All experiments were carried out at 4 °C employing a Julabo F25 thermostat (Labortechnik GmbH, Germany). This method was published in protocols [16,17].

2.5. Determination of Reduced and Oxidized Glutathione

Reduced (GSH) and oxidized (GSSG) glutathione were determined using high performance liquid chromatography with electrochemical detection (HPLC-ED) in a way as reported in reference [18,19]. The chromatographic system consisted of two solvent delivery pumps operating in the range of 0.001-9.999 ml/min (Model 582 ESA Inc., Chelmsford, MA, USA), Zorbax eclipse AAA C18 (150 × 4.6; 3.5 μm particle size; Agilent Technologies, Santa Clara, CA, USA) and a CoulArray electrochemical detector (Model 5600A, ESA). The electrochemical detector had three flow cells (Model 6210, ESA). Each cell consisted of four working carbon porous electrodes, each one with auxiliary and dry Pd/H_2 reference electrodes. Both the detector and the reaction coil/column were thermostated. The sample (20 μl) was injected using an autosampler (Model 542 HPLC, ESA). Samples were kept in the carousel at 8 °C during the analysis. Temperature in the column was adjusted at 32 °C. Mobile phase consisted of 80 mM TFA (A) and methanol (B). The compounds of interest were separated by linear gradient: 0 → 1 min (3 % B), 1 → 2 min (10 % B), 2 → 5 min (30 % B), 5 → 6 min (98 % B). Mobile phase had flow rate 1 ml/min. At the same time, applied voltage at working electrode was adjusted at 900 mV. Time of analysis was 20 min.

2.6. Determination of antioxidant activity

Spectrophotometric measurements of antioxidant activity were carried out using an automated chemical analyzer BS-400 (Mindray, China). The analyzer was composed of cuvette space tempered to 37 ± 1 °C, reagent space with a carousel for reagents (adjusted at 4 ± 1 °C), sample space with a carousel for preparation of samples and an optical detector. Transfer of samples and reagents was provided by robotic arm equipped with a dosing needle (error of dosage up to 5 % of volume). Cuvette contents were mixed by an automatic mixer including a stirrer immediately after addition of reagents or samples. Contamination was reduced due to its rinsing system, including rinsing of the dosing needle as well as the stirrer by MilliQ water. For detection itself, the following range of wave-lengths was used as 340, 380, 412, 450, 505, 546, 570, 605, 660, 700, 740 and 800 nm.

2.6.1. Determination of antioxidant activity by the ABTS test

Assay was carried out according to the following papers [20,21]. A 150 μl volume of reagent (7 mM $ABTS^{\bullet}$ (2,2′-azinobis 3-ethylbenzothiazoline-6-sulfonic acid and 4.95 mM potassium peroxodisulphate) was poured with 3 μl of sample. Absorbance was measured at 660 nm. For calculating of the antioxidant activity, difference between absorbance at the last 10^{th} minute and second minute of the assay procedure was used.

2.6.2. Determination of antioxidant activity by the ferric reducing antioxidant power (FRAP) method

Procedure for the determination was done in compliance with reported papers [20,21]. A 150 µl sized volume of reagent was injected into a plastic cuvette with subsequent addition of a 3 µl of sample. Absorbance was measured at 605 nm for 10 minutes. Difference between absorbance at the last 10^{th} minute and second minute of the assay procedure was used for calculating of the antioxidant activity.

2.6.3. Determination of antioxidant activity by the dimethyl-4-phenylenediamine (DMPD) method

The assay was done in a way as described in reported papers [20,21]. A 160 µl volume of reagent (200 mM DMPD, 0.05 M $FeCl_3$, 0.1 M acetate buffer pH 5.25) was injected into a plastic cuvette with subsequent addition of 4 µl sample. Absorbance was measured at 505 nm. Difference between absorbance at the 10^{th} minute and second minute of the assay procedure was used for calculating of the antioxidant activity.

2.6.4. Determination of antioxidant activity by the Free Radicals (FR) method

We used the previously optimized experimental protocol for the free radical method [22].A 150 µl volume of reagent was injected into a plastic cuvette and poured with 6 µl of sample. Absorbance was measured at 450 nm after two and 10 minutes and differences in the absorbancies were used for the further data processing.

2.6.5. Assay of uric acid

For the assay purposes, we poured 200 µl of 1 mM 2,4,5-tribromo-3-hydroxybenzoic acid (Greiner, Germany) in 100 mM phosphate buffer pH 7.0 with 4 µl of sample and 50 µl of a reagent consisting from 4-aminoantipyrine, 10 µM potassium ferrocyanide, peroxidase 2 kU/l, urikase 30 U/l and the same phosphate buffer as above. Absorbance was measured at 546 nm after an incubation lasting 6 minutes. The 2,4,5-tribromo-3-hydroxybenzoic acid solution was used for blank purposes.

2.7. Statistics

Data were processed using MICROSOFT EXCEL® (USA) and STATISTICA.CZ Version 8.0 (Czech Republic). Data are expressed as mean ± standard deviation (S.D.) unless otherwise noted (EXCEL®). Statistical significance of the measured data was determined using STATISTICA.CZ. Differences with $p < 0.05$ were considered significant and were determined by using one way ANOVA test (particularly Scheffe test), which was applied for means comparison.

3. RESULTS AND DISCUSSION

Different experimental methods were used for investigation of oxidative stress in medulloblastoma, neuroblastoma, glioblastoma and other brain tumors suffered childhood patients. Our principal interest was focused on electrochemical assay performed on screen printed electrodes in combination with SWV. Beside the SWV, some other oxidative stress markers such as GSH, GSSG [23], and a specific tumor marker metalothionein [24-26] were assessed in the samples by high performance liquid chromatography with electrochemical detection and differential pulse voltammetry Brdicka reaction. Antioxidant capacities were assessed as reference markers using standard protocols based on photometry.

3.1 Assay of low molecular weight level using square wave voltammetry

Square wave voltammetry of plasma samples on graphite screen printed electrodes provided two peaks marked as CVi_1 and CVi_2. Peaks CVi_1 and CVi_2 were positioned at 562 ± 20 mV and 839 ± 17 mV. Typical voltammograms of a plasma sample from medulloblastoma, neuroblastoma, glioblastoma and other brain tumors patients are shown in Figures 1, 2, 3 and 4, on the left. Average squares of the peaks were 6.88×10^{-3} µA/V for the peak CVi_1 and 4.24×10^{-3} µA/V for the peak CVi_2. Though the peak CVi_1 was detected in all tested samples, the peak CVi_2 was nearly depleted in some samples. We could appoint at significant pathology related to not all of the endogenous antioxidants but to only a specific group of it. The effect is not fully understood. However, it is supported by reports of the other scientists [27,28]. The heights of both detected peaks measured in medulloblastoma, neuroblastoma, glioblastoma and other brain tumors patients are shown in Figures 1, 2, 3 and 4, on the right.

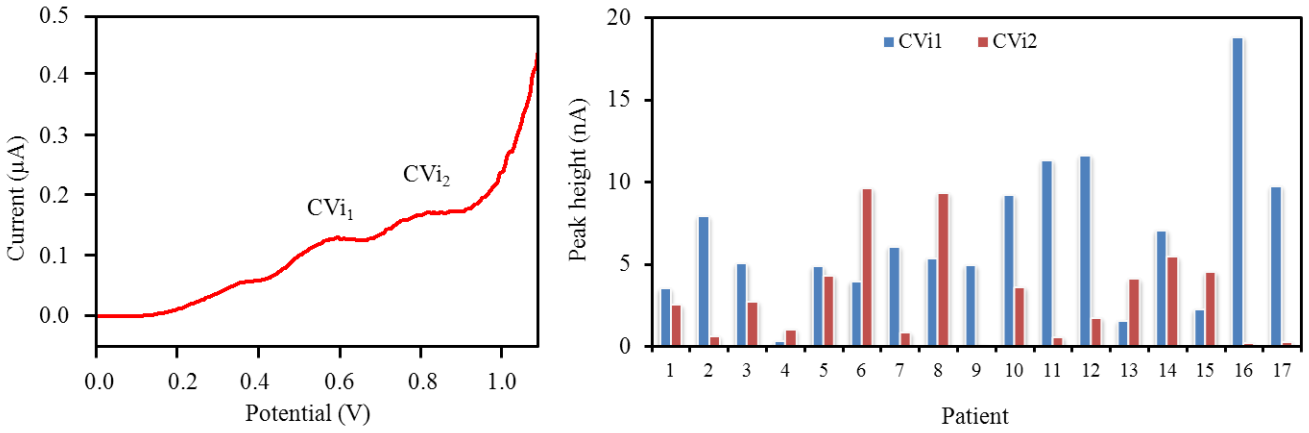

Figure 1. Typical SW voltammogram from a patient with diagnosed medulloblastoma. Screen printed sensors with graphite working, platinum reference and Ag covered with AgCl reference electrodes were used, electrolyte. The potential was applied in a range start from 0 to finish 1.1 V and potential step 5 mV, frequency 1 Hz, phosphate buffer pH 7. In a total 17 patients were assayed.

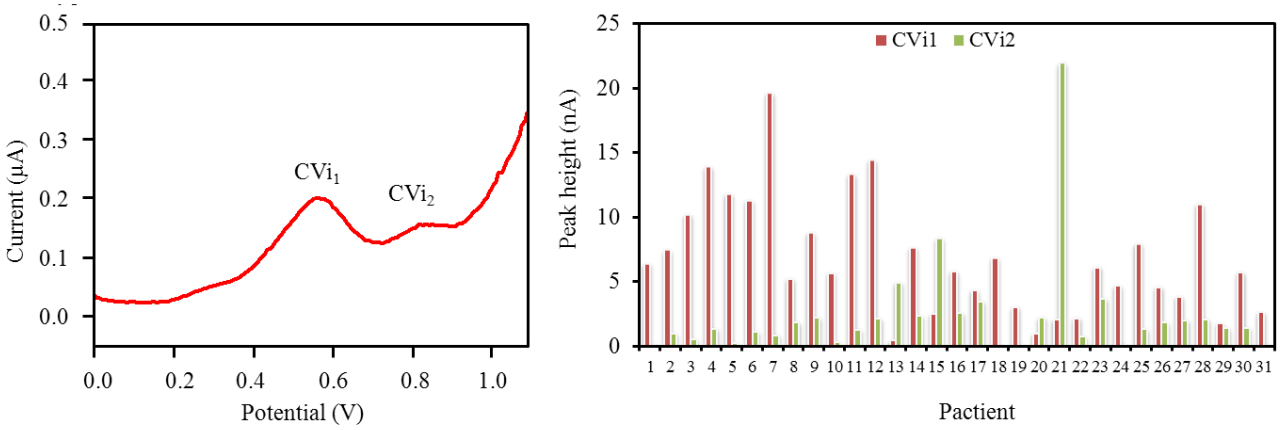

Figure 2. Typical SW voltammogram from a patient with diagnosed neuroblastoma. Other conditions see in Fig. 1. In a total 31 patients were assayed.

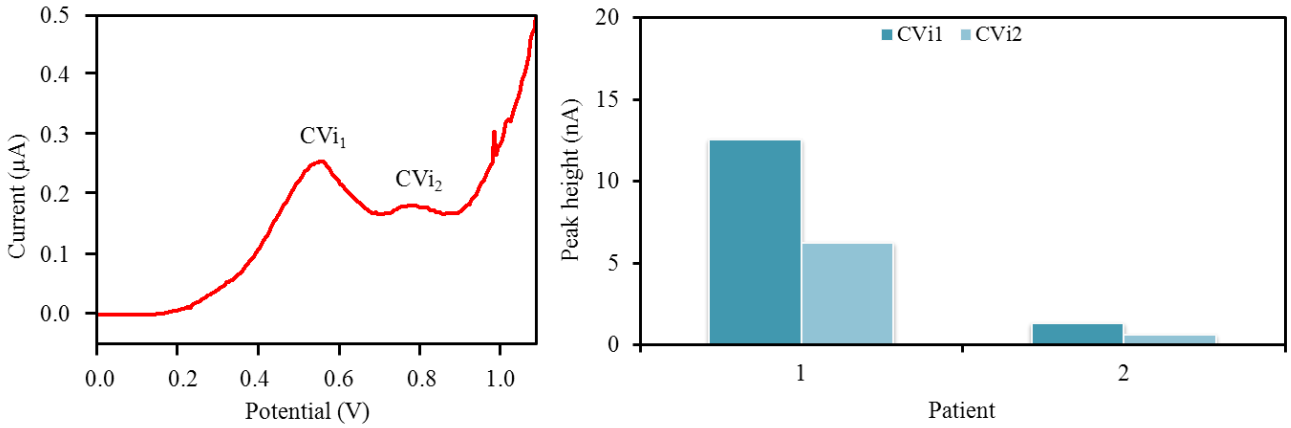

Figure 3. Typical SW voltammogram from a patient with diagnosed glioblastoma. Other conditions see in Fig. 1. In a total two patients were assayed.

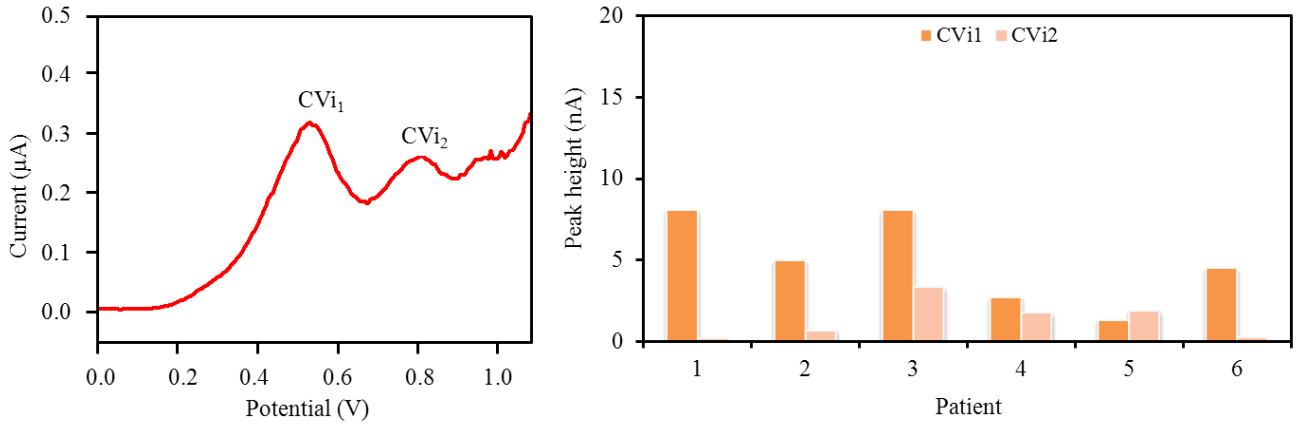

Figure 4. Typical SW voltammogram from a patient with other brain tumors. Other conditions see in Fig. 1. In a total 6 patients were assayed.

Based on the published papers and our experimental results, voltammetry can be used for measuring highly important LMWAs. The antioxidants are a wide group of compounds reacting with reactive oxygen and nitrogen species. GSH, reduced nicotinamide adenine dinucleotide, carnosine and lipoic acid can be exampled as typical endogenous antioxidants [29]. Here, voltammetry was also used to monitor the levels of antioxidant capacity in patients with tumors, in which the elevated concentration of reactive oxygen species can be expected due to tumor development and/or anticancer treatment. For measuring antioxidant capacity, we utilized carbon paste electrodes, which are easy to prepare. As a part of carbon paste, microparticles of sizes 2-5 mm (expanded carbon and carbon nanoparticles) were used. The electrodes proved reliability for the assay purposes. For the assessment of antioxidant capacity the sum of the areas of all signals measured in the samples were used. The signals were recorded in anodic area, in which the antioxidants can be simply measured [30]. The significant correlation to the assayed GSH and focusing of thiols peak in square wave voltammetry when considered the previous report confirms that the idea that the pathologies are related to depletion of thiol containing antioxidants [14]. The finding is in compliance with quoted papers as well [27,28,31,32].

3.2 Assay of GSH and GSSG

When considered determination of thiol containing reduced glutathione (GSH), the level was in a range 0.5 – 5.5 μg/mg of protein. The oxidized form of glutathione (GSSG) ranged in approximately 0.4 – 4 μg/mg of protein. The result point at fact that thiol containing compounds including GSH are responsible for formation of the CVi_2 peak, but the peak CVi_1 is formed by action of other LMWAs. GSSG does not significantly participate in the peak formation as the both peaks CVi_1 and CVi_2 are not well correlated with the marker (correlation coefficient under 0.2). GSH levels were observed in glioblastoma cells as decreasing regards to the increasing of ROS and lactate level as a result of TIGAR. As Iida et al. present, the intracellular concentration of GSH influenced the sensitivity of glioblastoma cells. It was confirmed the GSH increased resistance to H_2O_2 and other ROS [33]. The high levels of GSH in glioblastoma cells can be associated with a resistance to ionizing radiation and anticancer drugs. The rate-limiting enzyme for GSH synthesis is gamma-glutamylcysteine synthetase (gamma-GCS). It was investigated the continuous down-regulation expression influence on resistance to ionizing radiation and cisplatin. Hammerhead ribozyme against gamma-GCS was estimated having a potential to reduce the resistance of malignant cells to ionizing radiation and anti-cancer drugs [34]. Recently the protective influence of fatty acid oxidation for regeneration the GSH antioxidant system and protective effect on cancer cell against ROS, ATP depletion and death was published [35]. It is clear that GSH plays considerable role in tumor development.

3.3 Assay of metallothionein

Metallothioneins are a group of proteins rich in cysteine with molecular weights ranging from 6 to 10 kDa [36-38]. Metallothioneins play a key role in maintaining homeostasis of essential metals and

in protecting of cells against metal toxicity as well as oxidative damaging [37,39-42]. In this study, we applied differential pulse voltammetry Brdicka reaction (DPV Brdicka reaction) for determination of MT in blood serum of patients with various embryonal tumors. The principle of the detection technique called DPV Brdicka reaction bases in adsorption of thermostable metallothionein directly to the surface of the mercury electrode. The electrochemical analysis of MT resulted in appearing of three signals in the obtained voltammograms. The signal of MT complex with cobalt ions called RS2Co appeared at -1.0 V. Two other signals called Cat1 (-1.2 V) and Cat2 (-1.4 V) were catalytic. Cat2 height signal was proportional to concentration of MT in a sample. These signals correspond to the presence of free SH groups in MT molecule [43,44].

In our previous experiments we have studied association of metalothionein to cancer progression [17,25,26,41,45-51]. A monitoring of MT level at patients with a tumor disease could be useful from various points of view. One of the points of view is the role of MT as marker of chemoresistance to heavy metal based cytostatics [41]. Another possible point of view is comparison of MT level by various types of embryonal tumors, which is case of this experiment. Owing to conclusions from our previous papers, the elevated level of metallothionein in blood can be considered as a marker of cancer.

Table 1. Average levels of selected markers.

	peak CVi_1 (µA/V)	peak CVi_2 (µA/V)	GSH (µg/mg)	GSSG (µg/mg)	MT (µg/mg protein)
medulloblastoma	6.65	2.99	2.20	1.18	2.70
neuroblastoma	6.85	2.39	2.39	1.34	2.50
glioblastoma	9.44	1.02	4.50	1.13	2.07
other brain tumors	4.92	1.31	0.83	0.43	2.20

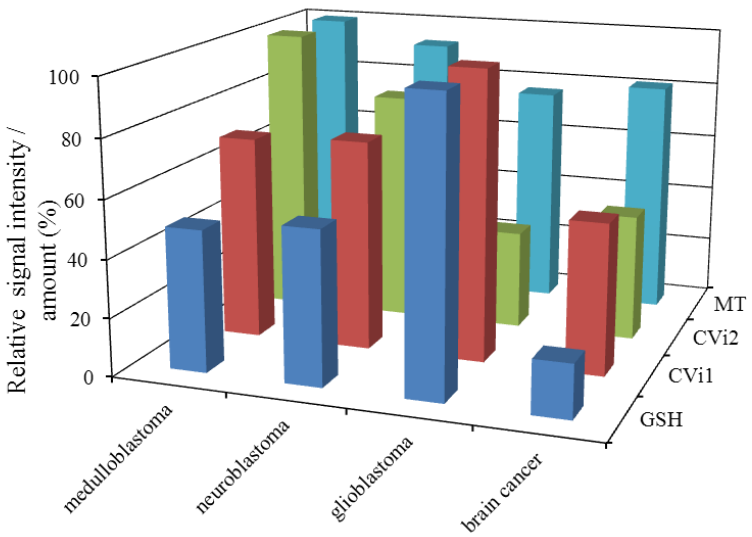

Figure 5. Correlation of various markers values. Relative change in square signal / amount of markers values with blastom variety; values are related to the maximum value of every marker.

Here reported experiment concerns on cancer type distinguishing using the metallothionein level and level of the other markers (indicated in Table 1). The upper levels of metallothionein were determined in medulloblastoma positive patients. Neuroblastomas and other brain tumors followed. The lowest levels of metallothionein were found in glioblastoma diagnosed patients.

Relative changes of markers levels in various blastomas patients are shown in Fig 5. Change of CVi1 peak corresponds to the change of GSH marker. Marker CVi1 and GSH have maximum values at glioblastomas and minimum at brain cancers. Change of CVi2 peak corresponds to the change of MT marker. Both markers have theirs maximum values at medulloblastomas and minimum at glioblastomas. It is clear from the obtained results that CVi2 peak determination is more sensitive to embryonal tumors present than changes in MT amount in blood serum determined by DPV Brdicka reaction. Opposite this CVi1 peak determination is less sensitive than GSH changes through embryonal tumors influence.

3.4 Assay of uric acid

Beside the other low molecular antioxidants, we decided to assay one of the most important antioxidants as uric acid (UA). It is an antioxidant formed in the body as a product of purine degradation [52]. Antioxidant potency of the uric acid is based on generation of urate radical being degraded by the other antioxidants. Moreover, uric acid can give stable complexes with iron ions, protects from iron mediated radical reactions, decrease peroxidation of lipids and participates in regeneration of vitamin E. Due to the mentioned facts, level of uric acid significantly influences antioxidant activity/capacity in organism and helps to reduce risk and rate of disparate disorders including Parkinson disease, myasthenia gravis and neuromyelitis [53-55].

Several researchers reported that uric acid level is depleted in patients with diagnosed cancer when compared to the health individuals [56,57]. On the other hand, uric acid can be increased due to some cancer events [58,59]. The increase can be caused by chemotherapy or irradiation during the therapy. An extensive study on issue of uric acid role in pathogenesis of cancer was made by Kolonel et al. [60]. They searched for alteration of uric acid level in Japanese men with positively diagnosed cancer and they did revealed no significant alteration in the uric acid level for total cancer (1544 cases), cancers of the stomach (214), colon (272), rectum (105), lung (223), bladder (89), or hematopoietic system (77). However, they proved significant positive association of uric acid level in prostate cancer (293 cases) suffering patients. The findings confirm the idea that uric acid level is altered in some pathogenesis only.

In here reported experiment, levels of uric acid were quite variable as the level ranged from 102 to 442 μM in the examined samples. The values were probably differing in such range due to age, disparate diagnoses, and phases of therapy. Correlation coefficients for the examined groups were made using the other assessed markers. The correlation coefficients were quite high for results from the other methods including SWV (Tables 2, 3 and 4). The results confirm implication of uric acid in the tested central nervous system cancers pathogenesis and appoint at good accessibility of uric acid as a diagnostic marker. In the course of the good correlation, uric acid level can be chosen as an

additional marker for cancer diagnosis. The markers can be simply measured with quite low costs and the assay is readily available to automation.

3.5 Assay of oxidative stress using standard photometric tests for assessment of low molecular weight antioxidants

Photometric assays of low molecular weight antioxidants level were used as a standard reference tools to the above mentioned methods. ABTS, FRAP, DMPD and FR were used for the purposes. The antioxidants levels were expressed as trolox equivalents (TE). The ABTS radical method is one of the most used assays for the determination of the concentration of free radicals. It is based on neutralization of a free radical arising from the one-electron oxidation of the synthetic chromophore 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid). The principle can be expressed by a simple equation: $ABTS^{\bullet} \rightarrow ABTS^{\bullet+} + e^{-}$. The FRAP method is based on the reduction of complexes of 2,4,6-tripyridyl-*s*-triazine with ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), which are almost colorless, providing slightly brownish coloration in the final. The compound *N*,*N*-dimethyl -1,4-diaminobenzene (DMPD) is converted in solution to a relatively stable and colored radical form ($DMPD^{\bullet+}$) by the action of ferric salt. After addition of a sample containing antioxidants, $DMPD^{\bullet+}$ radicals are scavenged and decolorized due to the scavenging [61]. The method FR is based on ability of chlorophyllin (the sodium-copper salt of chlorophyll) to mediate electron transfer accompanied with change in absorbance. This effect is conditioned by an alkaline environment and the addition of catalyst. The results of antioxidant capacities were correlated with values of peaks CVi_1 and CVi_2, GSH, GSSG and one to each other. The found correlation coefficients are summarized in table 2 for medulloblastoma, table 3 for neuroblastoma, table 4 for central nervous system ("brain") cancer. The most significant (the highest correlation coefficients) were found in samples from medulloblastoma diagnosed patients. It was recognized for GSH and GSSG (0.911) and for MT and GSH to GSSG ratio (0.604). The results point at good applicability of the markers for diagnostic purposes. Moreover, physiological coherences between the markers can be inferred.

Table 2. Correlation coefficients between results from the used methods when blood samples from medulloblastoma diagnosed patients performed in the assays.

Analyse	CVi_2	FR	FRAP	ABTS	DMPD	MT	GSSG	GSH	GSH/ GSSG	UA
CVi1	-0.377	-0.063	0.001	-0.187	0.267	0.009	0.187	0.074	-0.206	0.321
CVi2	x	-0.207	0.260	0.417	-0.345	-0.266	0.381	0.438	-0.373	0.369
FR		x	0.296	0.357	-0.073	0.381	0.365	0.433	0.473	0.546
FRAP			x	0.350	0.079	-0.001	0.155	0.211	0.119	0.499
ABTS				x	-0.447	0.358	0.531	0.484	0.107	0.489
DMPD					x	-0.073	-0.114	-0.151	-0.059	0.452
MT						x	-0.162	-0.050	0.604	0.368
GSSG							x	0.911	-0.417	0.401
GSH								x	-0.195	0.368
GSH/GSSG									x	0.225

Table 3. Correlation coefficients between results from the used methods when blood samples from neuroblastoma diagnosed patients performed in the assays. High correlation coefficients were proved for GSH and GSSG (0.773) and for FR and ABTS (0.690).

Analyse	CVi_2	FR	FRAP	ABTS	DMPD	MT	GSSG	GSH	GSH/ GSSG	UA
CVi1	-0.287	0.069	-0.186	-0.044	-0.341	0.347	-0.023	0.025	0.220	0.398
CVi2	x	0.113	0.288	-0.112	0.336	-0.102	0.126	0.103	-0.140	0.405
FR		x	0.341	0.690	0.049	-0.075	0.044	-0.002	0.055	0.587
FRAP			x	0.234	0.193	-0.641	0.144	-0.095	-0.336	0.452
ABTS				x	-0.012	-0.182	0.138	0.091	-0.100	0.541
DMPD					x	-0.360	-0.080	-0.286	-0.105	0.474
MT						x	0.061	0.113	0.144	0.308
GSSG							x	0.773	-0.639	0.385
GSH								x	-0.174	0.371
GSH/GSSG									x	0.294

Table 4. Correlation coefficients between results from the used methods when blood samples from other brain tumors diagnosed patients performed in the assays.

Analyse	CVi_2	FR	FRAP	ABTS	DMPD	MT	GSSG	GSH	GSH/ GSSG	UA
CVi1	0.016	0.633	-0.388	0.478	-0.434	0.884	-0.161	0.354	0.358	0.375
CVi2	x	-0.512	0.687	-0.668	0.554	0.343	-0.717	0.492	0.777	0.486
FR		X	-0.919	0.881	-0.807	0.236	0.410	0.090	-0.295	0.555
FRAP			x	-0.897	0.847	-0.012	-0.563	0.023	0.476	0.492
ABTS				x	-0.975	0.065	0.745	-0.335	-0.599	0.482
DMPD					x	-0.060	-0.760	0.359	0.578	0.494
MT						x	-0.522	0.545	0.703	0.355
GSSG							x	-0.823	-0.957	0.377
GSH								x	0.830	0.366
GSH/GSSG									x	0.312

For other brain tumors diagnosed patients, the highest correlations were detected for peak CVi_2 to MT (0.884), FR to ABTS (0.881), and GSH to GSH/GSSG (0.830). The findings support expectations that MT plays a significant role in the cancer related processes. The alterations in peak CVi_1represented antioxidants are inferred to be a related process. It is noteworthy that correlations for glioblastoma were not done as the disease is quite rare and we received only samples from two patients. The number of specimens is too low to make meaningful data processing. Considering the assessed markers were recommend to use SWV for a routine diagnosis of cancers. Though the findings are not specific to cancer only, it may be a supporting marker to provide more exact diagnosis of cancer type. A parameter such as electrochemical index would be introduced for the purposes. The index would consist from a peak CVi_1 and MT values. The simultaneous increase in peak CVi_1 and MT appoint at glioblastoma and other brain tumors rather than meduloblastoma and neuroblastoma.

4. CONCLUSIONS

In a conclusion, electrochemical detection using a simple square wave voltammetry is a suitable tool for monitoring of the antioxidant capacity in biological samples. The method was tested on an example of brain tumor suffered child patients. Reliability of the method was proved and it well correlated to the standard test. We consider the square wave voltammetry as a standard tool replacing the more elaborative protocols in some experiments. Especially, we are encouraged in application of SWV for distinguishing in type of cancers.

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