

## Determination of metallothionein, glutathione and thermostable protein fraction concentrations in tumour tissue from head and neck area

Natalia Cernei, Petr Majzlik, Ondrej Zitka, Dalibor Huska, Hana Binkova,  
Michal Masarik, Vojtech Adam, Rene Kizek\*

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### Abstract

Tumours of head and neck are one of the most incident tumour diseases. Well-timed diagnosis plays main role in their therapy. Aim of this work consisted in determination of metallothionein (MT), which could be new tumour marker. Determination of its concentration was carried out using electrochemical detection with use of differential pulse voltammetry. Concentrations of MT in samples of denaturated blood plasma of group of patients were about 2.3  $\mu\text{M}$  with slight differences in accordance with localization of tumours. Contents of glutathione and total proteins in denaturated were determined chromatographically and by the help of Biuret reaction. Rate of concentrations levels of GSH/GSSG was varying from 1.31 to 17.66.

**Keywords:** Metallothionein, GSH and GSSG, new tumour marker, thermostable proteins.

### Introduction

Annually, many millions of people suffer from different types of tumour disease; unfortunately, effective way of treatment is still

Natalia Cernei, Petr Majzlik, Ondrej Zitka, Dalibor Huska,  
Vojtech Adam, Rene Kizek\*

Department of Chemistry and Biochemistry Faculty of Agronomy,  
Mendel University in Brno, Zemedelska 1, CZ-613 00 Brno, Czech  
Republic

Hana Binkova

Department of Otolaryngology and Maxillofacial Surgery,  
University Hospital, Pekarska 53, CZ-656 91 Brno, Czech Republic

Michal Masarik

Department of Pathological Physiology, Faculty of Medicine,  
Masaryk University, Komenského náměstí 2, CZ-662 43 Brno,  
Czech Republic

\*Tel: +420 545 133 350, Fax: +420 545 212 044  
E-mail: kizek@sci.muni.cz

missing. In therapeutic procedures, well-timed diagnosis plays crucial role. Metallothionein, which is promptly and exactly determinable, was in our work used as potential marker of tumour disease. Metallothionein is small protein with high content of amino-acid cysteine, without aromatic amino-acids. Metallothionein consists of two domains  $\alpha$  and  $\beta$ , which are together able to bind up to seven ions of divalent metals. This protein is excellent transporter of metals, in physiological state especially of zinc and copper. Antioxidant activity of metallothionein was also determined. Its increased expression in some types of tumour diseases was documented before.

Metallothionein is supposed to participate in conception of resistance of tumour cells to certain types of chemotherapeutics, such as cisplatin. Structural characteristics make metallothionein to be excellent candidate for electrochemical detection. Prof. Brdicka was interested in similar ways of analysis more than 70 years ago. Result of analysis consists in curve with three signals, which the last is catalytic and depends on metallothionein concentration in sample. For analysis, we chose samples of blood from patients suffering by tumours in neck and head area. Moreover we studied a rate of concentrations of glutathione reduced and oxidized (GSH/GSSG) which provides information of oxidative stress. When this rate is less than value of 10 oxidation stress in organism is high. MT and glutathions are thiols which means that they are part of thermostable protein fraction of the sample of blood is denaturated (Kagi et al. 1988).

### Materials and methods

Samples were analyzed on apparatus 747 VA Stand in connection with 746 VA Trace Analyzer and 695 Autosampler (Metrohm, Switzerland) in classic three-electrode arrangement. Working electrode was hanging mercury drop electrode (HMDE) with drop area 0.4 mm<sup>2</sup>, reference electrode was Ag/AgCl/3M KCl and auxiliary was platinum electrode. Basic electrolyte (1 mmol/l [Co(NH<sub>3</sub>)<sub>6</sub>]Cl<sub>3</sub> and 1 mol/l ammonium buffer; NH<sub>3</sub>(aq) + NH<sub>4</sub>Cl, pH 9.6) was changed for each five measurements. DPV parameters were as follows: initial potential -0.7 V, end potential -1.75 V, modulation time 0.057 s, time interval 0.2 s, potential step 2 mV/s, modulation amplitude -250 mV, Eads = 0 V, temperature of basic electrolyte 4 °C. Samples of blood of patients suffering from

tumours in neck and head areas were obtained from Department of Otorhinolaryngology and Head and Neck Surgery of St. Anne's University Hospital Brno, all with permission of ethic commission. Samples were prepared by following procedure: firstly, they were 100x diluted by phosphate buffer (pH 6.9, 20mM) and subsequently denatured at temperature 99°C for 15 min (Eppendorf 5430, USA). After it, samples were centrifuged (16 000 g, 4 °C, for 30 min; Eppendorf 5402, USA) with subsequent supernatant sampling, where GSH and GSSG were analyzed by liquid chromatography with electrochemical detection using reversed phase chromatographic column Zorbax eclipse AAA C18 (150 × 4.6; 3,5 µm particles, Agilent Technologies, USA) and twelve-channel CoulArray electrochemical detector (Model 5600A, ESA, USA). Mobile phase consists of A: trifluoroacetic acid (80 mM) a B: 100% Met-OH. Detection potential was 900mV. Determination of thermostable proteins was carried out using Mindray BS 200 and Biuret reaction.

For determination of the total protein content the biuret solution (15 mM potassium sodium tartrate, 100 mM NaI, 15 mM KI and 5 mM CuSO<sub>4</sub>). As a standard albumin (1 mg in 1 ml of phosphate buffer, pH 7) was used. The measurement was done as follows: 180 µl of the biuret solution was mixed with 45 µl of real or standard sample; after stirring and incubating (10 min. at 37°C) the absorbance at 546 nm was measured.

## Results and discussion

Aim of this work was at electrochemical determination of metallothionein. Quantitative information may be obtained in electrochemistry by analysis of two group of electrochemical signals – redox, which connected with electrochemical reaction of analyte, and catalytic. Catalytic signals are connected with reaction of next compounds (for example hydrogen), which originate at given conditions in presence of analyte. Brdicka reaction and H peak technique belong to the group of methods monitoring cathodic signal of hydrogen (Breazeale et al. 1996). These methods are very sensitive and in view of sample preparation very unpretentious. We analyzed 65 samples of blood of patients with malignant tumour in oropharynx localization, 15 samples of full blood of patients with malignant tumour in buccal cavity, 14 samples of full blood of patients suffering from in hypopharynx localization, 5 samples of blood of patients with malignant tumour in paranasal sinuses localization, 29 samples of blood of patients with malignant tumour in larynx localization and 5 samples of blood of patients suffering from malignant tumour of parotid glands. All samples were of first capture. Resulting values of metallothionein concentrations was about  $2.3 \pm 0.3 \mu\text{M}$  with slight differences in accordance with localization of tumours (oropharynx  $2.8 \pm 0.4 \mu\text{M}$ , buccal cavity  $2.5 \pm 0.2 \mu\text{M}$ , hypopharynx  $2.2 \pm 0.4 \mu\text{M}$ , paranasal sinuses  $2.0 \pm 0.3 \mu\text{M}$ , larynx  $2.4 \pm 0.5 \mu\text{M}$  and parotid glands  $2.0 \pm 0.4 \mu\text{M}$ ). Concentrations were compared with average value of metallothionein concentration in samples of full blood of volunteers ( $0.5 \pm 0.1 \mu\text{M}$ ). Moreover we observed that rate of concentrations levels (GSH/GSSG) was varying from 1.31 to 17.66. That shows the significance differences between each patient caused probably by different stages of illness and different type of tumors.

In addition to analysis of full blood, levels of MT, total thermostable proteins and GSH/GSSG were analyzed directly in tumour tissue.

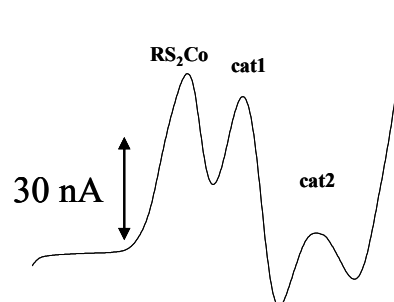


Figure 1: Voltammogram of sample of patients suffering from malignant tumour of larynx. The last signal (Cat2) corresponds to catalytical reduction of hydrogen ions of electrolyte and is directly proportional to concentration of metallothionein in sample.

## Conclusion

After comparison of metallothionein levels in patients and controls it is well evident, that metallothionein levels in case of patients are enhanced. By this fact we verified theoretical presumption for using of metallothionein as tumour marker; suitability of electrochemical techniques for its determination and analysis of proteins was also proved. Electrochemical methods would have in proteomics their position, especially thank to their low costingness, rapidity and sensitivity.

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