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Metallothionein and manganese concentrations in brain tumors

Bolesław Floriańczyk[1,2], Robert Kaczmarczyk[2], Jacek Osuchowski[2], Tomasz Trojanowski[2]

[1] *Department of Environmental Hygiene, Institute of Agricultural Medicine, Lublin, Poland*
[2] *Department of Neurosurgery and Children's Neurosurgery Clinic, Medical University of Lublin, Poland*

Abstract: Metallothioneins (MT) take part in the homeostasis of the metals which are necessary for the proper metabolism and they also take part in the detoxication of toxic metals from the tissues. They also protect the tissue from the effects of reactive oxygen species, radiation, electrophilic pharmacologic agents used in the cancer therapy and from mutagens. Manganese (Mn) – essential microelement – seems to be very important during development. Mn takes a part as cofactor of the enzymes regulating the metabolism. This microelement also participates in the reduction-oxidation reactions. An example of such reaction is the superoxide dismutase (MnSOD) activity with manganese as enzyme component. It is thought that manganese and manganese superoxide dismutase play an important role in the differentiating cells. The aim of this work was to determine the level of metallothionein and manganese in the brain neoplastic tissues, *astrocytoma* G-2 and malignant gliomas (*astrocytoma* G-4). The experimental materials were primary glial the brain neoplastic tissues resected during neurosurgical procedures. The level of the metallothionein was determined by the cadmium-hemoglobin affinity assay using the cadmium isotope (^{109}Cd), and the concentration of manganese was determined by atomic absorption spectrometry. The concentration of metallothionein and manganese was found to be higher in the *astrocytoma* G-4 (*glioblastoma multiforme*) patients. Negative correlation between the concentration of metallothionein and manganese ions was found in *astrocytoma* G-2 cells.

Key words: metallothionein, manganese, astrocytoma

INTRODUCTION

Metallothioneins (MT) are a widespread proteins in the animal world. These proteins are characterised by a great invariability of their structure. While isolated from various animals they only slightly differ from one another in the amino acid composition. The number of amino acids is constant in every animal group, and that is 60 (or 61) amino acids, 20 of which are the cysteines residues, which makes over 30% of the amino acids composition. Such a high amount of cysteins determines the metallothionein's functions [1-4]. Metallothioneins take part in the homeostasis of the ions of the metals which are necessary for the proper metabolism of the organism and they also take part in the detoxication of the tissue from toxic metals. Apart from these they also protect the tissue from reactive oxygen species, radiation, electrophilic pharmacological agents used in the cancer therapy and the mutagens [5-7]. The induction of metallothionein synthesis is influenced by many factors: heavy metals, inflammatory factors, reactive oxygen species, glycocortycoids and pharmacological agents [8].

Manganese (Mn) seems to be very important microelement during development. This microelement participates in the reduction-oxidation reactions due to its chemical properties (several oxidation stages). An example of such reaction is the superoxide dismutase (MnSOD) activity with manganese as enzyme component [9, 10]. It is thought that manganese and manganese superoxide dismutase play an important role in the differentiating cells [11].

Corresponding author: Bolesław Floriańczyk, MD, Phd, Department of Environmental Hygiene, Institute of Agricultural Medicine, Jaczewskiego 2, 20-090 Lublin, Poland. E-mail: bolkofs@poczta.onet.pl

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MATERIALS AND METHODS

Materials

The experimental materials were the brain neoplastic tissues resected during neurosurgical procedures. The brain tumors were divided into two groups: benign gliomas (*astrocytoma* G-2, n = 20) and malignant gliomas G-4 (*glioblastoma multiforme*, n = 23). The patients had not been exposed to any prior treatment for their tumor disease.

Methods

Determination of the metallothionein concentration

The concentration of the metallothionein was determined by the cadmium-haemoglobin affinity assay, using the cadmium ^{109}Cd isotope (Du Pont, USA) [12]. The tissues were homogenised in 4 volume of 10 mM Tris-HCl pH 7.4, then the homogenate was centrifuged at 10,000 g for 10 min. and the supernatant was heated for 2 min. at 100^{0}C on water bath. Samples were centrifuged at 10,000 g for 2 min. in order to remove precipitated proteins. Carrier-free ^{109}Cd was dissolved in 10 mM Tris-HCl buffer pH 7.4 adding $CdCl_2$ to yield a Cd concentration of 2.0 μg/ml and radioactivity of 1.0μCi/ml. Next, 200μl of this ^{109}Cd solution was mixed with 200μl of homogenate and incubated for 10 min. Then 100 μl of a 2% bovine haemoglobin solution (Sigma, USA) was added to the tube, heated at 100^{0}C on the water bath for 2 min and 100 μl of the 2% haemoglobin solution was again added. After 2 min.

heating, centrifugation at the same conditions was performed. The radioactivity of the supernatant samples (500μl) were measured using gamma-counter (Beckman LS6000TA, USA). Blank samples (buffer instead of the tissue sample) and total activity samples (buffer instead of haemoglobin) were run simultaneously [12].

Determination of the manganese concentration

Tissue samples were subjected to desiccation for 72 hours at 80°C, ashed at 450°C, and then dissolved in a concentrated HCl mixed with distilled water (1:1, v/v). The concentrations of manganese were determined by spectrophotometric method using atomic absorption spectrophotometer (Pye Unicam SP-192, U.K.) [13, 14].

Statistical Analysis

The statistical analysis of the results was conducted using SPSS 8,0 pack. To evaluate the significance of variable discrepancies of standard distribution we used t-Student test for independent trials. The interdependencies of particular parameters in the groups were examined by means of Pearson correlation coefficient and linear regression. The assumed hypotheses were verified on the significance level of $p < 0,05$.

RESULTS

Quantitative assay of metallothionein by means of cadmium-haemoglobin method provided information concerning their concentration in the tissues. The metallothionein concentration in the astrocytoma G-4 group was approximately 27% higher when compared to the astrocytoma G-2 group (these differences were statistically significant) (Tab. 1). A tendency of a higher concentration of manganese (about 11%) in the GM-4 group was found as well. The metallothionein concentration and manganese content was negatively correllated in G-2 group only ($r = -0.4722$, $p < 0.05$*).

DISCUSSION

Living organisms have developed mechanisms of utilising vital trace elements such as zinc and copper, and reducing to the minimum the toxic influence of heavy metals like cadmium, mercury and lead [15]. Metallothionein, intracellular proteins rich in cysteine residues, have the ability to bind excess of metal ions, thus regulating the number of their free ions [1, 2, 16]. The binding of metals goes on via cysteine residues.

In mammals, the highest concentration of MT can be found in the liver and kidney (10-15 μg of MT per gram of wet tissue). It indicates the important role of these organs in the detoxication processes and in the homeostasis of metal ions [17]. According to the literature data [2] in the other tissues, the concentration of MT is significantly lower (1-2 μg of MT per gram of wet tissue).

The concentration of metallotionein in the brain tumors is relatively high and in many cases is significantly higher their concentration in the liver and kidney [5, 18, 19]. Relatively high level of metallothionein in the neoplastic tissues indicates a very important role they play in the metabolism of cancer cell.

The synthesis and intracellular distribution of MT is a very important aspect in oncology, because these proteins not only indicate a protective role in environmental factors, but also are responsible for the cell's resistance to pharmacological medications [20].

The intensive expression of intracellular MT and manganese has been shown in many types of human and animal neoplasms [19, 21-28]. The studies have shown that the following factors induce the synthesis of MT in the neoplasm cell: tumor necrosis factor (TNF), interferon-α, interleukin-1, interleukin-2) [29]. The same factors induce increase concentration of manganese in the cancer cell.

Manganese seems to be very important microelement during development. Mn takes a part as co-factor of the enzymes regulating the metabolism of glucose and other carbohydrates, as well as lipids and proteins synthesis. Mn is also needed for the synthesis of proteoglycans and glycoprotein. It is a crucial component for the bone and nerves system formation [30, 31]. This microelement also participates in the reduction-oxidation reactions due to its chemical properties (several oxidation stages). An example of such reaction is the superoxide dismutase (MnSOD) activity with manganese as enzyme component [9, 10]. It is thought that manganese and manganese superoxide dismutase play an important role in the differentiating cells [11].

Negative correlation between the concentration of metallothionein and manganese ions found in brain neoplastic tissues indicates that their role are independent.

In normal conditions metallothionein isolated from the liver contains mainly zinc and copper. This observation leads to the conclusion that the primary function of metallothionein is the homeostasis of the microelements (Zn, Cu) necessary for correct growth and metabolism [32].

Although factors inducing the synthesis of MT are known, the mechanisms responsible for MT distribution have not yet been well documented. The synthesis of the MT is induced by the ions of metals, hormones, inflamatory factors, free radicals, physical stress and some pharmacological agents. The presence of the regulatory sequence in the gene for MT (metal responsive element – MRE and glycocortycoid responsive element – GRE), enables direct induction of the transcription with the participation of the metals ions and glycocortycoid. The other factors stimulate the synthesis of MT in an indirect way [33, 34]. In the case of all the factors there are differences in answer an idepending on the type of tissue and the kind of induced metallothionein [35].

Table 1 The concentration of metallothionein (μg/g of wet tissue) and manganese (mg/kg of wet tissue) in brain tumors.

	Metallothionein (μg/g of wet tissue)		Manganese (mg/kg of wet tissue)	
Investigated group	Mean value x	Standard deviation SD	Mean value x	Standard deviation SD
Astrocytoma G-2 (n = 20)	24.26	8.59	0.37	0.09
Astrocytoma G-4 (n= 23)	30.71	7.84	0.41	0.15
	Significance level $p < 0.05$*		Significance level $p = 0.29$	

CONCLUSSIONS

The cocentration of metallothionein and manganese differ in benign and malignant gliomas.

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