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Metallothioneins and microelements in brain tumours

Metallothioneins (MT) are the widespread proteins in animal world. While isolated from the different organs of different animals they only slightly differ from one another in the aminoacid composition. The number of aminoacids is fixed in every animal group, that is 60 (or 61) aminoacids, 20 of which are the cysteine radicals, which what makes over 30% of the amonoacid composition. Such a large ammount of cysteine, which include the reactive sulfhydryl groups -SH determines the metallothioneins' functions (1,3,4).

Metallothioneins take part in the homeostasis of metal ions which are the necessary for the proper metabolism of the organism (zinc, copper), regulation of the synthesis of the zinc proteins (for example the zinc-dependent transcription factors). They also take part in the removal of toxic metals from the tissue. Apart from al this, they also protect the tissue from free radicals, radiation, electrophilic pharmacological agents used in the cancer therapy and the mutagens (6,9).

The induction of metallothionein synthesis is influenced by many factors including heavy metals, inflammatory factors, free radicals, glucocorticoides and other hormones and the pharmacologic agents (11).

The aim of this work was to determine the levels of metallothionein, zinc and copper in brain neoplastic tissues. The research was initiated to determine whether the neoplastic process changes the values of metallothioneins, zinc and copper in those tissues.

MATERIAL AND METHODS

The experimental material consisted of brain tumors (brain tumours resected during neurosurgical procedures). The brain tumors were divided into two groups: benign gliomas (astrocytoma in G-2, n = 25) and malignant gliomas (astrocytoma in GM-4 — glioblastoma multiforme, n = 30) resected during neurosurgical procedures. The patients had not been exposed to any prior treatment for their tumor disease.

Metallothionein determination. The levels of metallothionein were determined by cadmium-hemoglobin affinity assay using the cadmium isotope (^{109}Cd) — (2).

Microelements determination. The weighed samples of each tissue were washed out in the physiological solution and then subjected to desication for 72 hours at 80^0C, ashed at 450^0C, and then dissolved in concentrated HCl, which had been mixed 1 : 1 with H_2O (v/v). The concentration of zinc and copper was determined spectrophotometricaly using Pye Unicam (SP-192) spectrophotometer (10).

The results were analyzed statistically by means of the Cohran-Cox test accepting the differences as intrinsic at the intrinsicity level of $p < 0.05$. The results are presented in the table.

RESULTS

The content of metallothionein in malignant gliomas is slightly higher (29.15 +/- 8.16 µg/g of tissue) than in the group of benign gliomas (23.10 +/- 7.28 µg/g of tissue) (Fig.1.); the difference is statistically significant ($p < 0.05$). In the group of benign neoplasms the level of zinc and copper was 12.56 +/- 3.56 mg/kg of tissue and 4.10 +/- 1.15 mg/kg of tissue respectively. In the group of malignant neoplasms the level of these microelements is 16.28 +/- 4.32 mg/kg for zinc and 3.40 +/- 0.93 mg/kg for copper. The differences in the zinc levels are statistcally significant ($p < 0.001$), but not in the case of copper content ($p = 0.52$).

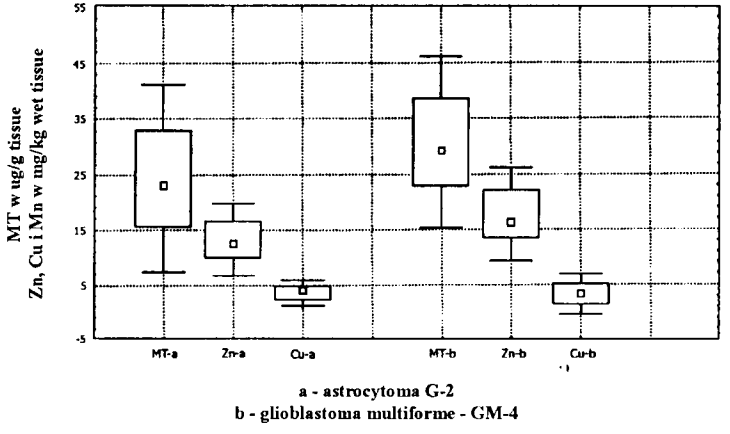

Fig. 1. The level of bioelements (in mg/kg wet tissue and metallothions (in ug/g tissue) in brain tumours

DISCUSSION

In the process of evolution living organisms have developed techniques allowing the resorption of zinc and copper, their transport and storage in the organism as well as systems protecting them against their toxic activity. These systems contain proteins of strictly determined funtions (5). The responsibility for the homeostasis of zinc and copper inside the cell is held by metallothioneins. Sulfhydryl groups frequently occuring in these proteins permit the binding of metallic ions. Although metallothioneins bind Zn and Cu with consxiderable affinity, microelements exchange is possible both between particular MT molecules as well as

other proteins and micro-molecular ligands. This exchange helps to supply proteins with zinc and copper, which they need in order to function properly.

An increased intracellular metallothionein expression and elevated zinc and copper was found in many human and animal neoplasms (7,8,13). Metallothionein synthesis induction is stimulated by such factors as metallic ions, free radicals, cytokines, lymphokines and stress. All the above mentioned factors can have their say in MT induction within a cancer cell (12,14). The mechanism that governs the induction of MT synthesis is not quite known. Research shows that cytokines like interleukin 1, interleukin 6, tumor necrosis factor (TNF) or interferon may induce MT synthesis (1). It is not impossible that neoplastic cells can release cytokines into the bloodstream, which induces MT synthesis both in the tumor and in the neoplasm-free liver.

It is very probable that the zinc accumulated in the cell induces metallothionein synthesis. The presence of a regulating MRE (metal response element) sequence in the gene for metallothionein enables direct induction of MT synthesis by means of metals (12).

In our studies, the level of metallothioneins in the group of malignant neoplasms was slightly higher than the level of these proteins in the group of benign neoplasms. In the group of malignant neoplasms, the level of zinc rises following an increase in metallothionein level, whereas the copper content is lower. For zinc contents in the studied neoplasm groups there is a statistically significant difference, but there is no significant difference between the levels of copper.

The experiments showed the function of metallothioneins as proteins taking part in the metabolism of metals that play an important role in the growth and development of the organism. In this case MT's are a reservoir of zinc and copper ions. Metallothioneins equip newly synthesised apoproteins and regulatory molecules with zinc and copper (14). This is the reason why these microelements concentrate in the area of hyperplasia, where an active process of proliferation is under way (15).

CONCLUSSION

1. In our studies, the level of metallothioneins in the group of malignant neoplasms was slightly higher than the level of these proteins in the group of benign neoplasms.
2. The level of zinc in the group of malignant neoplasms was higher than the level of these microelement in the group of benign neoplasms.

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SUMMARY

Metallothioneins take part in the homeostasis of the ions of the metals which are necessary for the proper metabolism of the organism (zinc, copper), biosynthesis regulation of the zinc containing proteins. They also take part in the detoxication of metals from the tissues. Besides,they protect the tissue from the effects of free radicals, radiation, electrophilic pharmacologic agents used in the cancer therapy and from mutagens. The experimental materials were brain astrocyto-mas, benign gliomas (astrocytoma G-2) and malignant gliomas (glioblastoma multiforme GM-4). The levels of the metallothionein were determined by cadmium-hemoglobin affinity assay using the cadmium isotope (^{109}Cd). The value of zinc and copper were determined by means of atomic absorption spectrophotometry. In our studies, the level of metallothioneins in the group of malignant neoplasms was slightly higher than the level of these proteins in the group of benign neoplasms. There was a statistical difference, but there is no significant difference in the levels of copper between malignant and benign groups.

Metalotioneiny oraz mikroelementy w nowotworach mózgu

Metalotioneiny biorą udział w homeostazie jonów metali niezbędnych do prawidłowego metabolizmu organizmu (cynk, miedź), w regulacji biosyntezy cynkoprotein oraz w procesach detoksykacji tkanek z metali toksycznych. Ponadto zabezpieczają komórkę przed wolnymi rodnikami, promieniowaniem jonizującym, elektrofilnymi środkami stosowanymi w terapii nowotworów i mutagenami. W niniejszej pracy materiał do badań stanowiły fragmenty zmian nowotworowych mózgu, pobierane podczas zabiegów operacyjnych. Guzy mózgu zakwalifikowane do dalszych badań podzielono na dwie grupy w zależności od stopnia zróżnicowania nowotworu: gwiaździaki w stopniu zróżnicowania G-2 (guzy łagodne) oraz glejaki wielopostaciowe GM-4 (guzy złośliwe). Poziom metalotionein oznaczono metodą kadmowo-hemoglobinową z zastosowaniem izotopu kadmu (^{109}Cd). Poziom cynku i miedzi oznaczono metodą atomowej spektrofotometrii absorpcyjnej. W badaniach stwierdzono wyższy poziom metalotionein oraz cynku w grupie glejaków wielopostaciowych w porównaniu z grupą gwiaździaków G-2 (różnice statystycznie znamienne). Różnicy statystycznie istotnej nie stwierdzono, porównując zawartość miedzi w obydwu grupach badanych.