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Laboratory Investigation

Quantitative analysis of copper, zinc and copper/zinc ratio in selected human brain tumors

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Summary

Serum copper and zinc concentrations and copper/zinc ratios have been shown to be increased in several types of human malignancies, including human brain tumors. In this study, copper and zinc levels and copper/zinc ratios were determined by atomic absorption analysis in tissue and serum from 29 primary and metastatic brain tumor patients. Metastatic carcinomas and malignant gliomas revealed significantly higher tissue copper concentrations than control tissues and meningiomas. Malignant gliomas demonstrated significantly higher tissue copper/zinc ratios. Both serum copper and copper/zinc ratio were significantly higher in the metastatic carcinoma group than control; however, serum copper levels in malignant glioma patients were not significantly different from control tissues. There were no differences both in the serum and the tissue concentrations of these trace elements in meningiomas and controls. These data suggested that copper, an important angiogenic factors, is accumulated within the malignant tissues of metastatic carcinoma and malignant glioma, but not meningiomas. These findings may have implications regarding angiogenesis in these tumors.

Introduction

Trace metals, such as copper and zinc, are important elements in homeostasis. Zinc (Zn) is a cofactor for many enzymes such as RNA and DNA polymerase [1]. Zinc interacts with nucleic acids to influence basepairing and DNA conformation. Such effects are known to result in somatic mutations. Nutritional deficiency of zinc results in delay of wound healing, growth retardation, anorexia, hyposmia and various skin dystrophies [2] Copper (Cu) is closely related with hematopoesis and the metabolism of osseous and connective tissue [3]. Prolonged copper deficiency is associated with anemia, leukemia, and osteoporosis [4]. In addition, copper is considered to be an important angiogenic cofactor [5–8].

Recently, several researchers have focused on the biological significance of these trace elements in malignant disease and have correlated abnormal trace element concentrations with prognosis of malignant diseases, rates of cellular growth, anaerobic glicolysis and concentration of nucleic acids in the tumor tissues [4, 9–11]. There are several reports concerning serum levels of Cu and Zn in patients with tumors; however, studies that measure tissue concentrations of these trace elements are rare [12, 13].

This study was undertaken to determine serum and tissue levels of copper and zinc and the copper/zinc ratio in selected human brain tumors. These values were compared to determine whether trace element concentrations could be correlated with histological malignancy.

Table 1. Serum levels of copper, zinc, and copper/zinc ratio

Tumor group	copper (μg/dl)		zinc (μg/dl)		copper/zinc ratio	
	mean	S.E.	mean	S.E.	mean	S.E.
control (N = 5)	98.20	11.47	119.60	8.00	0.85	0.13
meningioma (N = 5)	127.20	13.24	119.60	8.40	1.06	0.08
metastatic carcinoma (N = 8)	157.88	16.24	103.62	6.91	1.57	0.19
malignant glioma (N = 16)	128.50	9.88	117.00	3.87	1.15	0.10

Mean values and standard errors of the mean (S.E.).

Materials and methods

Tissue samples

Tissue samples from twenty-nine newly diagnosed brain tumor patients (13 males and 16 females) were evaluated. No patients had any symptoms of Cu or Zn deficiency and none had received chemotherapy or radiotherapy before this investigation (Tables 1 and 2). All metastatic tumors were from primary lung carcinoma. Control samples were obtained from 5 male patients during surgery for epilepsy. These samples did not include the epileptic focus.

Specimens of venous blood were obtained at the time of admission for surgery. Serum was separated by centrifugation (1000 g for 10 min) and the supernatants were stored at – 80° C. Tissue samples were obtained at the time of surgery and immediately stored in liquid nitrogen at – 80° C.

Cu and Zn assays

Tissue samples were weighed following vacuum-drying in a desiccator with phosphate pentoxide. Mixed acid (nitrate : perchlorate : distilled water = 1 : 1 : 1, volume/volume) was dripped on the sample for wet incineration and the sample was ustulated on a hot plate. Nitrate solution (2N) was added until the sample dissolved completely.

Copper and zinc standards were prepared by dissolving the pure metal (Kanto Chemistry) for stock solutions: (1, 2, 3 μg/ml). Optical densities from these samples were used to construct a standard concentration curve. The optic densities of the serum specimens were used to derive serum concentrations from the standard curve. Tissue copper and zinc levels were determined with a Hitachi 170 - 30 atomic absorption spectrophotometer. Tissue trace-metal concentrations (μg/g) were expressed as: μg trace-metal from spectrophotometry times capacity of 2N nitrate demanded for the solution/ the tissue weight (g) [14].

For the statistical analysis the significance of the mean values was established using the Student's *t*-test.

Results

Serum trace metal concentrations

There was no significant difference found in serum zinc levels among all groups (malignant gliomas

Table 2. Tissue levels of copper, zinc, and copper/zinc ratio

Tumor group	copper (μg/g)		zinc (μg/g)		copper/zinc ratio	
	mean	S.E.	mean	S.E.	mean	S.E.
control (N = 5)	5.37	0.08	48.57	4.06	0.11	0.007
meningioma (N = 5)	4.76	0.620	48.86	2.63	0.07	0.019
metastatic carcinoma (N = 8)	12.64	1.31	84.97	11.6	0.17	0.03
malignant glioma (N = 16)	22.69	3.36	68.36	7.35	0.29	0.042

Mean values and standard errors of the mean (S.E.).

117.00 μg/dl, metastatic carcinomas 103.62 μg/dl, meningiomas 119.60 μg/dl, control 119.60 μg/dl). Serum copper concentrations from patients with malignant gliomas (128.50 μg/dl) were not significantly different from control samples (98.20 μg/dl) or meningiomas (127.20 μg/dl). Serum copper concentrations from patients with metastatic carcinomas (157.88 μg/dl) were significantly higher than the control group ($p < 0.05$).

Serum copper/zinc ratios, were significantly higher in metastatic carcinoma patients (1.570 μg/dl) than control (0.850 μg/dl) ($p < 0.05$). There was no significant difference between the control group, meningioma group (1.060 μg/dl) and malignant gliomas (1.150 μg/dl). (Table 1 and Fig. 1.)

Tissue trace metal concentrations

Malignant gliomas (22.69 μg/g) and metastatic carcinomas (12.64 μg/g) had significantly higher values of tissue copper than both control samples (5.37 μg/g) and meningiomas (4.76 μg/g) (malignant gliomas; $p < 0.01$, metastatic carcinoma; $p < 0.05$). Metastatic carcinomas (84.97 μg/g) contained significantly higher zinc levels than both the control (48.57 μg/g) and meningioma groups (48.86 μg/g) ($p < 0.025$). We found a highly significant difference between the tissue copper/zinc ratio for the malignant gliomas (0.29) than for the control (0.11) and meningioma group (0.07) ($p < 0.01$) (Table 2 and Fig. 2).

There was no significant difference between control group and meningioma group with respect to tissue and serum levels of either copper, zinc, or the copper/zinc ratio (Tables 1, 2 and Figs 1, 2). In addition, there was no correlation found between the serum or tissue levels of these trace elements and patient age, tumor location, or tumor size.

Discussion

Trace elements play an important role in numerous biological systems through their action as activators or inhibitors, by competing with other elements and proteins for binding sites, by influencing the permeability of membranes, or through the other mechanisms [15]. In the nucleus, Zn is a well-known cofactor for nucleic acid polymerases and is associated with the high mitotic activity that has a possible role in cell growth. Animal experiments reveal inhibited tumor growth in mice fed with Zn-deficient diets [16, 17]. These reports suggest that zinc may be an important cofactor for growth of neoplastic cells. Copper is a necessary component of some metalloenzymes involving ceruloplasmin, cytochrome oxidase, uricase, dopamine hydroxylase, diamino oxydase, and tyrosinase [18]. In healthy humans, 96% of the total serum copper bound by ceruloplasmin, which forms part of alpha 2-globulin fraction, containing eight copper atoms and 12 terminal sialic acid chains per molecule [19]. Ceruloplasmin oxidizes DOPA, dopamine, adrenaline, noradrenaline, and serotonin [20]. Ionized copper induces synthesis of the adhesive proteins, fibronectin and collagen, in the extracellular space where these proteins are considered to stimulate the migration of neoplastic cells [21, 22]. This ion also binds with heparin and fibroblast growth factor, an angiogenic protein, and is considered to be capable of accelerating cell migration [7, 23].

Past reports indicate that serum levels of Cu and Zn are abnormal in patients with a variety of malignant tumors including, lung cancer [24], ovarian cancer [14], digestive tract cancer [9], and some malignancies in hematologic organs [25]. However, contradictory findings also have been reported [26]. Lightman *et al.* determined copper and zinc in ovarian malignancies with atomic absorption spectrophotometer [14]. They found highly significant differences between the tissue copper/zinc ratio for the benign and malignant lesions. However, the two groups did not differ significantly in copper concentration alone. The copper/zinc ratio might be of better prognostic value. Diez *et al.* found that the serum copper/zinc ratio had greater discriminating value than the copper and the zinc values alone and the serum copper/zinc ratio was progressively higher with advancing lung malignancy [24].

Abnormal copper levels also have been reported in primary brain tumors. In 1984, Turekÿ *et al.* reported that both serum copper level and ceruloplasmin level were increased in patients with primary

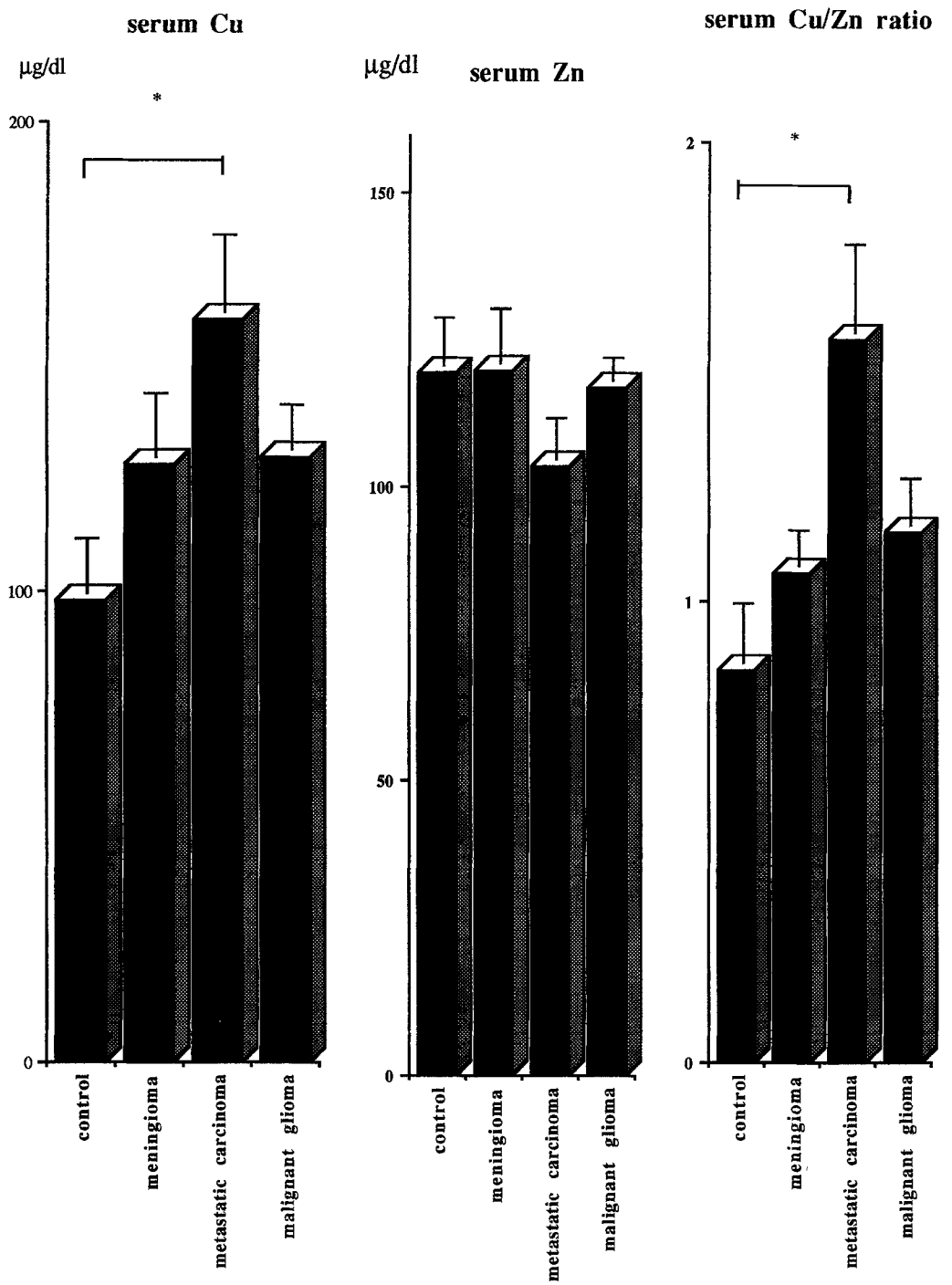

Fig. 1. Serum copper, zinc and copper to zinc ratio in patients with brain tumors according to patient group.
Cu: copper, Zn: zinc.
Results are given as mean values and error bar is standard error of the mean.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.025$.

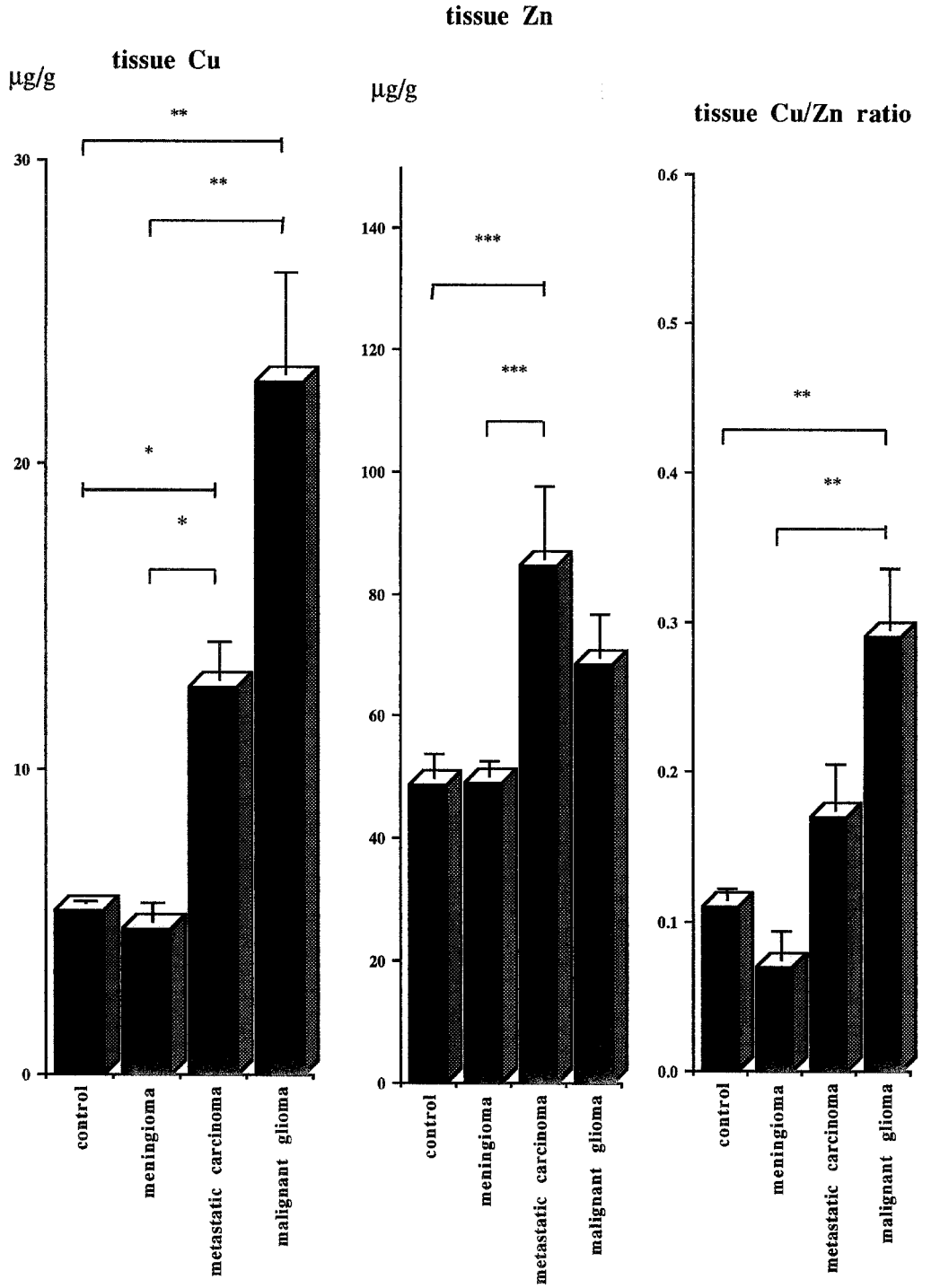

Fig. 2. Serum copper, zinc and copper to zinc ratio in patients with brain tumors according to patient group.
Cu: copper, Zn: zinc.
Results are given as mean values and error bar is standard error of the mean.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.025$.

brain tumors in comparison with healthy subjects or patients with non-tumorous neurological diseases [27]. Brem *et al.* have reported that copper depleted by diet and Penicillamine treatment (CDPT) could inhibit tumor growth in their experimental brain tumor models. They observed that copper in VX2 cells characteristically shifted from the cytoplasm into the nucleus in comparison with normal tissue cells. In addition to the large amount of copper deposited in the nucleus, they found the percentage of copper-positive cells in the experimental tumors was correlated with the angiogenesis. CDPT treatment diminished nuclear copper staining within the neoplastic cells, accompanied by the decrease in endothelial cell turnover [6]. Furthermore, they demonstrated that CDPT inhibited pseudopodial protrusion of 9L gliosarcoma cells [5]. They have emphasized the relationship between copper and tumor angiogenesis [5, 8].

Our investigation demonstrated that tissue copper and zinc levels in metastatic carcinomas and malignant gliomas are significantly higher than in brain tissue and meningiomas. Even though copper/zinc ratios in metastatic carcinomas were not significantly different from controls or meningiomas, the mean value was higher than the non malignant tissue. There was no significant difference between control tissues, and the meningioma group with respect to serum and tissue levels of either copper, zinc, or the copper/zinc ratio. In addition, there were no significant differences in serum concentrations of copper or zinc in malignant gliomas, meningiomas, or control samples and none of these patients had metastases of their primary brain tumors. Serum copper levels and copper to zinc ratios in patients with metastatic carcinomas were significantly higher than those in the control group. Since all of these metastatic brain tumors were resected prior to the identification of the primary tumor, it is reasonable to propose that the higher serum values for these trace elements may be a reflection of the systemic disease.

Malignant gliomas, metastatic carcinomas and meningiomas are considered to be well-vascularized tumors [28]. Our data support the observation that elevation of copper levels in malignant gliomas and metastatic lung carcinomas may be important in angiogenesis in these lesions. In contrast, the absence of elevated trace metal concentrations in meningiomas suggests that these non malignant tumors rely on a separate mechanism for angiogenesis.

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