

THE CIRCUIT OF ZINC AND COPPER IN THE BODY DURING CANCEROUS DISEASE

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Summary

The absorption of copper and zinc, their transport between plasma and tissues and their catching into cells are disturbed in a neoplastic process.

In order to quantify the distribution of copper and zinc in the body, we analyzed the metals concentrations in serum, urine and tumor/normal tissues in 30 patients with keratoacanthoma, 82 patients with basocellular carcinoma, 68 patients with spinocellular carcinoma and 35 healthy subjects. The measurement of copper and zinc concentrations were performed by spectrophotometrical methods and physical methods by activation with neutrons (INAA) and X rays emission induced by protons (PIXE).

The serum concentrations of copper and zinc discordantly ranged in a tumor process. The Cu/Zn report is correlated with aggressiveness of disease. The urine excretions of copper and zinc were in normal limits during the neoplastic disease. Moreover, we noted a significant accumulation of copper and zinc in tumor tissues.

Overexpression of copper and zinc in neoplastic tissues represents aggressive factor for the evolution of disease.

Keywords: copper, zinc, cutaneous carcinoma.

Rezumat

Absorbția cuprului și zincului, transportul acestora între plasmă și țesuturi și captarea lor în celule sunt alterate într-un proces neoplazic.

Pentru a cuantifica distribuția cuprului și zincului în organism, s-au analizat concentrațiile elementale în ser, urină și în țesuturi tumorale și normale la 30 cazuri cu keratoacantom, 82 cazuri cu carcinom bazocelular, 68 cazuri cu carcinom spinocelular și 35 subiecți sănătoși. Dozarea concentrațiilor de cupru și zinc s-a efectuat prin metode spectrofotometrice și prin activarea cu neutroni (INAA) și emisia de raze X indusă de protoni (PIXE).

Concentrațiile serice ale cuprului și zincului variază discordant într-un proces tumoral. Variația raportului Cu/Zn se corelează cu severitatea bolii. Eliminările urinare ale cuprului și zincului în cursul bolii canceroase se mențin în limite normale. În plus, s-a remarcat acumularea considerabilă a zincului și cuprului în țesuturile neoplazice.

Supraexpresia cuprului și zincului în țesuturile neoplazice reprezintă factori agravanți ai evoluției bolii.

Cuvinte cheie: cupru, zinc, carcinoame cutanate.

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Introduction

In the human body the total quantity of copper is between 100-150 mg. The distribution of copper (1, 2, 3) in the body is very different individual and it depends on external and internal factors: diet, age, antagonists, genetic modifications, pathological conditions, transporters.

The quantity and distribution of zinc in the body is influenced also by: diet, antagonists, transporters, temperature, inflammatory and cancerous processes. All tissues from our body contain zinc (4, 5, 6). About 85% of total zinc is stored in the muscles and bones and about 11% is distributed in the liver and skin. Zinc concentration into the skin is about 5-6 times higher in epidermis than in dermis. It seems that 30-40% from intracellular zinc is localized in the nucleus, 50% in the cytoplasm, organelles and enzymatic vesicles and the rest of zinc at the level of cellular membranes. Zn ions don't take part in redox reactions. These ions don't cross the membranes by passive diffusion, but they cross with the help of transporters (ZIP, ZnT and metallothioneine family).

The copper and zinc have a similar electronic structure that could explain the biological antagonism between them (1, 2, 4, 7, 8, 9):

- Zinc is competitive with copper at the absorption level for binding the same sites of metallothioneine;
- Copper and zinc absorptions are fast. In the plasma they bind reversibly to the serum albumins and after that they migrate to tissues;
- Metallothioneine is a cysteine rich protein with low molecular mass, which binds the copper and zinc in order to control them;
- In the body, copper and zinc are found as metalloproteins. About 93% from serum copper is bound to ceruloplasmin and the rest is bound to albumin and amino acids. Copper is constituent of enzymes: tyrosinase, superoxide-dismutase, ceruloplasmin, cytochrome c oxidase, dopamine β -hydroxylase, lysyl oxidase. In the serum, zinc is bound to α 2-macroglobulin and to complexes with low molecular mass. Zinc is a part of different enzymes: matrix metalloproteinases (MMPs), ADAMs molecules, superoxide-dismutases, phos-

photransferases, carbonic anhydrases, transferases, isomerases, carboxypeptidase, polymerases (DNA, RNA), thymidine kinases;

- The tissue variations of copper and zinc in the pathological processes haven't been completely elucidated yet;
- Zinc is excreted by excrement, urine, saliva, skin. Copper is eliminated from the body by bile, urine, excrement.

The redistribution of zinc and copper during the neoplastic process could explain the implication of these elements in carcinogenesis. There are proofs that the copper and zinc absorptions, their transport between plasma and tissues and their catching into cells differ due to the mineral pool of each subject (calcium, manganese, iron, cadmium, cobalt, mercury, magnesium).

Taking into account the recent researches, we present in this study some experimental proofs regarding the circuit of copper and zinc during the neoplastic process.

Materials and Methods

The experiments were performed on the following samples:

- blood and urine samples which were got in the initial moment and after each therapeutic cure;
- normal and tumoral tissue fragments which were surgically obtained at the Clinical Hospital "Prof. Dr. Scarlat Longhin" Bucharest.

The serum and urinary copper and zinc levels were analyzed by spectrophotometrical methods in patients with clinical diagnosis which was confirmed by paraclinical examinations:

- 30 *keratoachantoma* cases: 16 men and 14 women with ages between 34 and 65 years;
- 82 *basocellular carcinoma* cases: 42 men and 40 women with ages between 45 and 72 years;
- 68 *spinocellular carcinoma* cases: 37 men and 31 women with ages between 50 and 76 years;
- 35 healthy subjects (*control group*): 19 men and 16 women with ages between 19 and 68 years.

The tissue concentrations of copper and zinc were analyzed by spectrophotometrical methods and activation with neutrons INAA and X rays emission by protons action PIXE (IFIN „Horia Hulubei”).

Results and discussions

Our results showed (table no. I) a significant correlation between serum copper and zinc and the progression of tumoral process.

The serum copper increased significantly in patients with cutaneous cancer in comparison with benign tumors lot and control group. The statistical analysis of serum copper values in control group and keratoachantoma didn't show significant differences (test "U"): $u_c = 0,56 < u_{0,05} = 1,96$; $p > 0,05$. However, the average of serum copper differed significantly between control and BCC: $u_c = 4,03 > u_{0,05} = 1,96$; $p < 0,05$ and between control and SCC: $u_c = 3,92 > u_{0,05} = 1,96$; $p < 0,05$.

The serum zinc was correlated inversely with tumoral development which was in contrast with serum copper variations. The statistical tests were

performed to show the zinc variation among lots (test "U"): between control and keratoachantoma $u_c = 0,14 < u_{0,05} = 1,96$; $p > 0,05$ the average of zinc didn't significantly differ; between control and BCC: $u_c = 2,08 > u_{0,05} = 1,96$; $p < 0,05$ and between control and SCC: $u_c = 2,42 > u_{0,05} = 1,96$; $p < 0,05$ were statistical variations.

The rapport of serum averages Cu/Zn increased in cutaneous cancer in comparison with control (table no. II) which noted the antagonism between these metal ions. The statistical calculation of linear correlation factor "r" (Pearson) between serum zinc and copper showed: in control group $r = | -0,76 | > r_{0,05; 34} \sim 0,325$; in keratoachantoma $r = | -0,79 | > r_{0,05; 29} \sim 0,355$; in basocellular cutaneous carcinoma $r = | -0,87 | > r_{0,05; 81} \sim 0,217$ and in spinocellular cutaneous carcinoma $r = | -0,88 | > r_{0,05; 67} \sim 0,232$ which reflected a negative correlation between serum copper and zinc.

All patients didn't show variations of urinary copper and zinc in comparison with control group (table no. III).

Table no. I. The serum levels of copper and zinc (average \pm standard deviation) in cutaneous cancer

Lots	Cases no.	Serum copper ($\mu\text{g/dL}$)	Serum zinc ($\mu\text{g/dL}$)
Control Group	35	104,23 \pm 34,18	92,12 \pm 37,51
Keratoachantoma	30	109,86 \pm 45,04	90,81 \pm 36,12
Basocellular cutaneous carcinoma (BCC)	82	137,78 \pm 54,24	77,49 \pm 27,11
Spinocellular cutaneous carcinoma (SCC)	68	141,12 \pm 61,08	75,11 \pm 24,89

Table no. II. The rapport serum Cu/Zn in patients with cutaneous tumors

Lots	Cases no.	Cu/Zn
Control Group	35	1,13
Keratoachantoma	30	1,20
Basocellular cutaneous carcinoma (BCC)	82	1,77
Spinocellular cutaneous carcinoma (SCC)	68	1,87

Table no. III. Urinary excretions of copper and zinc in patients with cutaneous proliferations

Lots	Cases no.	Copper ($\mu\text{g}/24$ hours)	Zinc ($\mu\text{g}/24$ hours)
Control Group	35	43,1 \pm 18,6	680,5 \pm 305,4
Keratoachantoma	30	47,6 \pm 21,3	564,3 \pm 403,1
Basocellular cutaneous carcinoma (BCC)	82	42,4 \pm 29,2	701,8 \pm 209,7
Spinocellular cutaneous carcinoma (SCC)	68	41,8 \pm 18,5	635,5 \pm 311,5

The urinary zinc level didn't differ between groups. The urine elimination of zinc was between 150 and 1000 µg/24 hours among studied subjects. The absence of oscillation for urinary zinc was confirmed by statistical analysis (Test "U"): between control and keratoachantoma: $u_c = 1,29 < u_{0,05} = 1,96$; $p > 0,05$; between control and BCC: $u_c = 0,37 < u_{0,05} = 1,96$; $p > 0,05$ and between control and SCC: $u_c = 0,70 < u_{0,05} = 1,96$; $p > 0,05$.

Copper excretion didn't show significant differences between groups (15-70 µg/24 hours). The statistical analysis point out (Test "U"): between control and keratoachantoma: $u_c = 0,9 < u_{0,05} = 1,96$; $p > 0,05$; between control and BCC: $u_c = 0,15 < u_{0,05} = 1,96$; $p > 0,05$ and between control and SCC: $u_c = 0,26 < u_{0,05} = 1,96$; $p > 0,05$.

The copper level increased in tumoral cutaneous tissues in comparison with their concentration in normal skin tissue (table no. IV). The copper concentrations between keratoachantoma and normal tissue haven't been significant differences, but higher values for copper were detected in basocellular and spinocellular cutaneous carcinoma (figura no. 1). Statistically, variations of tissue copper averages among studied groups weren't remarkable (Test "t" Student): between control and keratoachantoma: $t_c = 0,129 < t_{0,05,23} = 2,069$, $p > 0,05$; between control and BCC: $t_c = 0,62 < t_{0,05,33} = 2,036$, $p > 0,05$; between control and SCC: $t_c = 0,605 < t_{0,05,34} = 2,034$, $p > 0,05$.

There was noted a significant increase of zinc in tumoral tissue, also (figura no. 1). The averages

Tabel no. IV. The averages of copper and zinc in cutaneous tumors vs. normal tissue

Lots	Cases no.	Cu (ppm)	Zn (ppm)
Control Group	17	27,3 ± 23,7	82,4 ± 47,3
Keratoachantoma	8	26,8 ± 15,8	127,9 ± 21,2
Basocellular cutaneous carcinoma (BCC)	18	32,3 ± 25,4	135,1 ± 41,2
Spinocellular cutaneous carcinoma (SCC)	19	31,8 ± 21,1	126,6 ± 39,1

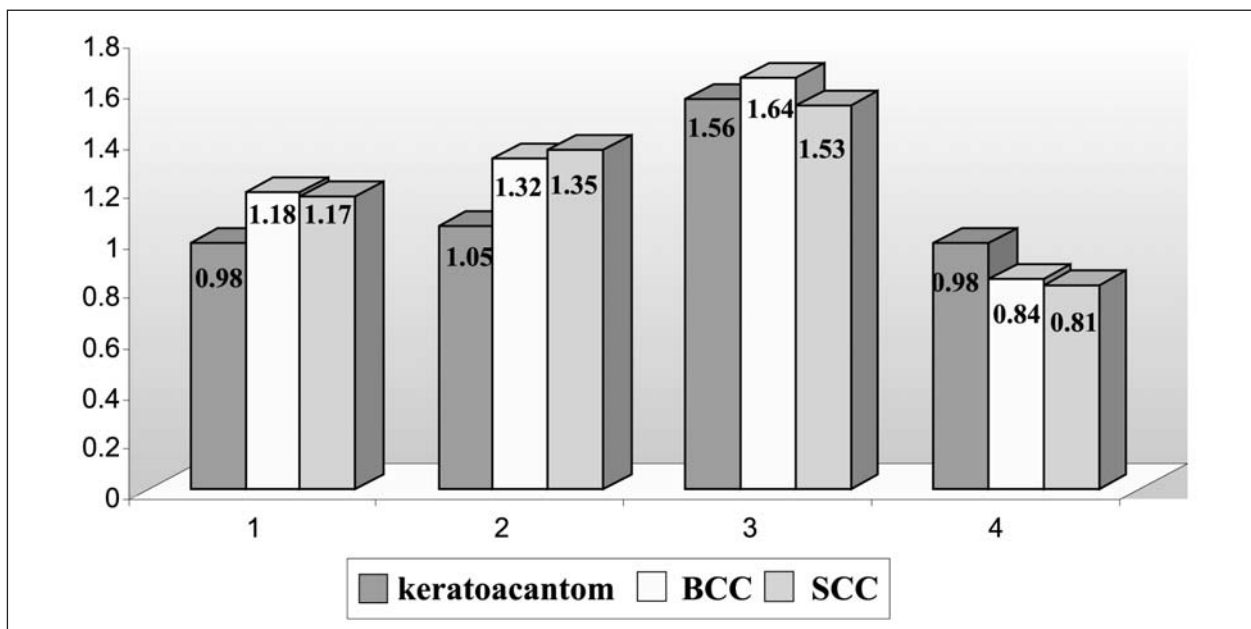


Figure nr. 1. The rapport of tissue and serum copper and zinc in keratoachantoma, basocellular carcinoma, spinocellular carcinoma vs. normal tissue

of tissue zinc differed significantly among studied lots (Test "t" Student): between control and keratoachantoma: $t_c = 2,58 > t_{0,05,23} = 2,069$, $p < 0,05$; between control and BCC: $t_c = 3,53 > t_{0,01,33} = 2,733$, $p < 0,01$; between control and SCC: $t_c = 3,08 > t_{0,01,34} = 2,728$, $p < 0,01$. The calculation of linear correlation coefficient "r"- (Pearson) between tissue copper and zinc evidenced for the normal tissue $r = | 0,88 | > r_{0,05;16} \sim 0,482$; keratoachantoma $r = | 0,93 | > r_{0,05;7} \sim 0,707$; basocellular carcinoma $r = | 0,92 | > r_{0,05;17} \sim 0,468$ and spinocellular carcinoma $r = | 0,91 | > r_{0,05;18} \sim 0,456$ that showed a positive correlation.

Zinc inhibits the malign cells proliferations without any effect on the healthy cells (10, 11, 12, 13, 14, 15). This action is argued by:

- Zinc is implicated in cells growth regulation by means of ERK;
- Zn^{2+} influences the genes expression for proteins which are implicated in cell cycle control;
- Zinc is an inhibitor of apoptosis. The depletion of this element determines the programmed cell death in many cell lines, but the excess of tissue zinc has cytotoxic role;
- Extracellular zinc may inhibits the malign cells growth by activation of $p21^{Cip/WAF1}$;
- Zinc isn't competitive with cytostatics;
- Zinc promotes keratinocytes proliferations and migrations;
- Zinc affects the intercellular adhesion by means of integrins;
- The excess of zinc prolongs the malign cells life;
- Zn^{2+} ions can replace the Cu^{2+} and Fe^{2+} ions from active site of antioxidant enzymes;
- Zn^{2+} influences the metallothioneine synthesis which protects nucleic acids against ROS.

Intracellular excess of copper is very toxic (16, 17, 18, 19):

- Copper ions participates to H_2O_2 production;
- Copper ions determines the activation of antiapoptotic Akt kinase/ protein kinase B complex by means of lysyl oxidase;
- Copper modifies the genetic material by its binding to DNA in G1/S phase of cell cycle;
- Copper is implicated in cells survival by depletion of GSH stores, ROS production and increasing of malign cells lipoperoxidation;
- Extracellular copper interferes in promotion of tumoral cells invasion by activation of lysyl oxidase. Lysyl oxidase is a copper dependent amino-oxidase, which is expressed in tumoral cells and which participates to malign cells proliferation and differentiation, connective tissue remodeling, extracellular matrix maturation, monocytes, smooth muscle cells, fibroblasts mobility and migration.

The accumulation of zinc and copper in tumoral cutaneous tissues could be influenced by inflammatory reactions from neoplastic process (19). Because of these influences, serum zinc decrease with its accumulation in cells. The acute phase reactivities production is responsible for this pathological distribution. IL-1, IL-6, TNF cytokines induces metallothioneine synthesis which influences the transport of zinc and copper excess to cellular level. The copper level increases in an inflammatory area due to acute phase molecules. The serum copper increases due to IL-1 mediated-acute response, also. It's supposed that ceruloplasmine increases during an inflammatory process as a reaction against ROS. The tissue excess of zinc and copper in cutaneous tumors determines the ROS generation. Oxidative stress is strongly expressed in malign cells. When defensive systems were exhausted, the oxidative aggression determines the apoptosis and necrosis of cells. In this way cells die that induces the stimulation of inflammatory response and neutrophiles infiltration.

Conclusions

- Serum copper and zinc concentrations are opposite in a neoplastic process. The evaluation of serum zinc decreasing and the analysis of rapport Zn/Cu are utile for detection of disease aggressivity.
- Excess of tissue copper and zinc levels could be factors which aggravate the disease evolution. The high quantities of copper and zinc influence oxygen reactive species generation and the activation of defensive systems against oxidative aggression.
- Urinary excretions of copper and zinc during cancer are in normal limits even if in the body exists an excess or a deficit of these elements;
- Consequently, it's proposed that copper and zinc economy is realized by accommodation of absorption mechanisms.

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