Exposure to Heavy Metals in Plastics Industry and Dyes - Risk Factor in the Development of Skin Cancer

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In the industry of plastics and dyes, pharmaceuticals and agriculture, the toxic effects of heavy metals have been reported during prolonged professional exposure. Heavy metals in the environment can enter the body orally, inhaled or by skin absorption. Unlike other toxins present in the environment, heavy metals accumulate in tissues because the body has no ability to remove them. Heavy metals in excess cause numerous metabolic imbalances. In cancer, heavy metal overload could be a stimulus for secretion of chemokines, for promoting oxidative stress and antioxidants inactivation, for altering cell signaling, for promotion and progression of tumorigenesis. Strategies to prevent accumulation of toxic metals, to decipher the pathophysiological mechanisms by which heavy metals affect cell metabolism, to increase excretion of toxic metals without removing essential minerals in the body is the subject of current researches.

Keywords: heavy metals, dyes , plastics , skin cancer

An important concern of scientists is represented by knowledge of the dangers caused by the accumulation of heavy metals in the body. Heavy metals have many commercial and industrial applications, are present in plastics and ceramics, paints and dyes, pigments, pharmaceuticals and metallurgy, in insecticides and pesticides. Excess heavy metals become toxic and determine loss of cell functions by production of free radicals, by inducing oxidative alterations of biological macromolecules (nucleic acids, lipids, proteins) and by inactivation of the repair enzymes of DNA, by promoting angiogenesis and inflammation, by alteration of cell signaling [1, 2].

There have been carrie numerous worldwide researches based on developing strategies to prevent the cumulation of toxic metals in tissues and increasing excretion of these metals without removing essential minerals in the body.

In the plastics industry, many toxic substances are used, as follows: dyes, catalysts, initiators, regulators, emulsifiers, antioxidants, solvents, stabilizers. In the latest years, plastic materials are used more and more [3-5]. The coloring of plastic materials is made with pigments (organic, anorganic) and colorants with metallic complexes in order to obtain a pleasant appearance. Pigments with special effects (metal powders, pearl pigments, photoluminescent pigments) improve the optical properties of plastics.

The main practical application of metallic colorants is inking textile fibers. Typically, metallic dyes are used for coloring animal fibers (wool, silk, fur), vegetal fibers (cotton, linen, silk), synthetic fibers (nylon, polyethylene , polyvinyl chloride). Some dyes are used for coloring tanned leather, paper, food, printing inks, rubbers, gasoline and mineral oils, in photography and cinematography, in paint manufacture, in biology and medicine.

Artificial colorants contain heavy metals and other chemicals that raise concerns about the dangers of their use. Persons engaged in plastics industry are exposed to increased risk of developing cancer, endocrine and immune dysfunctions, of developing allergic diseases, pruritic manifestations, eye and respiratory irritations, skin and mucosal lesions [3-5].

Heavy metals from plastics may act as allergens. It is suggested that metal ions can bind to albumin and can act as haptens to which the immune system reacts specifically. The greatest allergenic potentials were observed in nickel ions, gold, mercury, palladium, silver, cobalt, platinum, titanium, copper, iridium. Some studies reported allergic contact dermatitis, chronic urticaria, itching, and hypersensitivity type 1 at metals from plastics. People who are exposed to potentially allergenic substances develop skin disorders associated with the production of cytokines responsible for the inflammatory phenomena.

People involved in the production of plastics are exposed to hormonal disturbances. It was reported that occupational exposure to chemicals used in this industry interferes with receptors of hormones, with sex hormone synthesis and conversion. Chronic inhalation of particles of heavy material formed in the different plastic manufacturing processes could lead to toxic systemic effects associated with asthma.

By definition, heavy metals are chemical elements that have a specific gravity of at least 5 times higher than water. The heavy metals known at this moment are: antimony, arsenic, bismuth, cadmium, cerium, chromium, cobalt, gallium, gold, iron, lead, manganese, mercury, nickel, platinum, silver, tellurium, thallium, tin, uranium, vanadium, zinc. Not all heavy metals are harmful to the human body. Complexes of metals (chromium, manganese, iron, cobalt, copper, zinc, molybdenum, aluminum) are used as mordant for vegetable dyes. Some metals are essential in the development of metabolic processes (copper, iron, zinc, manganese, magnesium, chromium). Iron and copper are toxic when the body cannot eliminate the excess. Yellow iron oxide FeO (OH) . H_2O, red iron oxide Fe_2O_3 and black iron oxide FeO - Fe_2O_3 are included in the compositions of synthetic dyes. Under these conditions, iron is prooxidant, has low biodisponibility and is toxic in large quantities. Iron hydroxides are used as surface colorants for chemically

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processed foods, as food additives, as anti-caking agents. Pathophysiological mechanisms of toxicity caused by the accumulation of heavy metals in tissues can be explained by the ability of these chemicals to bind oxygen, nitrogen and sulfhydryl groups of organic structures and to induce structural alterations of proteins, nucleic acids and enzymes.

Heavy metals toxic to cell metabolism come from different sources: contaminated air, water, soil, food. They are transition metals with multistage valences,have a weak electropositive character and a low affinity for oxygen . Both redox - active metals that participate in Fenton reactions (Fe, Cu, Cr , Co, V , Ni) and redox - inactive metals (Pb, Cd, Hg) increase oxygen reactive species (ROS), increase nitrogen reactive species (RNS) in tissues, based on a Fenton transformation:

$Metal^{3+} + .O2 \longrightarrow Metal^{2+} + O_2$ Haber-Weiss

$Metal^{2+} + H2O2 \longrightarrow Metal^{3+} + OH^- + .OH$ Fenton reaction

The most important changes mediated by RNS are:

NO (nitric oxide) + $O_2^{\cdot-}$ (superoxide) → $ONOO^-$ (peroxynitrite)
$ONOO^- + H^+ \rightarrow$ **ONOOH** (peroxynitrous acid) → $\cdot NO_2$ (nitrogen dioxide) + **·OH** (hydroxyl radical)
$ONOO^- + CO_2$ (carbon dioxide) → $ONOOCO_2^-$ (nitrosoperoxycarbonate)
$ONOOCO_2^- \rightarrow \cdot NO_2$ (nitrogen dioxide) + $O{=}C(O\cdot)O^-$ (carbonate radical)
$\cdot NO + \cdot NO_2 \rightleftharpoons N_2O_3$ (dinitrogen trioxide)

The most important changes mediated by ROS are:

$O_2 \longrightarrow O_2^- \longrightarrow H_2O_2 \longrightarrow \cdot OH \longrightarrow H_2O$

Oxygen — Superoxide anion — Hydrogen peroxide — Hydroxyl radical — Water

Carcinogenic effect of heavy metals is a subject discussed in many studies, but the results are contradictory.

All the data related to the complexity of biochemical phenomena induced by heavy metals in tumor metabolism increased authors interest of to conduct researches in skin cancer [6 - 11]. It is interesting to investigate the relationship between the accumulation of heavy metals in people with tumors and tumor angiogenesis.

Experimental part

Laboratory measurements

-hematologic determinations were made in automatic system using ABX Pentra C60 (France);

-biochemistry determinations (liver function tests, blood sugar, kidney tests, lipid profile, copper, iron) were made with automatically photometric system using HumaStar300 (GmbH Germany). Determinations of transferrin, ferritin, soluble transferrin receptor (sTfR), ceruloplasmin were made by immunoturbidimetric method using HumaStar300.

-interleukin 8 (IL8) dosage was done by ELISA using TECAN system (GmbH Austria).

The present study was based on prospective analysis of 128 subjects with cutaneous malignant melanoma. All the subjects were followed clinically and laboratory.

All experimental determinations were reported to a control group without neoplastic pathology.

Statistical analysis

The statistical analysis was done using SPSS soft. Laboratory parameters were expressed by the mean and standard deviation. Statistical significance of parameter variations was considered for $p<0.05$.

Results and discussions

Evaluation of subjects with cutaneous malignant melanoma on iron status (hemoglobin, sideremia, soluble receptors for transferrin, ferritin) led to the following results:

-iron deficiency in 23.5% of studied subjects (low values for hemoglobin, sideremia and ferritin and elevated soluble transferrin receptors);

-anemia associated with cronic diseases to 7 % of cases (low values for hemoglobin, sideremia, transferrin, normal values of ferritin and soluble receptors for transferrin);

-iron overload in 36 % of cases (elevated hemoglobin, sideremy, transferrin, low soluble transferrin receptors);

-normal metabolism of iron in 33.5 % of subjects.

Evaluation of subjects with cutaneous malignant melanoma on cooper status (copraemia, ceruloplasmin) showed:

-copper deficit to 8.6 % of cases;

-copper overload in 41.4 % of cases;

-normal metabolism of copper in 50.0 % cases.

Based on these results, we selected 18 subjects divided in to two groups: group A with normal status for iron and copper (group) and group B with iron and copper overload. The groups were similar, as follows:

-clinical and demographic characteristics: age, sex, habitat, nutritional status, anatomical location of the tumor, presence/absence of ulceration, clinical stage of tumor;

-histological characteristics (Breslow index, Clark level), immunohistochemical characters (positive HMB45 , S100 protein, Melan A and vimentin);

-biological parameters (leukocytes, platelets count, liver tests, kidney tests, lipid profile, serum glucose).

A special attention was given to define the relationship between biochemical indicators of iron and copper status in the body and the tumor angiogenesis, assessed by serum IL8 (table 1 and 2).

The authors observed in subjects with malignant melanoma and normal iron (94.3+37.2 ug/dl) and copper (88.90+31.9 ug/dL) status compared to controls (89.9+29.7 ug/dL, 90.4+22.7ug/dL):

-significantly increase of ferritin (77.6+11.4 ng/mL melanoma, 36.3+8.2 ng/mL control, $p<0.05$) ceruloplasmin (36.6+42.1 mg/dL melanoma, 32.1+0.9 mg/dL, control, $p<0.05$) and IL-8 (26.8+8.3 pg/mL melanoma, 13.2+1.0 pg/mL, control, $p<0.05$) (figs. 1, 4, 5);

-low levels without statistical significance for transferrin (237.5+26.4 mg/dL melanoma, 259.8+46.5 mg/dL

Variables	Malignant melanoma		Control
Iron (ug/dl)	94.3±37.2	174.5±28.8	89.9±29.7
Copper (ug/dl)	88.9±31.9	181.1±38.2	90.4±22.7
Ferritin (ng/ml)	77.6±11.4(1)	199.2±101.2(1.2)	36.3±8.2
Transferrin (mg/dl)	237.5±26.4	166.4±31.2(1.2)	259.8±46.5
sTfR (mg/l)	3.8±0.6	2.2±1.2 (1.2)	4.1±0.4
Ceruloplasmin (mg/dl)	36.6±4.21(1)	47.2±3.2(1.2)	32.1±0.9
IL8 (pg/ml)	26.8±8.3(1)	88.3±16.1(1.2)	13.2±1.0

(1) $p<0,05$ statistical significance melanoma versus control;
(2) $p<0,05$ statistical significance melanoma normal iron and copper versus melanoma increased iron and copper

Table 1
LABORATORY MEASUREMENTS IN SUBJECTS WITH MELANOMA AND CONTROL.

Variables	Malignant melanoma				Control	
	Normal iron/copper		Increased iron/copper			
	r	p	r	p	r	p
Iron (ug/dl)	0.15	0.23	0.26	0.02	0.08	0.26
Copper (ug/dl)	0.26	0.09	0.47	0.00	0.06	0.18
Ferritin (ng/ml)	0.22	0.04	0.77	0.00	0.10	0.00
Transferrin (mg/dl)	-0.08	0.00	-0.44	0.00	-0.06	0.42
sTfR (mg/l)	-0.11	0.05	-0.53	0.00	-0.16	0.12
Ceruloplasmin (mg/dl)	0.09	0.05	0.22	0.00	0.11	0.19

r-regression coefficient, p-statistical significance

Table 2
STATISTICAL CORRELATIONS BETWEEN SERUM LEVELS OF IL8 (PG ML) AND PARACLINICAL FACTORS THAT DEFINE IRON AND COPPER STATUS IN SUBJECTS WITH MELANOMA AND CONTROL

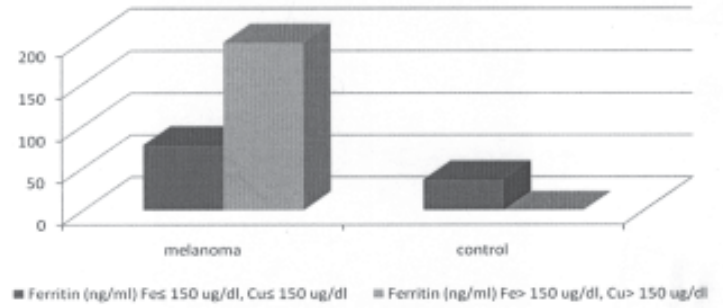

Fig. 1 Graphical representation of serum ferritin in subjects with melanoma and control by iron and copper levels

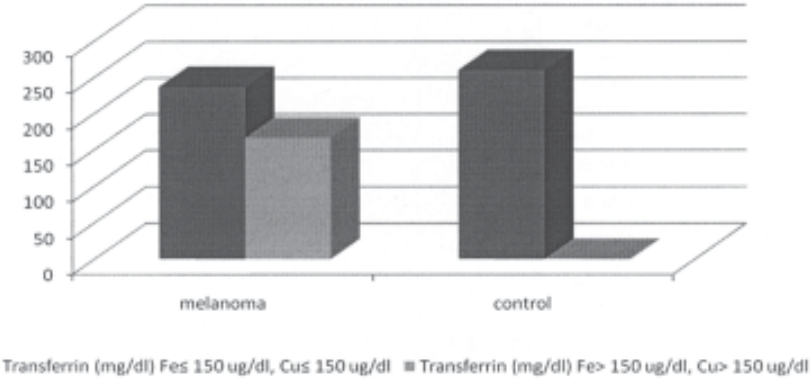

Fig. 2 Graphical representation of serum transferrin in subjects with melanoma and control by iron and copper levels

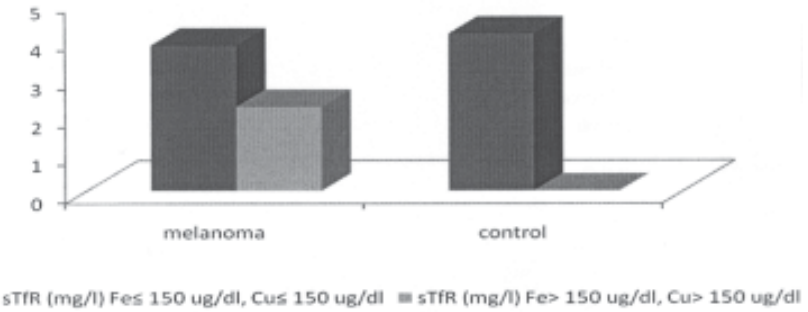

Fig. 3. Graphical representation of serum sTfR in subjects with melanoma and control by iron and copper levels

control, p>0.05), soluble receptors for transferrin (3.8+0.6 mg/L melanoma, 4.1+0.4 mg/L control, p>0.05) (figs. 2, 3).

In subjects with malignant melanoma and high levels of iron (174.5±28.8 ug/dl) and copper (181.1+38.2 ug/dL) were observed:

-high levels of ferritin (199.2±101.2 ng/mL), ceruloplasmin (47.2±3.2 mg/dL) and IL8 (88.3±16.1 pg/mL) compared to subjects with melanoma and normal iron/copper status (77.6±11.4 ng/mL ferritin, 36.6±4.21 mg/dl ceruloplasmin, 268.±8.3 pg/mL IL8,p<0.05), compared to control (36.3±8.2 ng/mL ferritin, 32.1±0.9 mg/dL ceruloplasmin, 13.2±1.0 pg/mL IL8, p<0.05) (figs. 1, 4, 5);

-significant lower levels for transferrin (166.4±31.2 mg/dL) and soluble receptors for transferrin (2.2±1.2 mg/L) compared to subjects with melanoma and normal iron/copper status (237.5±26.4 mg/dL transferrin and 3.8±0.6 mg/L soluble receptors for transferrin, p<0.05) compared to control (259.8±46.5 mg/dl transferrin and 4.1±0.4mg/L soluble receptors for transferrin, p<0.05) (fig. 2, 3).

Statistical correlations between serum levels of IL8 and biochemical parameters defining the iron and copper status in the body showed:

-positive relations with statistical significance between IL8 and iron, IL8 and copper in subjects with melanoma and copper and iron overload; between IL8 and ferritin in all groups studied (table 2);

-negative relationships with statistical significance between IL8 and transferrin, respectively, IL8 and soluble receptors for transferrin in subjects with malignant melanoma (table 2);

Study of heavy metals in tumor pathology is a challenge for researchers. Some studies showed that heavy metals

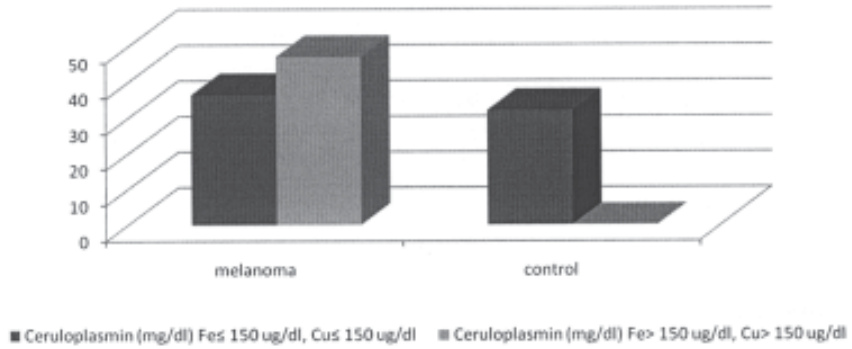

Fig. 4. Graphical representation of serum ceruloplasmin in subjects with melanoma and control by the iron and copperlevel

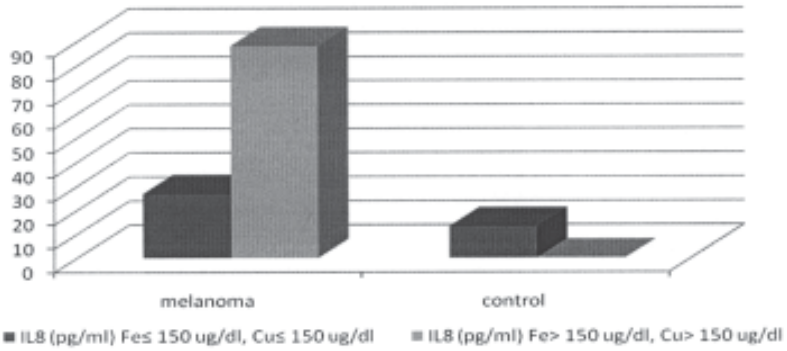

Fig. 5. Graphical representation of serum IL8 in subjects with melanoma and control by the iron and copper level

accumulate in tumor cells and produce disturbances on the expression of genes related to stress, inflammation, tumor suppression, angiogenesis, apoptosis [1, 2, 6, 10, 13, 14]. Tumor cells synthesize and secrete certain growth factors with autocrine and paracrine action that influence proliferation, migration, metastasis, apoptosis of cells and angiogenesis [6 - 12]:

-malignant melanoma , a severe cancer, synthesizes: VEGFs/VEGFRs, PIGF, uPA/uPAR, IL -8, FGF/FGFR, neuropilin 1/2, CXCR-1/2, TGFalfa/beta, integrine receptors (alfa5beta3, alfa5beta1, alfa6/beta4, alfa2/beta2, alfa3beta1, alpha4beta1), gangliosides (GM1, GM2, GM3, GD2, GD3, O- AcetilGD3, GD1a, GD1b, GT1b, GQ1b), neurohormones;

-extracellular matrix components synthesize MMPs (-1, -2, -3, -7, -9, -10), TIMPs (-1, -2, -3), integrins [6, 14].

-endothelial cells of the tumor microenvironment produce and store FGF2/FGFR, IFNalfa, IL2, IL1beta, NO, PGE2, VEGF/VEGFR, osteopontin, COX2, neuropilin1/2, uPA, CXCR1/2, integrins receptors (alfa5beta3, alfa5beta1, alfa6beta4, alfa2beta2, alpha4beta1) [6, 13].

-inflammatory cells elaborate a molecular factors that affect tumour angiogenesis [6, 13, 14], Neutrophils elaborate VEGF, IL6, IL8. Monocytes / macrophages produce VEGF, TGF- beta, FGF2, PDGF, P substance, IL8, TNFalpha, prostaglandins, angiogenin, proteases. Mast cells elaborate VEGF, FGF2, TGF-beta, TNFalpha, IL-8, histamine, NGF, tryptase, heparin, adrenomedulin, MMPs -2 and -9, alpha 5 integrin, leptin, PDGF, IFNalfa, beta, gamma, IL-4, IL-12, IL-18, PAI -1, TIMPs. Eosinofiles elaborate VEGF, TGF2, TNFalpha, PDGF, GM -CSF, NGF, IL6, IL8, eotaxin.

The pathophysiology of disorders caused by iron and copper in the context of neoplasia could be explained by induction of the oxidative stress and by inactivation of some biological compounds. Such metals can bind oxygen, nitrogen and sulfhydryl groups of organic compounds. Increased serum levels of metaloproteinase linking iron and copper as response to their accumulation in the body, could be a primary body mean of defense against the heavy metal toxicity.

Conclusions

The serum level of chemokines in subjects with aggressive skin cancer, such as melanoma, may be explained by the ability of tumor cells (malignant melanocytes, endothelial cells, epithelial cells, fibroblasts, neutrophils, monocytes, macrophages, lymphocytes) to secrete autocrine and paracrine growth factors in response to various stimuli. In the current study, the authors assume that the accumulation of heavy metals in the context of neoplasia could induce tumor progression and neo-vascularization. Assessment of angiogenesis in subjects with cutaneous malignant melanoma by serum interleukin 8 in relation with biochemical factors that establish iron and copper status in the body, proved an interdependent relationship between the level of heavy metals in the body and metabolic disorders installed in individuals with cancer. The authors estimate that avoiding prolonged occupational exposures in plastics industry, at dyes and pharmaceuticals, in agriculture, could be means of reducing the undesirable effects caused by the accumulation of heavy metals in the body, especially in people with cancer.

Acknowledgements. All authors have equally contributed to this work: conceived and designed the experiments; performed the experiments; analyzed the data; wrote the paper. The authors have no conflicts of interest that are directly relevant to the content of this paper.

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Manuscript received: 24.04.2014