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ORIGINAL PAPERS: CLINICAL OR BASIC RESEARCH

Breast tumours strongly accumulate transition metals

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ABSTRACT

Objectives: *Increased levels of transition metals like iron, nickel, chromium, copper and lead are closely related to free radical generation, lipid peroxidation, formation of DNA-strand breaks and tumour growth in cellular systems. In order to determine the correlation to malignant growth in humans, we investigated the accumulation of heavy metals in 8 healthy and 20 breast cancer biopsies by means of a standardized Atomic Absorption Spectrophotometry (AAS) methodology with acid hydrolysis for sample preparation. Additionally, heavy metal analysis in all control biopsies was also performed with an Inductive Couples Plasma – Mass Spectroscopy (ICP-MS) technique. For statistical analysis of the results, the Mann-Whitney U-Test was used.*

Results: *A highly significant accumulation of iron ($p < 0.0001$), nickel ($p < 0.00005$), chromium ($p < 0.00005$), zinc ($p < 0.00001$), cadmium ($p < 0.005$), mercury ($p < 0.005$) and lead ($p < 0.05$) was found in the cancer samples when compared to the control group. Copper and silver showed no significant differences to the control group whereas tin, gold and palladium were not detectable in any biopsies.*

Conclusions: *These data suggest that pathological accumulation of transition metals in breast tissue may be closely related to the malignant growth process and explain the anti-tumoural effects of current therapies with high doses of vitamin C or substituted phenols, respectively.*

Keywords: breast cancer, heavy metals, iron, nickel, chromium, zinc, mercury, lead, cadmium, copper, AAS

Abbreviations list

EDDA – Ethylendiamine N,N'-diacetate
NTA – Nitrilotriacetic Acid
AAS – Atomic Absorption Spectrophotometry
ICP-MS – Inductive Coupled Plasma – Mass Spectroscopy
MELISA – Memory Lymphocyte Immunostimulation Assay

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INTRODUCTION

Reports in the last two decades closely relate the presence of transition metals like iron (Fe) or copper (Cu) to free radical generation via Fenton / Haber-Weiss-reactions, ascorbate autoxidation, lipid peroxidation processes and formation of DNA strand breaks (1,2,3,4). As previously published, lipid peroxidation-induced malondialdehyde-DNA adducts can accumulate and reach high levels in the breast tissue of women with breast cancer, leading to endogenous DNA modifications (5). Furthermore, ferric-ethylendiamine N,N'-diacetate (EDDA)- and nitrilotriacetic acid (NTA)-complexes were shown to induce free radicals and renal carcinomas in Wistar rats, demonstrating the key role of transition metals in the abnormal proliferation process (6,7). Since repeated mithochondrial and nuclear DNA mutations may lead to malignant growth, we investigated the heavy metal content in breast cancer biopsies and in healthy breast tissue biopsies. ❑

MATERIAL AND METHODS

Heavy metal analyses have been performed in 20 frozen breast cancer biopsies and in 8 frozen healthy breast tissue samples, supplied by the Dept. of Oncology, 1st Faculty of Medicine, Charles University, Prague, Czech Republic and by Caritas Hospital St. Josef, Regensburg, Germany. Biopsies in the patients group belong to women aged 23-49 years, the control biopsies belong to healthy women aged 21-43 years. None of our patients received cytostatic drugs before surgery. The study was approved by the local ethic committee and all participants gave their informed written consent before being enrolled in the study.

The concentrations of iron, cadmium, lead, chromium, tin, nickel, copper, silver, gold, palladium and zinc in the final solution have been measured by a standardized furnace-AAS-technique using a Perkin Elmer Sima 6000 AA-spectrophotometer and acid hydrolysis as pulping procedure for sample preparation (8). The concentration of mercury was assessed by means of a Perkin-Elmer FIMS mercury analyzer. Additionally, heavy metal analysis in all control biopsies has been done by using an ICP-MS technique in the Laboratory for Micro Trace Minerals, Hersbruck, Germany. All biopsies have been taken from the core of the tumour nodules, and metal concentration in 1 g of tumour breast tissue was measured and compared to metal concentration in the same amount (1 g) of healthy breast tissue. All tests have been performed three times and the final result per sample is expressed in μg/kg breast tissue, recording the mean value of three determinations. The Mann-Whitney U test was used for statistic analysis of the results. ❑

RESULTS

Data analysis shows a highly significant accumulation of Fe, Ni, Cr, Zn, Hg, Cd, and, to a less extend, of Pb in malignant breast tissue, when compared to healthy breast tissue (Table 1).

Iron levels were dramatically increased in the breast cancer biopsies (median: 53173.5 μg/kg, range: 14664-205930 μg/kg) when compared with the control group (median: 10937 μg/kg, range: 5331-21646 μg/kg) ($p < 0.0001$).

A highly significant **nickel** accumulation (median: 994.5 μg/kg, range: 469-3361 μg/kg) was recorded in the patient biopsies. Control biopsies showed measurable levels (median: 21 μg/kg, range: 11-33 μg/kg), but at more than one order of magnitude lower ($p< 0.00005$).

Similar results have been noticed for **chromium** (median: 815.5 μg/kg, range: 313-5978 μg/kg) when compared to the control group (median: 38.5 μg/kg, range: 19-119 μg/kg) ($p< 0.00005$).

A surprisingly high accumulation of **zinc** (median: 17075 μg/kg, range: 1326-97895 μg/kg) was recorded in the cancer biopsies, the difference to the control group (median: 3741 μg/kg, range: 2548-9339 μg/kg) being again highly significant ($p < 0.001$).

Mercury was found moderately increased in 11 out of 20 cancer samples (median: 6.9 μg/kg, range: 1.8-45.9 μg/kg); a highly significant difference was recorded when compared to the control group (median: 2.1 μg/kg, range: 0.1-6.6 μg/kg) ($p < 0.005$).

Increased **cadmium** concentrations have been found in 18 out of 20 cancer biopsies (median: 42 μg/kg, range: 9-551 μg/kg), the difference to the control group (median: 15,6 μg/kg, range: 5,2-30 μg/kg) was highly significant ($p< 0.005$) (Table 1).

Lead was also increased in 12 out of 20 tumour biopsies (median: 104.5 μg/kg, range:

Breast cancer biopsies						
Patients	Fe μg/kg	Ni μg/kg	Cr μg/kg	Zn μg/kg	Hg μg/kg	Cd μg/kg
1	27.381	893	655	6.268	2.1	165
2	205.930	733	410	6.420	4.1	33
3	14.664	530	316	6.022	1.8	168
4	29.813	760	513	9.594	8.2	43
5	48.573	1.001	366	1.068	6.1	35
6	32.347	921	701	8.965	33.4	62
7	47.796	949	855	5.929	7.8	120
8	29.385	1.230	838	8.197	7.3	9
9	37.154	469	313	32.642	9.1	142
10	142.391	1.285	968	56.838	4.9	20
11	80.164	1.152	4.415	22.888	11.9	124
12	58.453	1.433	1.786	97.895	12.5	40
13	106.350	3,361	5,978	32.917	2.2	551
14	28.723	490	458	1.326	5.2	22
15	65.112	988	793	50.516	9.0	96
16	84.816	1.057	906	21.082	7.6	16
17	76.608	1.277	1.362	53.336	45.9	42
18	72.376	1.528	1.389	53.709	6.5	42
19	42.254	624	708	6.953	2.8	34
20	57.774	1.142	1.562	27.319	4.1	29
Median	53.174	995	816	17.075	6.9	42
Healthy breast tissue biopsies						
Controls	Fe μg/kg	Ni μg/kg	Cr μg/kg	Zn μg/kg	Hg μg/kg	Cd μg/kg
1	5.331	32	29	2.548	2.5	6
2	11.448	11	19	3.509	6.6	8
3	21.646	19	36	3.973	2.3	23
4	11.424	32	119	2.940	1.9	5
5	10.138	33	.70	4.032	2.5	8
6	10.450	23	54	5.600	0.2	28
7	17.200	12	41	9.339	0.1	27
8	8.261	15	25	2.607	0.1	30
Median	10.937	21	39	3.741	2.1	16
Significance	$p<0.0001$	$p<0.00005$	$p<0.00005$	$p<0.001$	$p<0.005$	$p<0.005$

TABLE 1. Heavy metal content in breast cancer (n=20) and healthy breast tissue (n= 8) biopsies
All results represent the mean of three determinations

9-976 μg/kg). The statistical difference to the control group (median: 63.5, range: 1-92 μg/kg) was still significant ($p < 0.05$).

Surprisingly, lower **copper** levels were found in 11 out of 20 patient biopsies median

919 μg/kg, range 320-44687 μg/kg), when compared to the control samples (median):

1279.5 μg/kg, range: 261-3049). The other 9 cancer samples showed 7 increased values and 2 in the normal range, documenting a different accumulation pattern, possibly related to the tumour aetiology or growth stage. All in all, no significant difference was recorded between the cancer group and the controls ($p = 0.65$).

Silver was detected in only for 4 out of 20 cancer samples (range: 34-91 μg/kg), but in none of the control biopsies (data not shown). **Tin**, **gold** and **palladium** were detectable neither in cancer nor in control biopsies.

When compared by two different techniques (AAS and ICP-MS), there was no statistical

difference in the heavy metal content of the control biopsies (data not shown). ❑

DISCUSSION

To our knowledge, this is the first report describing a large accumulation of Fe and other transition metals like Ni, Cr, Cd, Zn, Hg and Pb in the breast cancer tissue. These findings may have an implication in the breast cancer pathogenesis. The etiology of human breast cancer is still controversial, although hormonal influences and toxic compounds inducing oxidative stress and lipid peroxidation have been suggested to play a role in breast carcinogenesis.

In biological systems, the concentration of redox-active transition metals capable of catalysing / generating free radicals like superoxide, hydrogen peroxide and hydroxyl radical appears to be relatively low. However, under certain pathological conditions (haemochromatosis, Wilson disease, collagenoses and different malignancies), transition metals and their transport proteins may accumulate in different target organs, inducing cellular lipid peroxidation and DNA-attack. In this respect, the ability of excess Fe in mediating the formation of hydroxyl radicals, suppression of cellular immune functions and promotion of tumour growth is well established (2,6,7,9) and increased Cu concentrations were also found in human lung cancer biopsies (10) and in other tumours (11).

Ni, Cr and Cd have been recognized as mutagens and carcinogens through their ability to inhibit the repair of damaged DNA. Besides, another general feature is their property to enhance the mutagenicity and carcinogenicity of directly acting genotoxic agents (12). At the same time, carcinogenic effects of Ni, directly or in association with organic compounds, have been described in the literature (13,14) and, recently, slightly increased concentrations of Fe and Ni have been found in the malign human prostate (15). Inhaled particulate forms of hexavalent Cr cause lung cancer and at cellular levels, Cr exposure may lead to cell cycle arrest, apoptosis or neoplasic transformation (16). Occupational exposure to Cd is associated with lung cancer in humans and high Cd concentrations were found in proliferative prostate lesions (17).

Macromolecular compounds (dextrans) substituted with Hg containing side-chains were reported to promote fibrosarcoma growth in mice (18).

Interestingly, Zn as an essential element, was shown to mediate and increase tumour growth, and Zn depletion was shown to suppress the tumour growth in mice and rats (19,20,21).

None of our patients were occupationally exposed to metals. However, all were exposed to metals through dental restorations such as amalgam, gold bridges or metallic retainers. Another source of metal exposure is cigarette smoke. About half of our patients were smokers and virtually all of them have been passively exposed to cigarette smoke.

The higher heavy metal concentration encountered in various tumour cells may be used for therapeutical interventions with ascorbic acid or phenolic compounds, as already reported (22,23,24,25). Reduction and mobilization of transition metals from their storage or transport proteins renders them extremely reactive in catalysing free radical reactions according to the equations:

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + {}^*OH + OH^-$$

$$H_2O_2 + {}^*O_2^- \xrightarrow{Fe^{3+},\ Cu^{2+}} {}^*OH + OH^- + O_2$$

The described Fenton- and Haber-Weiss-reactions are strong generators of hydroxyl radical leading to lipid peroxidation, DNA strand breaks and apoptosis (2,7,22).

In turn, bioactivation of phenolic / quinonic compounds at the tumour site may lead to a significant generation of superoxide and semiquinone radicals, with deleterious effects for the metal rich malignant cells (23,24,26)

Preventive diagnostic procedures should include 2 / 16-OH-oestrogen ratio, Phase II detoxification assessment besides medical imaging and current tumours markers. Since inflammation often precedes the development of cancerous lesions, the MELISA test ® (27) might be useful for the determination of metal-induced inflammation in individual patients. ❑

Conclusion

The above data suggest that unphysiological accumulation of transition metals in tumour tissue may be closely related to the malignant growth process, and allows new therapy concept with prooxidant Vitamin C or phenolic compounds, respectively to be considered in the future.

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