Increased levels of transition metals in breast cancer tissue

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Abstract

OBJECTIVES: High levels of transition metals such as iron, nickel, chromium, copper, and lead are closely related to free radical generation, lipid peroxidation, formation of DNA-strand breaks, and tumor growth in cellular systems. In order to determine the correlation to malignant growth in humans, we investigated the accumulation of heavy metals in 20 breast cancer biopsies and compared the findings to the levels found in 8 healthy biopsies.

METHODS: The concentration of transition metals in breast cancer and control biopsies was assessed by a standardized Atomic Absorption Spectrofotometry (AAS) technique with acidic hydrolysis for sample preparation. Additionally, heavy metal analysis in control biopsies was also performed with an Inductive Coupled Plasma – Mass Spectroscopy (ICP-MS) technique. For statistical analysis of the results, the Mann-Whitney U Test was applied.

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: The data suggest that pathological accumulation of transition metals in breast tissue may be closely related to the malignant growth process.

ORIGINAL ARTICLE

Abbreviations & Units

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

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- and Haber-Weiss-reactions, ascorbate autoxidation, lipid peroxidation processes, and formation of DNA strand breaks [2,12,14,19]. As published previously, 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 [24]. 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 [9, 16]. Since repeated mitochondrial 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 & Methods

Heavy metal analyses were performed on 20 frozen breast cancer biopsies and 8 healthy breast tissue samples supplied by the Institute of Pathophysiology and Oncology, Charles University, Prague, Czech Republic, and the Caritas Hospital St. Josef, Regensburg, Germany.

The concentrations of Fe, cadmium (Cd), lead (Pb), chromium (Cr), tin (Sn), nickel (Ni), Cu, mercury (Hg), silver (Ag), gold (Au), palladium (Pd), and zinc (Zn) in the biopsy materials were measured in the Spezialklinik Neukirchen, Germany, by a standardized furnace-atomic absorption spectrophotometry (AAS)-technique using a Perkin Elmer Sima 6000 AA-spectrophotometer and acidic hydrolysis as pulping procedure for sample preparation [17].

Additionally, heavy metal analysis in control biopsies was done using an inductive coupled plasma-mass spectroscopy (ICP-MS) technique in the Laboratory for Micro Trace Minerals, Hersbruck, Germany. Each analysis was performed three times. The final result per sample is the mean value of three determinations expressed in µg/kg. The Mann-Whitney U Test was used for statistical analysis of the results.

Results

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

Iron levels were dramatically increased in the breast cancer biopsies when compared to the control group (median: 53,174 µg/kg, range: 14,391–205,930 vs 10,937 µg/kg, range: 5,331–21,646) ($p<0.0001$).

A highly significant Ni accumulation was recorded in the patient biopsies (median: 995 µg/kg, range: 469–3,361). Control biopsies showed measurable levels (median: 21 µg/kg, range: 11–33), but at more than one order of magnitude lower ($p<0.00005$). Similar results were found for Cr when compared to the control group (median: 816 µg/kg, range: 313–5,978 vs 39 µg/kg, range: 19–119) ($p<0.00005$).

A surprisingly high accumulation of Zn was recorded in the cancer biopsies (median: 17,075 µg/kg, range: 1,326–97,895), the difference to the control group (median: 3,741 µg/kg, range: 2,548–9,339) again being 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), a highly significant difference when compared to the control group (median: 2.1 µg/kg, range: 0.1–6.6) ($p<0.005$).

Increased Cd concentrations were found in 18 out of 20 cancer biopsies (median: 42 µg/kg, range: 9–551), the difference to the control group (median 16 µg/kg, range: 5–30) being highly significant ($p<0.005$).

Lead was also increased in 12 out of 20 tumor biopsies (median: 105 µg/kg, range: 9–976). The statistical difference to the control group (median: 64 µg/kg, range: 1–92) was still significant ($p<0.05$) (data not shown).

Surprisingly, lower Cu levels were found in 11 out of 20 patient biopsies (median: 919 µg/kg, range: 320–44,687), when compared to the control samples (median: 1,280 µg/kg, range: 261–3,049). Of the remaining nine cancer samples, seven showed increased values and two were in the normal range, documenting a different accumulation pattern possibly related to the tumor aetiology or growth stage. Taken together no significant difference was recorded between the cancer group and the controls ($p=0.65$) (data not shown).

Only four out of the 20 cancer samples, but none of the control biopsies, showed detectable levels of Ag (range: 34–91 µg/kg) (data not shown). Tin, Au, and Pd were not detectable in either cancer or control biopsies. When the content of heavy metals in control biopsies was analysed by two methods (AAS and ICP-MS) the values were not significantly different (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 for the pathogenesis of breast cancer. 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 cancerogenesis.

Table 1. Heavy metal content in breast cancer (n = 20) and healthy breast tissue (n = 8) biopsies

	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	13,068	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

All results represent the mean of three determinations.

In biological systems, the concentration of redox-active transition metals capable of catalyzing and/or generating free radicals like superoxide, hydrogen peroxide, and hydroxyl radical is relatively low. However, under certain pathological conditions (haemochromatosis, Wilson disease, collagenoses, and various 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, suppressing cellular immune functions, and promoting tumor growth is well-established [9,12,16,25]. Increased Cu concentrations were also found in human lung cancer biopsies [1] and in other tumors [5].

Nickel, Cr, and Cd have been recognized as mutagens and carcinogens because of their ability to inhibit the repair of damaged DNA. In addition, they can enhance the mutagenicity and carcinogenecity of directly-acting genotoxic agents [4]. At the same time, carcinogenic effects of Ni, directly or in association with organic compounds, have been described in the literature [6,15]. Recently, increased concentrations of Fe and Ni have been found in the malignant human prostate [26]. Inhaled particulate forms of hexavalent Cr cause lung cancer, and at cellular level, Cr exposure may lead to cell cycle arrest, apoptosis, or neoplastic transformation [20]. Occupational exposure to Cd is associated with lung cancer in humans, and high Cd concentrations have been found in proliferative prostate lesions [23].

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 tumor growth, and Zn depletion was shown to suppress tumor growth in mice and rats [11, 13,22].

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 have been exposed passively to cigarette smoke.

The higher heavy metal concentrations encountered in various tumor cells may be used for therapeutic interventions with metal chelators, ascorbic acid or phenolic compounds as already reported [3,7,8,10]. Reduction and mobilization of transition metals from their storage or transport proteins renders them extremely reactive in catalyzing free radical reactions according to the equations:

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

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

These Fenton- and Haber-Weiss-reactions are strong generators of hydroxyl radicals leading to lipid peroxidation, DNA strand breaks, and apoptosis [3,12,16]. Bioactivation of phenolic/quinonic compounds at the tumor site may lead to a significant generation of superoxide and semiquinone radicals with deleterious action for the metal-rich malignant cells [7,8]. Preventive diagnostic procedures should include, besides current tumor markers, 2/16-OH-estrogen ratio and Phase II detoxification assessment. Since inflammation often precedes the development of cancerogenic lesions, the MELISA® Test [21] might be useful for the determination of metal-induced inflammation in an individual patient.

In conclusion, the presence of transition metals in breast cancer tissue might be closely related to the malignant growth process.

REFERENCES

1 Adachi S, Takemoto K, Ohshima S, Shimizu Y, Takahama M. Metal concentrations in lung tissue of subjects suffering from lung cancer. Int Arch Occup Environ Health 1991; **63**:193–197.

2 Aust SD, Morehouse LA, Thomas CE. Role of metals in oxygen radical reactions. J Free Radic Biol Med 1985; **1**:3–25.

3 Baader SL, Bruchelt G, Carmine TC, Lode HN, Rieth AG, Niethammer D. Ascorbic-acid-mediated iron release from cellular ferritin and its relation to the formation of DNA strand breaks in neuroblastoma cells. J Cancer Res Clin Oncol 1994; **120** (7):415–421.

4 Beyersmann D. Effects of carcinogenic metals on gene expression. Toxicol Lett 2002; **127**(1–3): 63–68.

5 Ebadi M, Swanson S. The status of zinc, copper and methallothionein in cancer patients. Prog Clin Biol Res 1988; **259**:161–175.

6 Hartwing A. Recent advances in metal carcinogenicity. Pure Appl Chem. 2000; **72**:1007–1014.

7 Ionescu JG. New evidence-based therapies for cancer. Proceedings of the 17th Int. Symposium on Integrative Medicine, p.1–21, Tenerife, Spain, June 2005.

8 Ionescu JG. Transition metals and cancer. Presented at the 12th MELISA Study Group Conference, Prague, 10th–11th September 2005.

9 Liu M, Okada S. Induction of free radicals and tumors in the kidney of Wistar rats by ferric ethylendiaminbe-N,N'-diacetate. Int J Sports Med 1996; **17**:397–403.

10 Lode HN, Bruchelt G, Zinsser D, Baader SL, Rieth AG, Schade UF, et al. Ascorbic acid induces lipid peroxidation on neuroectodermal SK-N-LO cells with high endogenous ferritin content and loaded with Mab-ferritin immunoconjugates. Anticancer Res 1994; **14**(5A):1903–1906.

11 McQuitty JT Jr, DeWys WD, Monaco L, Strain WH, Tob CG, Apgar J, et al. Inhibition of tumor growth by dietary zinc deficiency. Cancer Res. 1970; **30**(5):1387–1390.

12 Mello FA, Meneghini R. In vivo formation of single-strand breaks in DNA by hydrogen peroxide is mediated by the Haber-Weiss-reaction. Biochem Biophys Acta. 1984; **781**: 56–63.

13 Mills BJ, Broghamer WL, Higgins PJ, Lindeman RD. Inhibition of tumor growth by zinc depletion of rats. J Nutr 1984; **114**(4):746–752.

14 Minotti G, Aust SD. The requirements for iron (III) in the initiation of lipid peroxidation by iron (II) and hydrogen peroxide. J Biol Chem 1987; **262**:1098–1104.

15 Ohmori T, Okada K, Tabei R, Shibata T. Effects on tumor induction, growth, metastasis and histology of concurrent administration of putrescine and its metabolising inhibitor alpha-defluoromethylornithine in nickel tumorigenesis in soft tissue. Carcinogenesis 1994; **15**(4):647–652.

16 Okada S. Iron-induced tissue damage and cancer: the role of reactive oxygen species and free radicals. Pathol Int 1996; **46**:311–332.

17 Pierini G, Fini M, Giaveresi G, Dallari S, Brayda BM, Rocca M, et al. Atomic absorption spectrophotometry (AAS) for the evaluation of metallosis in prostheses and artifical organs: a new approach. Int J Artif Organs 1999: **22**(7):522–527.

18 Pitha J, Kociolek K, Apffel CA. Opposite effects of dextrans substituted with sulfhydryls or mercury on tumor growth. Cancer Res 1979; **39**(1):170–173.

19 Scarpa M, Stevanato R, Viglino P, Rigo A. Superoxide ion as active intermediate in the autoxidation of ascorbate by molecular oxygen. J Biol Chem 1983; **258**:6695–6697.

20 Singh J., Carlisle DL, Pritchard DE, Patierno SR. Chromium-induced genotoxicity and apoptosis: relationship to chromium carcinogenesis (review). Oncol Rep 1998; **5** (6):1307–1318.

21 Stejskal VD, Danersund A, Lindvall A, Hudecek R, Nordman V, Yaqob A, et al. Metal-specific lymphocytes: biomarkers of sensitivity in man. Neuroendocrinol Lett 1999; **20**:289–298.

22 Takeda A, Goto K, Okada S. Zinc depletion suppresses tumor growth in mice. Biol Trace Elem Res 1997; **59**(1–3):23–29.

23 Waalkes MP, Coogan TP, Carter RA. Toxicological principles of metal carcinogenesis with special emphasis on cadmium. Crit Rev Toxicol 1992; **22**:175–201.

24 Wang M, Dhingra K, Hittelman WN, Liehr JG, de Andrade M, Li D. Lipid peroxidation-induced putative malondialdehye-DNA adducts in human breast tissue. Cancer Epidemiol Biomarkers Prev 1996; **5**:705–710.

25 Weinberg ED: The role of iron in cancer. Eur J Cancer Prev 1996; **5**:19–36.

26 Yaman M, Atici D, Bakirdere S, Akdeniz I. Comparison of trace metal concentrations in malign and benign human prostate. J Med Chem 2005; **48**:630–634.