Concentration of cadmium, nickel and aluminium in female breast cancer

Hanna Romanowicz-Makowska[1], Ewa Forma[2], Magdalena Bryś[2], Wanda Małgorzata Krajewska[2], Beata Smolarz[1]

[1]Department of Pathology, Research Institute of Polish Mother's Memorial Hospital, Lodz, Poland
[2]Department of Cytobiochemistry, University of Lodz, Lodz, Poland

Aims: The aim of this study was to investigate the cadmium (Cd), nickel (Ni) and aluminium (Al) concentrations in female breast cancer and normal tissue.
Materials and methods: The concentration of metals in 16 non-cancerous breast tissues and 67 breast cancer samples was measured by flame atomic absorption spectrometry.
Results: In the case of normal breast tissue the concentrations were 0.61 ± 0.24 μg Cd/g dry tissue, 1.84 ± 0.67 μg Ni/g dry tissue, and 3.63 ± 1.00 μg Al/g dry tissue, whereas in breast cancer concentrations of metals were 0.76 ± 0.38 μg/g dry tissue, 2.26 ± 0.79 μg/g dry tissue, and 4.40 ± 1.82 μg/g dry tissue, respectively. The concentration of Cd and Al in normal breast tissue was significantly lower than in breast cancer. In the case of Ni concentration, we did not observe statistically significant differences between normal and cancerous tissue. There were no significant differences in concentration of studied metals, in breast cancer, in the context of age, menopausal status, and cancer histological grading.
Conclusion: The data obtained show higher concentration of cadmium and aluminium and support a possible relationship between those metals and breast cancer

Key words: cadmium, nickel, aluminium, female breast cancer.

Introduction

Cadmium (Cd) and nickel (Ni) are transition metals, which have been classified as human carcinogens by the International Agency for Research on Cancer. This classification is based on epidemiological studies in occupationally exposed workers and animal studies [1-5]. Anthropogenic emission of these metals exceeds emission from natural sources by two- to tenfold [3, 6]. As to Ni, it can be combined with other metals, such as iron, copper, chromium, and zinc, to form alloys, like stainless steel [5, 7-10]. Human exposure to Ni and Cd occurs primarily via inhalation and ingestion. In addition, absorption of Ni can occur through skin contact with metals containing this element, as well as by wearing jewellery and using coins that are produced from Ni alloys [9, 11]. In the case of Cd, absorption is strongly dependent on the route of exposure. Only about 5% of its oral dose is absorbed by the gastrointestinal tract and more than 90% is absorbed from the lung [6]. Carcinogenesis is also considered as a critical effect of Cd. There are studies that indicate a role of this metal in renal, liver, haematopoietic system, bladder and stomach cancer in humans. Some evidence indicates that environmental cadmium exposure can also be associated with prostate, pancreatic and breast cancer in humans [12, 13]. Approximately 10-20% of the population is sensitive to nickel. Its accumulation in the body through chronic exposure could lead to lung fibrosis, cardiovascular and kidney diseases and carcinogenesis [5, 8, 14]. Epidemiological studies have clearly implicated Ni compounds as human carcinogens based on a higher incidence of lung and nasal cancer among nickel mining, smelting and refinery workers.

There are some reports suggesting that exposure to nickel could also cause other types of neoplasms such as carcinoma of the larynx, kidney, prostate, stomach, and soft-tissue sarcomas. However, the statistical significance of these findings is unproven [2, 5, 9, 15].

Aluminium (Al) is the third most abundant element in nature after oxygen and silicon, comprising approximately 8% of the Earth's crust [16, 17]. Several substances containing Al are used as food packaging materials [17-19]. Salts of Al are used as the active antiperspirant agent in underarm cosmetics. Their mode of action is thought to involve blocking of the sweat duct which prevents the secretion of sweat onto the skin surface, probably through the formation of a physical plug composed of precipitated salts and dead cells at the top of the sweat duct [20, 21]. The metal is absorbed through several routes. These include oral, intranasal, transdermal, and parenteral pathways. Absorption of this metal is increased by low pH which enhances the solubility of aluminium species. In humans, Al plays the causal role in dialysis encephalopathy, osteomalacia and microcytic anaemia. Furthermore, several studies have suggested a possible link between Al neurotoxicity, Alzheimer's disease and breast cancer [17, 21].

The aim of this study was to examine the concentration of Cd, Ni, and Al in normal and cancerous breast tissue and to evaluate relationships between the concentration of these metals and clinicopathological parameters of cancer.

Materials and methods

Samples of ductal breast carcinoma were obtained from 67 women (age range 32-78 years, mean ± SD 52.83 ± 6.41) undergoing surgery for breast neoplasms in the Polish Mother's Memorial Hospital, Lodz, Poland. No distant metastases were found in any of the patients at the time of treatment onset. The median follow-up of patients at the time of analysis was 39 months (range: 2-71 months). The average tumour size was 20 mm (range 17-32 mm). All the tumours were graded by a method based on the criteria of Bloom-Richardson [22]. Histological grades were evaluated in all the cases: grade I – 21 cases, grade II – 40 cases and grade III – 6 cases. Additionally, all these women were not especially exposed to carcinogenic metals.

Non-cancerous tissue samples were collected from 16 breasts of the same patients and excised from non-affected areas of mammary gland about 5 cm away, or as far from the neoplasm as possible. These tissues were also histologically diagnosed and used for metal detection (control) only if there was no trace of cancer tissue.

Immediately after resection, samples were placed in polyethylene containers and stored at –20°C for subsequent analysis of their Cd, Ni and Al content.

Reagents and solutions

All used reagents and chemicals were analytical grade. Bidistilled and freshly deionized water, resistance 18.2 MΩ/cm, Milli-Qplus (Millipore, Billerica, MA, USA) was used throughout this procedure. HNO_3, HCl and H_2SO_4 (Suprapure, Merck, Darmstadt, Germany) were used for the preparation of solutions. All glassware (borosilicate) and plasticware (low-density polyethylene) were soaked in 2% (v/v) HCl for 2 h, rinsed with deionized water, soaked in 2% (v/v) HNO_3 for 2 h, and then rinsed several times with deionized water.

Instrumentation and sampling procedure

A flame atomic absorption spectrometer (AAnalyst 100, Perkin–Elmer Instrument, CT, USA) was used to determine metal concentrations. For the calibration, working solutions of metals were prepared based on TraceCERT® (Sigma-Aldrich Corp, St. Louis, MO, USA).

Thawed tissues were dried by incubation at 80°C until tissues achieved a constant weight. Then, the tissue samples, weighing 0.2–0.5 g, were mineralized in aqua fortis (mixture of concentrated HNO_3 and H_2SO_4). After mineralization, each sample was diluted to 10 ml of deionized water. The accuracy and precision of analysis for tissue Cd, Ni and Al contents were assessed by a simultaneous analysis of a standard reference bovine liver sample (SRM 1577b, The National Institute of Standards and Technology, USA). In the range of the samples analysed in the study, the precision for Cd, Ni, and Al were ± 4, 3 and 5%, respectively. For each sample, three parallel independent determinations were made. The final concentrations were reported in μg metal/g dry tissue.

Statistical methods

The STATISTICA 5.0 software (StatSoft, Inc. USA) made the statistical calculations. For the concentrations of various metals in the investigated samples, mean and standard deviations (SD) were computed. None of the metal concentrations passed the test for being normally distributed (Kolmogorov-Smirnov test) and therefore nonparametric statistical tests were used for analysing the results (Mann-Whitney U test; Spearman's rank analysis). P-values < 0.05 were considered to be significant.

Results

The presence of Cd, Ni and Al was detected in all control and breast cancer samples. All mean and SD values for each of the three metals were expressed in micrograms per gram dry tissue weight. The results of the analysis of cadmium, nickel and aluminium in nor-

mal breast tissue and female breast cancer are presented in Fig. 1. Concentration of cadmium in normal breast tissue ranged from 0.09 to 1.24 μg/g dry tissue and the mean value (0.61 ± 0.24 μg/g dry tissue) was statistically lower than in breast cancer (Mann-Whitney U-test, $P < 0.05$). The concentration of Cd in breast cancer was between 0.16 and 1.56 μg/g dry tissue. Similar differences were observed in the case of Al concentration (Mann-Whitney U-test, $P < 0.05$). In noncancerous tissue concentration of Al ranged from 0.32 to 6.59 μg/g dry tissue, whereas in breast cancer, the concentration of Al was contained within the broad range 0.28–8.32 μg/g dry tissue. Mean Al content in normal tissue was 3.63 ± 1.00 μg/g dry tissue. In normal tissues as well as in breast cancer samples Al concentration was the highest among investigated metals (Fig. 1). In the case of Ni, concentration of this metal was nearly significantly lower in normal tissues than in breast cancers (Mann-Whitney U-test, $P = 0.057$). Concentrations of Ni in normal tissues were in the range 0.37-2.85 and mean was 1.84 ±0.67 μg/g dry tissue. In breast cancer samples, the concentration of Ni was 0.59-4.80 μg/g dry tissue. The observed mean Cd concentration in analysed cancers was threefold lower than the mean Ni content (Mann-Whitney U-test, $P < 0.0001$). Interestingly, there was a significant inverse correlation between the mean concentration of cadmium and nickel in female breast cancers (Spearman's rank correlation coefficient $R = -0.298$, $P < 0.05$). Mean concentration of aluminium in breast cancer was over fivefold higher than cadmium concentration (Mann-Whitney U-test, $P < 0.0001$) and nearly twice as high as Ni content (Mann-Whitney U-test, $P < 0.0001$). There was no correlation between concentration of aluminium and other metals.

Quantitative analysis of Cd, Ni, and Al in the context of breast cancer and clinicopathological parameters is shown in Table I. Statistical analysis showed no significant differences of studied metals in the context of age, menopausal status, and cancer grade.

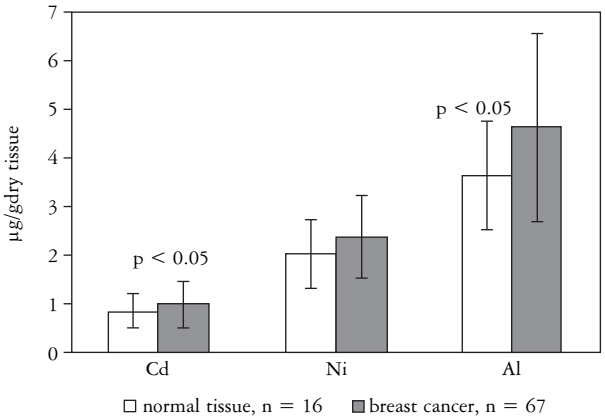

Fig. 1. The content of cadmium, nickel, and aluminium in normal tissue compared to female breast cancer. Each bar represents a mean ± SD value. Statistical analysis by the Mann-Whitney U test indicates significant differences in the content of cadmium and aluminium between normal tissue of mammary gland and breast cancer ($P < 0.05$). In the case of Ni concentration, there was no statistical difference between normal and cancerous samples

Discussion

The main causes of breast cancer development are sex, age, lack of childbearing or breastfeeding, higher hormone levels, race, economic status and dietary iodine deficiency. High-fat diet, alcohol intake, obesity, environmental factors such as tobacco use, radiation, endocrine disruptors and shift work and genetic factors increase the risk of cancer development.

The relationships between risk factors and breast cancer development are not exactly known. Therefore, the identification of new risk factors for breast cancer is ur-

Table I. Quantitative analysis of Cd, Ni, and Al (μg/g dry tissue) in the context of female breast cancer and clinicopathological parameters

	Cd (mean ±SD)	Ni (mean ±SD)	Al (mean ±SD)
Total (n = 67)	0.76 ±0.38b	2.26 ±0.79	4.40 ±1.82
Age			
< 40 (n = 21)	0.72 ±0.30	2.30 ±0.85	4.32 ±1.77
40-60 (n = 39)	0.79 ±0.40	2.33 ±0.74	4.36 ±1.93
> 60 (n = 7)	0.77 ±0.33	2.29 ±0.80	4.42 ±1.80
Menopausal status			
Premenopausal (n = 31)	0.77 ±0.38	2.13 ±0.60	4.62 ±1.73
Postmenopausal (n = 36)	0.75 ±0.38	2.37 ±0.93	4.15 ±2.01
Scarf-Bloom-Richardson grade			
I (n = 21)	0.75 ±0.39	2.16 ±0.86	4.09 ±1.91
II (n = 40)	0.77 ±0.37	2.31 ±0.78	4.55 ±1.87
III (n = 6)	0.73 ±0.45	2.26 ±0.61	4.56 ±1.22

gently needed, and an analysis of some gene polymorphisms could be an interesting option. In our earlier study we investigated the association between DNA repair gene polymorphism and the incidence of breast cancer in Polish women [23, 24]. In the present work we investigated whether the concentrations of Cd, Ni, and Al in breast tissues were associated with the risk of breast cancer in Poland.

In our study we found an association between breast cancer occurrence and concentration of Cd, Ni, and Al in this study population. The concentration of Cd, Ni, and Al was associated with an elevated risk of breast cancer in the Polish population. The concentration of Cd and Al in normal breast tissue was significantly lower than in breast cancer. In the case of Ni concentration, we did not observe statistically significant differences between normal and cancerous tissue.

We also analysed the concentration of Cd, Ni, and Al in groups of patients suffering from breast cancer according to different cancer grading by the Bloom-Richardson classification. In the present study, concentration of Cd, Ni, and Al was not related to cancer grade. The reason for this may be the relatively small group of grade I, II and III enrolled in our study.

The present work was performed on an ethnically homogeneous population, which may improve our knowledge regarding to what extent the genotype-phenotype relationship variations are population-related.

In the light of numerous studies, the investigated metals have multiple effects on cells and can be involved in carcinogenesis. Cadmium affects cell proliferation, differentiation, apoptosis and signal transduction by enhancement of protein phosphorylation and activation of transcription and translation factors. Cd reduces activities of proteins involved in antioxidant defences and stimulates the production of reactive oxygen species, which may act as signalling molecules in the induction of gene expression and apoptosis. Several studies have shown that Cd interferes with DNA repair and DNA methylation. The inhibition of DNA repair processes by Cd represents a mechanism by which this metal enhances the genotoxicity of other agents and may contribute to tumour initiation by cadmium. This metal may also play a role in the progression of cancer, by increasing the metastatic potential of existing cancer cells. However, the mechanisms underlying these effects have not yet been elucidated. Some studies have shown that cadmium can disrupt the tight junctions between many types of epithelial cells by interfering with the normal function of E-cadherin, a Ca^{2+}-dependent cell adhesion molecule that plays a key role in epithelial cell-cell adhesion. The disruption of E-cadherin-mediated cell adhesion can trigger the β-catenin-mediated activation of oncogenes in epithelial cells and increase the invasive potential of epithelial-derived cancers [3, 6, 12, 25, 26].

In the case of Ni, the mechanisms of carcinogenesis are likely to involve genetic and epigenetic routes. The molecular mechanisms of nickel-induced carcinogenesis include production of oxidative DNA damage and inhibition of DNA repair. Additionally, Ni induces oxidative stress that depletes glutathione, as well as activation or silencing of certain genes and transcription factors, especially those involved in the cellular response to hypoxia. The epigenetic effects of Ni include alteration in gene expression resulting from DNA hypermethylation and histone hypoacetylation. However, the exact molecular mechanisms of Ni carcinogenesis are not known and have been the subject of numerous epidemiological and experimental investigations [5, 9].

At a molecular concentration, any effects of Al would be most likely to involve DNA damage followed by aberrant signalling of growth pathways [21]. Al has been suggested to contribute to oxidative stress [17, 21]. The established role of oestrogen in the development and progression of breast cancer raises questions concerning a potential contribution from the many chemicals in the environment which can enter the human breast and which have estrogenic activity [20]. Cd, Ni and Al belong to the class of environmental endocrine disrupters and have a significant effect on ERα expression and activity [21, 27-29].

Because exposure to metals is widespread, the elucidation of their roles in the aetiology and development of hormone-related diseases, such as breast cancer, may have significant implications in risk reduction and disease prevention [27].

Acknowledgements

This work was supported by a grant of the President of the City of Łódź (2007).

References

1. IARC (International Agency for Research on Cancer). Beryllium, cadmium, mercury, and exposures in the glass manufacturing industry. In: Monographs on the evaluation of carcinogenic risks of humans, 1993, 58, Lyon, France.
2. IARC (International Agency for Research on Cancer). (1990) Chromium, nickel and welding. In: Monographs on the evaluation of carcinogenic risks of humans, 1990, 49, Lyon, France.
3. Waisberg M, Joseph P, Hale B, et al. Molecular and cellular mechanisms of cadmium carcinogenesis. Toxicology 2003; 192: 95-117.
4. Waalkes MP. Cadmium carcinogenesis in review. J Inorg Biochem 2000; 70: 241-244.
5. Kasprzak KS, Sunderman FW Jr, Salnikow K. Nickel carcinogenesis. Mutat Res 2003; 533: 67-97.
6. Filipic M, Fatur T, Vudrag M. Molecular mechanisms of cadmium induced mutagenicity. Hum Exp Toxicol 2006; 25: 67-77.
7. Martelli A, Rousselet E, Dycke C, et al. Cadmium toxicity in animal cells by interference with essential metals. Biochemie 2006; 88: 1807-1814.
8. ATSDR (Agency for Toxic Substances and Disease Registry) US Public Health Service. Toxicological profile of nickel. Atlanta, GA: US Department of Health and Human Services, Public Health Services, 2005.

9. Denkhaus E, Salnikow K. Nickel essentiality, toxicity, and carcinogenicity. Crit Rev Oncol Hematol 2002; 42: 35-56.
10. Barceloux DG. Nickel. Clin Toxicol 1999; 37: 239-258.
11. Godt J, Scheidig F, Grosse-Siestrup C, et al. The toxicity of cadmium and resulting hazards for human health. J Occup Med Toxicol 2006; 1: 22-27.
12. Satarug S, Baker JR, Reilly PE, et al. Changes in zinc and copper homeostasis in human livers and kidneys associated with exposure to environmental cadmium. Hum Exp Toxicol 2001; 20: 205-213.
13. Antila E, Mussalo-Rauhamaa H, Kantola M, et al. Association of cadmium with human breast cancer. Sci Total Environ 1996; 186: 251-256.
14. Vahter M, Berglund M, Akesson A, et al. Metals and women's health. Environ Res A 2002; 88: 145-155.
15. Sivulka DJ. Assessment of respiratory carcinogenicity associated with exposure to metallic nickel: A review. Regul Toxicol Pharmacol 2005; 43: 117-133.
16. ATSDR (Agency for Toxic Substances and Disease Registry). US Public Health Service. Toxicological profile of aluminum. Atlanta, GA: US Department of Health and Human Services, Public Health Services, 2006.
17. Becaria A, Campbell A, Bondy SC. Aluminum as a toxicant. Toxicol Ind Health 2002; 18: 309-320.
18. Soni MG, White SM, Flamm WG, et al. Safety evaluation of dietary aluminum. Regul Toxicol Pharmacol 2001; 33: 66-79.
19. Yokel RA, McNamara G. Aluminium toxicokinetics: an update minireview. Pharmacol Toxicol 2001; 88: 159-167.
20. Darbre PD. Environmental oestrogens, cosmetics and breast cancer. Best Pract Res Clin Endocrinol Metab 2006; 20: 121-143.
21. Darbre PD. Aluminium, antiperspirants and breast cancer. J Inorg Biochem 2005; 99: 1912-1919.
22. Bloom H, Richardson W. Histological grading and prognosis in breast cancer; a study of 1409 cases of which 359 have been followed for 15 years. Br J Cancer 1957; 11: 359-377.
23. Sobczuk A, Smolarz B, Romanowicz-Makowska H, Pertyński T. Rola polimorfizmów pojedynczych nukleotydów (SNP) w obrębie genów mechanizmów naprawy DNA przez rekombinację RAD51, XRCC2, XRCC3 i XRCC4 w patogenezie raka piersi u kobiet w wieku pomenopauzalnym. Prz Menopauz 2009; 4: 228-232
24. Romanowicz H, Smolarz B, Baszczyński J, et al. Genetics polymorphism in DNA repair genes by base excision repair pathway (XRCC1) and homologous recombination (XRCC2 and RAD51) and the risk of breast carcinoma in the Polish population. Pol J Pathol 2010; 61: 206-212.
25. Pearson CA, Prozialeck WC. E-cadherin, β-catenin and cadmium carcinogenesis. Med Hypotheses 2001; 56: 573-581.
26. Beavon IRG. The E-cadherin-catenin complex in tumour metastasis: structure, function and regulation. Eur J Cancer 2000; 36: 1607-1620.
27. Martin MB, Reiter R, Pham T, et al. Estrogen-like activity of metals in Mcf-7 breast cancer cells. Endocrinology 2003; 144: 2425-2436.
28. Brama M, Gnessi L, Basciani S, et al. Cadmium induces mitogenic signaling in breast cancer cell by an ERα-dependent mechanism. Mol Cell Endocrinol 2007; 264: 102-108.
29. Stoica A, Katzenellenbogen, Martin MB. Activation of estrogen receptor-? by the heavy metal cadmium. Mol Endocrinol 2000; 14: 545-553.

Address for correspondence

Beata Smolarz
Department of Pathology
Research Institute of Polish Mother's Memorial Hospital
ul. Rzgowska 281/289
93-316 Łódź, Poland
phone: +48 42 271 12 80
fax: +48 42 271 20 74
e-mail: smolbea@wp.pl