A Comparison of Concentrations of Selected Heavy Metals in Neoplastic and Peri--Neoplastic Lung Tissues in Inhabitants from Wielkopolska and Upper Silesian Industrial District in Poland

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Abstract

This paper presents results of a study on determination of the concentration of Cd, Pb, Cu and Zn in neoplastic and peri-neoplastic lung tissues from 110 subjects of two regions of different degrees of environmental pollution: Wielkopolska (75) and Upper Silesian Industrial District (35). The concentrations of the metals were determined by the method of differential pulse anodic stripping voltammetry (DPASV). The mean values (in µg/g wet tissue) obtained for the inhabitants from Wielkopolska were: Cd - 0.56; Pb - 0.45; Cu - 1.98; Zn - 12.93 in the neoplastic tissue and Cd - 0.88; Pb - 0.40; Cu - 1.96; Zn - 12.80 in the peri-neoplastic tissue. For the inhabitants from the Upper Silesian Industrial District (USID) the corresponding values were: Cd - 0.63; Pb - 0.25; Cu - 1.61; Zn - 20.63 in the neoplastic tissue and Cd - 1.37; Pb - 0.06; Cu - 1.66; Zn - 19.20 in the peri-neoplastic tissue. Concentrations of Cd were higher in peri-neoplastic tissues than in the neoplastic ones in the subjects from both groups. The highest concentrations of Pb and Cu were found in tissues of the inhabitants from Wielkopolska, while the highest concentrations of Cd and Zn were in tissues of those from Upper Silesian Industrial District.

Keywords: cadmium, lead, copper, zinc, lung, neoplastic tissue, peri-neoplastic tissue, differential pulse anodic stripping voltammetry.

Introduction

Progress in recognition of the mechanism of carcinogenesis has been achieved in part due to the identification of chemical factors affecting or favouring the development of cancer. Among these factors particular attention has been paid to metals commonly met in the form of inorganic carcinogenic compounds in the environment [1,2, 3]. It seems that the systems most susceptible to the carcinogenic influence of the environment are those in closest contact with it, such as lungs and alimentary tract.

Determination of the concentrations of metals in lung cancer tissues has not been performed on a large scale and therefore the kinetics, diagnostic value and prognostic value of such measurements have not been established [4]. The significance of metals in carcinogenesis is mainly assessed on the basis of differences between their concentrations in neoplastic, peri-neoplastic and healthy tissues. Results of the relevant studies of lung tissues have been presented by many authors [5, 4, 6, 7, 8, 9, 10, 11, 12].

The studies seem highly relevant, all the more so in conditions of increasing and diversified exposure to environmental pollution and increasing number of lung cancer cases [13]. A study was undertaken to assess the content of Cd, Pb, Cu and Zn in neoplastic and peri--neoplastic lung cancer tissues in subjects from two regions of Poland characterised by different degrees of environmental pollution: Wielkopolska and the Upper Silesian Industrial District (USID). Wielkopolska is the industrial and agricultural region in which environmental pollution is related to heavy industry, transportation and modern agriculture. USID is the region with Poland's highest density of industrial plants hazardous for people and is known as one of the most polluted areas in Europe.

The choice of determination of the concentrations of the above-mentioned metals followed from the fact that according to Gilman classification, Cd is suspected to have a carcinogenic effect, as do Pb, Cu and Zn [2].

Experiment

Collection of Samples

The neoplastic and peri-neoplastic lung tissues were taken from 110 subjects: 20 women and 90 men. Of these individuals, 75 were from Wielkopolska and 35 from USID. The age of the subjects varied from 35 to 94 years (x= 57.76 ± 11.78). In the investigated group from Wielkopolska 5 persons did not smoke, whereas in the group with USID 2 were non-smokers.

The samples were collected before or during operations in 72 cases and during autopsies in 38 cases. The tissues placed in foil bags were stored at -4°C. The samples were cut and frozen with plastic knives.

Sample Preparation

The weighed portions (1.0 to 1.5 g) of the samples were dried in an oven at 95°C for 1 h. Then the samples were combusted at 495°C. After 1 h a portion of H_2O_2 was added and combustion was continued. These procedures were repeated when needed. The dry residue from the mineralised tissue was dissolved in hot 1 mol/dm^3 hydrochloric acid.

The concentrations of Cd, Pb, Cu and Zn were determined by DPASV (differential pulse anodic stripping voltammetry) method using a pulse polarograph PP-04 made by Unitra-Telpod [14].

The concentrated acids, ammonium hydroxide, and standard solutions of the metals made by Merck were suprapure grade and the other reagents made by POCh were pure for analysis. All solutions were prepared with water deionised in a Milli-Q System apparatus. The solutions were kept in quartz cells. Glass was washed with HNO_3(1 + 1)*.

* The metals, concentration tests were carried out by the Laboratory of Applied Analytical Chemistry, University of Warsaw, Department of Chemistry.

Statistical Analysis

The results of determinations of the concentrations of Cd, Pb, Cu and Zn were subjected to statistical analysis. Arithmetic means (X), standard deviations (SD) and extreme values (min., max.) were calculated. A comparison of the data for particular groups was made. The significance of the differences was assessed by the nonparametric Mann-Whitney test applied for two samples from different environments [15]. The limiting value of the level of significance was $p < 0.05$.

Results and Discussion

Results of the determinations of the concentrations of Cd, Pb, Cu and Zn in neoplastic and peri-neoplastic lung tissues from subjects coming from two regions of different levels of environmental pollution (Wielkopolska and Upper Silesian Industrial District) are collected in Tables 1 and 2.

In the tissues from the subjects from Wielkopolska, the concentration of Cd was 57.14% higher in the peri-neoplastic ($p < 0.02$) than in the neoplastic tissues, while the concentrations of Pb, Cu, Zn was at a similar level in these two kinds of tissues.

The concentrations of Cd in the peri-neoplastic tissues of the inhabitants from USID were 2.2-fold higher than in the neoplastic tissues ($p < 0.001$), while the concentrations of Pb were 4.2-fold lower. The concentrations of Cu and Zn in the neoplastic and peri-neoplastic tissues were similar.

The concentrations of Pb and Cu in the neoplastic tissues of the subjects from Wielkopolska were 1.8-fold ($p < 0.0001$) and 1.2-fold ($p < 0.02$) higher than in the corresponding tissues from USID inhabitants. No significant differences were found in the concentrations of Cd and Zn in these tissues. Cd content was by 12.50% and that of Zn by 59.55% lower in the tissues from the Wielkopolska subjects than in the corresponding tissues from the inhabitants from USID.

The concentrations of Pb and Cu in the peri-neoplastic tissues of the subjects from Wielkopolska were by 6.6-fold ($p < 0.00001$) and 18.07% ($p < 0.03$) higher than the corresponding concentrations in the USID tissues. On the other hand, the concentrations of Cd and Zn in the peri-neoplastic tissues of the USID subjects were 1.5-fold higher than in the corresponding tissues from the Wielkopolska inhabitants. The differences were significant at the levels $p<0.02$ and $p<0.001$, respectively.

As follows from the results, the accumulation of Pb, Cu and Zn in the neoplastic and peri-neoplastic tissues is at a similar level, except for the content of Pb in the USID tissues. An interesting observation was a significant increased concentration of Cd in the peri-neoplastic tissues than in the neoplastic ones, in particular in the tissues from USID inhabitants. This result is in agreement with that obtained by Hart [7], who reported an insignificant increase (1.4-fold) in the concentration of Cd in the peri-neoplastic relative to the neoplastic tissues. This result can be related to the fact that the peri-neoplastic tissues we studied contained mainly Cd-thionein, while the general concentration of metal-

Table 1. Concentrations of Cd, Pb, Cu and Zn in neoplastic lung tissues from inhabitants living in Wielkopolska and the Upper Silesian Industrial District (USID).

Regions of inhabitation	n	Statistical measures	Concentrations of the metals [μg/g wet tissue]			
			Cd	Pb	Cu	Zn
Wielkopolska	75	$\bar{x}$	0.56	0.45	1.98	12.93
		SD	0.60	0.55	0.86	5.89
		min.	0.01	0.004	0.23	3.80
		max.	2.90	2.40	5.30	29.20
USID	35	$\bar{x}$	0.63	0.25	1.61	20.63
		SD	0.74	0.26	1.78	9.83
		min.	0.01	0.01	0.10	3.00
		max.	2.90	2.30	2.40	41.30

Table 2. Concentrations of Cd, Pb, Cu and Zn in peri-neoplastic lung tissues from the inhabitants studied living in Wielkopolska and the Upper Silesian Industrial District (USID).

Regions of inhabitation	n	Statistical measures	Concentrations of the metals [μg/g wet tissue]			
			Cd	Pb	Cu	Zn
Wielkopolska	75	$\bar{x}$	0.88	0.40	1.96	12.80
		SD	0.89	0.66	0.79	5.53
		min.	0.03	0.02	0.54	3.20
		max.	5.81	3.70	4.70	32.10
USID	35	$\bar{x}$	1.37	0.06	1.66	19.20
		SD	1.08	0.05	0.92	9.90
		min.	0.06	0.01	0.50	6.10
		max.	3.60	0.25	5.30	41.00

lothioneins in these tissues was significantly (3.2-fold) lower than in the tumor. The tissues studied by Hart contained mainly Cu-thionein. The accumulation of metals in lungs, also in neoplastic tissues, can be explained by the presence of proteins which can bond them. The relatively great differences in the concentrations of metallothioneins can be accounted for by a great diversity of cell populations in neoplastic tissues. Aggregations of the cells containing metallothioneins can be surrounded by tumor cells, necrotic areas, and stromal cells, and can be in the inflammed regions and proteins may not occur in all these sites. Metallothioneins form complexes with metals (Zn, Cu, Cd), and the complexes can undergo dissociation and can be bonded by proteins in the tumor tissue, thus taking part in carcinogenesis. They can be involved in growth and differentiation of cells. For example, Cu-thionein can supply copper ions for the enzymes engaged in the new formation of blood vessels, a necessary reqiurement for tumor growth [7]. An increased concentration of Cu-thionein was found in the lungs of newborn babies. Its level decreases to the values typical of mature lungs within the first week of life. This protein appears at an increased level as a factor accompanying development of lung cancer, in the tumor and in the serum [7].

The results obtained by Tietz et al. proved that the concentrations of the metals studied (including Cd and Pb) were lower in the tumor tissues than in the peri-neoplastic tissues [12]. As the author explains, higher concentrations of metals in lung tissues appear prior to the formation of a tumor, whereas the tumor tissue itself does not accumulate metals. Also, according to Molokhia and Smith, the concentrations of the metals studied (Cu included) in lung neoplastic tissues were lower than in the peri-neoplastic tissues and healthy ones [9]. The results obtained by Tietz [12] and Molokhia et al. [9] prove that the lung neoplastic tissue shows a reduced capability of absorption and retention of the metals studied. This conclusion is only partly in agreement with the results of our study, in which the concentration of Cd was found to be lower in the neoplastic than in the peri-neoplastic tissues. The concentrations of metals determined in our study can only be compared with the results reported by Hart and Tietz [7, 12]. According to their results, the concentrations of heavy metals were much higher in the peri-neoplastic than in the neoplastic tissues. An interesting observation made in this study was a significantly increased level of Pb and Cu in the neoplastic and peri-neoplastic tissues of the inhabitants from Wielkopolska and a significantly increased level of Cd and Zn in the peri-neoplastic tissues of the USID subjects. The results can be related to the degree of environmental pollution with these metals in the respective regions.

The increased concentrations of Cd, mainly in the peri-neoplastic tissues may indicate its involvement in the development of tumors; however, this supposition has to be confirmed by further studies on the mechanism of this metal role in carcinogenesis.

It should be emphasised that analyses of pairs of tissues (neoplastic and peri-neoplastic) taken from the same individual eliminates the influence of the age, hormone level, diet and environmental factors on the differences in the concentration of heavy metals in these two kinds of tissue [6].

Conclusions

The above-presented results of the study lead to the conclusion that the content of Cd in the peri-neoplastic tissue is significantly higher than in the neoplastic tissue, in the samples taken from the subjects from both Wielkopolska and the Upper Silesian Industrial District. The concentrations of Cd, Pb, Cu and Zn in the studied tissues revealed differences according to the region in which the patients lived. Concentrations of Pb and Cu are higher in the tissues from the inhabitants living in Wielkopolska, while those of Zn and Cd were higher in the tissues from the subjects living in Upper Silesian Industrial District. The method of DPASV assures high accuracy and reproducebility of results and can be used for determining concentrations of metals in human lung tissue.

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