The Influence of Atmospheric Chromium on Selenium Content and Glutathione Peroxidase Activity in Blood of Tannery Workers

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The concentration of selenium and thiobarbituric acid reactive substances (TBARS) and activity of glutathione peroxidase (GSH-Px) were determined in blood of 34 workers of a tannery in Gniezno, Poland, who worked in an area containing chromium compounds. Fourteen workers were exposed to chromium compounds at concentrations of 0.11 ± 0.07 mg Cr/m³ (mean ± SD) and 20 at concentrations 5–10 times lower i.e., 0.022 ± 0.009 mg Cr/m³. Excretion of Se in urine was measured in all of the investigated workers. Decreased Se concentration in whole blood and blood plasma and elevated TBARS concentration in blood plasma were found in the whole group of investigated tanners as compared to controls. Tanners working in areas with high chromium concentrations had a statistically significant decrease in Se concentration in blood and plasma and decreased urinary excretion of the microelement as compared with other tanners. TBARS concentration was 2.5 times lower in workers exposed to higher chromium concentrations (p<0.005) than in other workers. Positive linear correlations were found between the concentration of Se in blood and the amount of the element excreted in urine (r = 0.48; p<0.005), the concentration of Se in blood plasma and in urine (r = 0.46; p<0.01), and the concentration of Se in blood and erythrocyte GSH-Px activity (r = 0.42; p<0.02). The observed differences between Se concentration in blood and urine of tannery workers and people who are not employed in the industry may indicate a kind of specific adaptation of the body to the working environment containing chromium compounds. Key words: blood, chromium, glutathione peroxidase, selenium, tannery workers, TBARS, thiobarbituric acid reactive substances, urine.

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The concentration of trace elements in organisms is apt to vary considerably, depending on the geographic region, the extent of environmental pollution, the presence of agonists and antagonists in the environment, and the diet. Occurrence of certain diseases of unclear etiology is sometimes attributed to deficiency of microelements or lack of balance between their intake and excretion. Among the microelements that are necessary for humans (1), selenium (Se) is the most toxic (2). Unlike the case of other microelements, the borderlines between deficiency, normal status, and toxic level are very narrow. Epidemiological studies carried out in the Scandinavian countries and in the United States showed that low levels of Se in blood may contribute to neoplastic (3), neurological (4), or cardiovascular (5) diseases.

Many researchers studying Se metabolism believe that, in order to clearly define the role of this element in pathogenesis of certain diseases in people, it is important not only to determine its level in the diet and concentration in blood but also its biochemical activity, which is expressed by the activity of the selenium-dependent enzyme glutathione peroxidase (GSH-Px, EC 1.11.1.9) (6). GSH-Px catalyzes the reduction of hydrogen peroxide to water and of organic hydroperoxides, including hydroperoxides of polyunsaturated fatty acids, to less toxic alcohols.

According to some researchers, insufficient attention has been given to investigation of inhabitants of industrial centers with respect to the differences in the concentration of necessary trace elements, their metabolites, and their influence on the people living in the polluted area (7).

Lieback and Ruden (7) suggested that the concentration of Se in the blood of people employed in industrial plants differs from that of people living in areas where industry is not developed because industrial smoke contains high Se concentrations. Snoop et al. (8) demonstrated that rural subjects had significantly lower whole blood, erythrocyte, and plasma Se levels, as well as lower blood GSH-Px activity, than urban residents. It is also worth noting that Se plays a protective role in heavy metal poisoning, e.g., people exposed to mercury compounds accumulate Se (9,10). Similar processes may occur when people are exposed to other metals.

Chromium has been reported to cause pulmonary disorders in humans exposed to its dust and is considered to be a potential carcinogen in water discharged from industries involved in metal plating, anodizing, and chrome tanning processes. Chromate salts are recognized human carcinogens of the lung, nasal cavity, and paranasal sinus, and are animal carcinogens of the stomach and larynx (11). In 1986, the National Institute Occupational Safety and Health Conference of Governmental Industrial Hygienist on limit values and biological exposure indices recommended limits for chromic acid at 0.05 mg/m³ as chromium trioxide (unpublished data).

Chromium compounds are widely used in many other branches of industry, including tanning technology. The concentration of chromium in particulate dust (particles below 10 μm) at different workstations in the tanning industry is 10–25 times higher than in the surrounding environment (75 ± 25 ng/m³ in buffing of chrome crust and 39 ± 14 ng/m³ in chrome tanning (mean ± SD)) (12). In the tanning process, Cr(III) is the most common form of chromium in the working environment; however, Cr(VI) may occur as well. Harmful effects of chromium compounds depend on the oxidation state of the metal; these vary so much that they have to be considered independently. The compounds of trivalent chromium are difficult to absorb, whereas those of Cr(VI) easily penetrate physiological barriers such as cellular membranes. Further metabolism of chromium compounds in humans has not been well examined. Although an increase of chromium concentration in some organs has been observed (13), there are no reports on distinct dependence between exposure to chromium and the amount of the element that is excreted.

The purpose of this study was to establish the effect of a long-term exposure of tannery workers to chromium compounds on the status of selenium in their bodies and whether Cr(VI) compounds, with their strongly oxidizing properties, influence the intensity of oxidative processes (expressed as concentration of thiobarbituric acid reactive substances (TBARS)) in the blood of the examined tannery workers.

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Materials and Methods

A group of 34 polishers of chromium-tanned leather in a tannery in Gniezno, Poland, were examined. The workers were 22–55 years of age and were exposed to different concentrations of chromium compounds from 3 to 16 years, depending on the workstation.

The control group consisted of 104 healthy residents of central Poland, 17–69 years of age, who were not employed in industry (students, administration workers, farmers). All subjects completed medical histories and were free of any acute or chronic disease. The protocol of the study was approved by the Ethical Committee of the Medical Academy in Lodz.

The concentration of chromium in the material taken was determined according to Polish Standard PN-79/Z-04126.01, Air Quality Criteria. Chromium from workstations that was collected on membrane filters during a working shift and mineralized using nitric and perchloric acid was determined to be Cr⁺⁶ by the colorimetric method using diphenylcarbazide (14).

Blood samples were collected into heparinized tubes (free of trace elements) by cubital venipuncture during the workshift. Methods for obtaining and preparing the samples were described previously (15).

Samples of urine were stored at -20°C in polystyrene tubes until analysis. Whole blood, serum, and urine Se concentrations were determined fluorometrically with 2,3-diaminonaphthalene as a complexing reagent, according to Watkinson (16). The methods of determination of Se in blood and urine were previously described (17). Levels of TBARS in plasma were analyzed spectrophotometrically with 2-thiobarbituric acid using the method of Yagi (18).

Erythrocyte and plasma GSH-Px activities were assayed according to the method of Paglia and Valentine (19) as modified by Hopkins and Tduhope (20), with β-nicotinamide adenine dinucleotide phosphate (NADPH) oxidized per minute at 25°C by 1 ml of plasma or 1 g of hemoglobin (Hb). The Hb concentration was measured with Drabkin reagent (0.61 mM potassium ferricyanide, 0.77 mM potassium cyanide, 11.9 mM sodium bicarbonate) by the cytochrome method. Creatinine levels in urine samples were determined by the method of Hawk et al. (21).

The data obtained are presented as arithmetic mean ± SD. Statistical significance was analyzed by the Student's t-test. Differences at p<0.05 were considered significant. Correlation between different values was tested by simple linear regression using the method of least squares.

Results

Determination of Cr concentration in the Gniezno tannery carried out over the years 1978–1990 showed considerable differences in concentrations of Cr compounds at different workstations. At the fluffing machines, the Cr concentration was 0.11 ± 0.07 mg Cr/m³ of air (range 0.06–0.36 mg/m³) and at other workstations of the same department, it was 0.023 ± 0.009 mg Cr/m³ (range 0.01–0.35 mg/m³). Thus, the examined workers were divided into two subgroups: fluffers, with 5 times the different exposure, and finishing workers.

Table 1 presents Se and TBARS concentrations in blood of both tannery workers and controls. A statistically significant decrease of Se concentration in blood (p<0.001) and a statistically significant increase of TBARS concentration (p<0.004) were found in the tannery workers as compared with the control group. No differences were found in the variables under investigation, depending on the period of employment in the tannery (not shown).

Table 1 presents the values of the parameters determined in the tannery workers, depending on the concentration of chromium compounds at their workstations. Workers exposed to high concentrations of chromium in the air exhibited increased Se concentration in blood and increased TBARS concentration in blood plasma. Differences related to the concentration of chromium compounds at the workstation were observed in Se concentration in whole blood and plasma and the amounts of Se excreted in urine. Increased erythrocyte and plasma GSH-Px activity was also observed in the workers who were exposed to high chromium concentration.

The analysis of TBARS concentration in the blood plasma of workers demonstrated considerable individual differences in this parameter, which were not related to the duration of their exposure to chromium compounds (Fig. 1). No differences depending on the duration of exposure were observed in Se concentration in plasma or whole blood of the workers.

| Parameters             | Control group (n = 104) | Tannery workers |               |               |
|------------------------|-------------------------|-----------------|---------------|---------------|
|                        | All (n = 34)            | Low chromium (n = 20) | High chromium (n = 14) |               |
| Blood Se (µg/l)        | 104.2 ± 23.2 (68.1–148.2) | 86.5 ± 14.9 (59.2–118.5) | p<0.001* | p<0.0001* |
|                        |                         | 91.0 ± 14.2 (52.0–115.9) |               |               |
|                        |                         | 79.2 ± 12.1 (32.0–110.9) |               |               |
| Plasma Se (µg/l)       | 80.1 ± 18.9 (50.1–134.7) | 68 ± 11.5 (47.2–93.5) | p<0.02* | p<0.001* |
|                        |                         | 72.0 ± 12.1 (41.2–108.5) |               |               |
|                        |                         | 63.3 ± 8.4 (32.0–100.9) |               |               |
| Urine Se (ng/g creatinine) | 10.81 ± 3.50 (7.66–18.21) | 9.28 ± 2.47 (5.66–18.78) | p<0.04* | p<0.001* |
|                        |                         | 10.38 ± 2.59 (5.66–18.78) |               |               |
|                        |                         | 7.80 ± 1.41 (3.66–15.2) |               |               |
| Plasma TBARS (µmol/l)  | 4.66 ± 0.97 (2.86–5.69) | 9.67 ± 6.83 (4.71–16.53) | p<0.004* | p<0.005* |
|                        |                         | 13.1 ± 6.6 (4.71–16.53) |               |               |
|                        |                         | 5.61 ± 1.7 (2.31–6.36) |               |               |
| Erythrocyte GSH-Px activity (U/g Hb) | 16.7 ± 2.3 (12.7–23.2) | 17.9 ± 3.4 (12.2–27.4) | p<0.05* | p<0.05* |
|                        |                         | 17.8 ± 4.0 (12.2–27.4) |               |               |
|                        |                         | 18.0 ± 1.9 (12.2–24.0) |               |               |
| Plasma GSH-Px activity (U/ml) | 0.213 ± 0.039 (0.113–0.288) | 0.237 ± 0.077 (0.155–0.579) | p<0.05* | p<0.05* |
|                        |                         | 0.212 ± 0.038 (0.155–0.579) |               |               |
|                        |                         | 0.245 ± 0.055 (0.155–0.579) |               |               |

Data are expressed as a mean ± SD (range).

*Statistical significance in comparison to control group.

Statistical significance between investigated group of tannery workers.

Figure 1. Level of thiobarbituric acid reactive substances (TBARS) in plasma of tannery workers compared to the duration of chromium exposure.
A positive linear correlation was found in the tannery workers between Se concentrations in blood and in urine (Fig. 2; \( r = 0.48; p<0.005 \)) and between the concentrations of Se in plasma and in urine (not shown; \( r = 0.46; p<0.01 \)). There was also a positive linear correlation between the concentration of Se in blood and GSH-Px activity in erythrocytes (not shown; \( r = 0.42; p<0.02 \)).

**Discussion**

Modern tanning technology uses chromium compounds, primarily Cr\(^{3+}\), but some Cr\(^{6+}\) is always present. In the examined tannery, the addition of Cr\(^{6+}\) is stable and equals 2 mg Cr\(^{6+}\)/kg Cr\(^{3+}\).

The methods of determining Cr described in this paper were developed in the early 1970s and have been used in industrial laboratories for over 20 years. Compared with the modern methods of determining total Cr concentration, it may yield lower results. Although this colorimetric method is less sensitive than the modern methods used to measure Cr (higher minimum detection level), we chose to present results of Cr determination in the air at different workstations of the tannery, assuming that each measurement shows the same error in the comparison with real values. Hence, the Cr concentrations under consideration are proportionally lower than the real values.

Chromium appears in compounds at a +6 oxidation state, which is particularly toxic when it enters the body with inhaled air (13). The workers of the tannery in Gniezno were reported to have constantly increasing chromium concentrations in urine and specific skin reactions as a result of long-term exposure to chromium compounds (22). The incidence of type II diabetes mellitus and cardiovascular disorders were described in a group of tannery workers; 80% of the investigated tannery workers were obese and 40% exhibited hyper tension (12).

The molecules of soluble chromium compounds are inhaled into the body and are absorbed by blood through the alveoli. Cr\(^{3+}\) compounds are generally unable to diffuse through the plasma membrane, and Cr\(^{6+}\) has been found to accumulate on the cell surface. Cr\(^{6+}\) is extracellularly reduced to Cr\(^{3+}\) with the participation of a variety of compounds (glutathione, ascorbic acid, serum proteins). This process may initiate a series of free radical reactions and may lead to the formation of a number of active metabolites. Increased peroxidation of biologically active compounds may result in permanent damage of the structure or function of vulnerable organs in the body.

In the blood, Cr\(^{3+}\) penetrates the red blood cell and is bound by Hb or its other components. In other cells, similar processes probably occur. At a pH close to neutral or slightly alkaline such as that in body fluids, most of Cr\(^{3+}\) compounds form colloidal complexes with proteins and are accumulated in cells (23).

It has been reported that, in cases of heavy metal poisoning (Pb, Hg, Cd), Se protects organisms from harmful effects of these elements. Animals to which cadmium or lead in different forms was administered in different ways excreted less Se in urine, feces, or exhaled air (24, 25). So far, the mechanism of protection against toxic effects of Pb, Hg, and Cd by Se has not been explained. It is likely that part of the interaction between Se and divalent cations of heavy metals is related to the affinity of divalent cations for sulfhydryl groups and therefore with Se–H groups.

Seeking an analogy with its interaction with heavy metals, we analyzed Se concentrations in whole blood and blood plasma and its urinary excretion in the tannery workers exposed to different levels of chromium compounds to determine whether an interaction occurs between Se and Cr.

Significant decreases in Se concentrations in whole blood and plasma observed in the tannery workers exposed to chromium compounds may confirm the hypothesis about the interaction between Se and Cr. Moreover, workers with high exposures had significantly lower Se concentrations in blood and they excreted lower amounts of Se in urine than workers with low exposures. A possible explanation is that biological selenides are formed with Cr and accumulate in some organs.

In the group of examined tanners, a linear correlation was found between the concentration of Se in blood or plasma and its concentration in urine (\( r = 0.48; p<0.005 \) and \( r = 0.46; p<0.02 \), respectively).

Significant positive correlation between urinary Se (concentration or daily excretion) and whole blood, plasma, or serum have been observed, which suggests an interconnection between Se burden and urinary or total Se excretion. Strongly significant correlation between urinary Se concentrations (\( \mu g/day \)) and Se intake (\( \mu g/day \)) was observed in some studies (26). Epidemiological studies have shown that the intake of Se has a major influence on Se concentration in blood, but other factors (e.g., protein turnover) may also influence plasma Se. Some authors (26) suggested that urinary Se may therefore be a more convenient indicator of Se status than the plasma Se. This may allow the reduction of the number of measurements of Se concentration in blood (which is invasive) and use the level of the microelement in urine as the sole indicator of Se pool in the body.

Neve (27) and Wasowicz (28) believe that the concentration of Se in blood and its components depends on the amount of the element in the diet. The workers of the tannery and residents of the same region, who are employed outside industry probably consume similar amounts of Se (29); therefore, differences in the diet of the examined group are probably not the cause of different concentrations of Se in their blood. Furthermore, the increased excretion of the element in urine is not the cause of decreased Se concentration. On the contrary, when the exposure to chromium increased, excretion of Se in urine significantly decreased (7.80
± 1.40 ng/g creatinine vs. 10.38 ± 2.59 ng/g creatinine; p<0.001). It was also demonstrated that the tannery workers who were exposed to air with high concentrations of chromium compounds at their workplace exhibited significantly higher erythrocyte and plasma GSH-Px activity than workers of other departments. Assuming that Se content in diet is similar in tannery workers and in controls living in the same region, blood and urine Se levels provide information on other sources of intake of Se, and urinary excretion is the main route of elimination of this microelement (30). It can be supposed that the group of workers in this study exhibits increased demand for selenium as a result of exposure to the action of the compounds (29). It is also possible that chemical oxidant stress is responsible for low Se status of the exposed workers (31). A number of papers have been published concerning long-term exposure to certain elements and different responses of Se in the bodies of the examined individuals (9,15,32). In rubber factory workers exposed to Se during rubber production, the concentrations of Se in blood and urine were found to be lower than in subjects not connected with industry. Zachara et al. (15) attempted to explain these statistically significant differences by increased excretion of the microelement with perspiration (15). In children with dental fluorosis living in regions with high fluorine concentration in soil and drinking water, urinary excretion of Se was reported to be 87% higher than in healthy children (32). The mechanism of selenium–fluorine interaction is not known, although a straight antagonistic interaction between them has been indicated (32). In miners exposed to Hg, simultaneous accumulation of Se and Hg in the body has been reported; as a result, excretion of Se in urine decreased. When mice were given mercury compounds via different routes (subcutaneously, per os, intraperitoneally), selenoprotein, which can fix Hg, was isolated from their kidneys. In this model, however, the Se–Hg complex was catabolized faster in the kidney and caused an increase of excretion of Se in urine (9).

In the exposure to chromium compounds of different oxidation numbers, among other factors, the action of Cr as an oxidant should be considered: in certain conditions, Cr⁴⁺, in contact with biological compounds, may be reduced to Cr⁰ (13). This may lead to peroxidation of biological compounds that are present in the cell or on its surface. In effect, certain negative changes may occur: cell membranes may be damaged due to peroxidation of unsaturated fatty acids, genetic material may be modified, or hormonal composition of a given individual may be changed. This may be confirmed by the observed statistically significant increase in TBARS concentration, which seems to be a manifestation of increased lipid peroxidation in blood plasma of tannery workers exposed to chromium compounds in the air. At the same time, this explanation cannot be treated explicitly because TBARS are very sensitive to a number of factors and the increase of TBARS concentration may result from a combination of chemical compounds to which the person is exposed in different simultaneous processes. Thus, in the workers who are exposed to lower amounts of chromium compounds in the air, a significant increase of TBARS concentration is observed. TBARS levels in plasma in some workers exposed to chromium compounds at concentrations of 0.023 ± 0.007 mg/m³ were found to be several times higher than in the control group; this is interesting from the point of view of prevention of occupational diseases. In the examined group of workers employed in the tannery for over 5 years, extremely high TBARS concentrations (range 12.4–36.6 μmol/l) were found in six individuals (about 20% of the examined population). This may be a manifestation of a special individual sensitivity to oxidative stress or lowered antioxidative capacity. The high level of TBARS in the blood of tanners should not be attributed to their exposure to chromium compounds alone, but concentration or activity of antioxidants in plasma should be carefully analyzed; nutritional antioxidant supplementation might be introduced for the purposes of prevention.

The manifestation of biochemical function of Se in the body is GSH-Px activity. No differences in this parameter were found between the examined group of tannery workers and the control group either in erythrocytes or in blood plasma. However, a linear correlation was found between Se concentration in whole blood and GSH-Px activity in erythrocytes. Thomson et al. (33) suggest that Se concentrations above 100 ng/ml of whole blood, no proportional increase of GSH-Px activity is observed. This may indicate that an optimum Se concentration (expressed as the optimum GSH-Px activity) has been reached. In the examined group of tannery workers, the average Se concentration was 86.4 ± 14.9 μg/l, but big individual differences (59.2–118.5 μg/l) might lower the coefficient of correlation between the blood selenium concentration and erythrocyte GSH-Px activity (33). Apart from data from New Zealand, extensive studies on optimum concentration of Se in blood for different groups of healthy people, which were conducted in recent years in many countries, do not show a correlation between Se concentration and GSH-Px activity in the blood. One of the possible explanations of this may be that the organism has already achieved the optimum activity of the selenoenzyme (6). It also may be due to the fact that a very little part of Se in blood is bound with GSH-Px—about 10% of erythrocyte Se and only 1% of plasma Se (27).

In conclusion, it may be said that in tannery workers, who during their working shift are exposed to an atmosphere containing chromium compounds, concentration of Se in blood and blood plasma is lower than in people who are not occupationally exposed to chromium compounds. Their urinary excretion of this microelement is also lower. Assuming that the diet of the residents of the region under study employed in the tannery and people who are not connected with industry contains similar amounts of the microelement, the observed differences in Se concentration may result from specific adaptation of the body to working conditions in which the atmosphere contains chromium compounds.

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