Do works concerning ferrochromium alloys mean risk for the inhabitants living in their surrounding? A cytogenetic study

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In the present study cytogenetic markers as chromosomal aberrations, sister chromatid exchanges and micronuclei were evaluated in peripheral lymphocytes of 26 foundry metallurgists exposed to increased level of chromium, 26 inhabitants living in the surroundings of the works within a distance of 5 km, and 26 inhabitants living in uncontaminated area in a distance more than 30 km from the works. Our goal was to verify whether side-products arising during the production of ferrochromium alloys and released into the air in the surroundings of the works do have a cytogenetic effect on the there living population. All of the cytogenetic markers were significantly increased \((P < 0.001)\) only in the group of the workers, in comparison with the other two groups. We did not find increased levels of the observed cytogenetic markers in peripheral lymphocytes neither of inhabitants living in an uncontaminated area nor of inhabitants living in the neighbourhood of the works. Smoking proved to be a significant positive predictor for SCE frequency in all investigated groups. There was no significant difference in frequency of chromosomal aberrations and micronuclei between smokers and non-smokers. We can conclude that only occupational exposure to chromium increases the frequency of chromosomal aberrations, sister chromatid exchanges, and micronuclei in peripheral lymphocytes.

Key words: chromosomal aberration, micronuclei, sister chromatid exchange, chromium, environmental exposure.

Introduction

In the middle of the 50’s the works in Istebné have started with production of the ferrochromium al-

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monar tumours is especially chromium, which constitutes a part of the exhalants emitted by the works.

Chromium (III), an essential metabolic trace nutrient (BAARNHART, 1997) is less toxic (MAC KENZIE et al., 1958) and much less efficient contact allergen (POLAK et al., 1973) than chromium (VI), which can cause allergic contact dermatitis (BRUYNZEEL et al., 1988) and lung cancer (HUGHES et al., 1994). Occupational studies, including those from the United Kingdom (DAVIES, 1991), the United States (PASTIDES et al., 1994) and from Slovakia (BUCHANCOVÁ et al., 1994) have reported increased risk of lung cancer and other illnesses of respiratory system by chromate workers.

However, there is no clear evidence of relationship between exposure to environmental level of chromium, and the adverse health effects. Therefore in an attempt to evaluate the environmental risk of chromium, we performed a study of inhabitants living in the neighbourhood of works producing ferrochromium alloys, using the cytogenetic analysis of peripheral lymphocytes. This method is traditionally used to evaluate an exposure to clastogens (NATARAJAN & OBE, 1980). Cytogenetic parameters such as chromosomal aberrations, sister chromatid exchanges and micronucleus test are known as biomarkers and an increase in these markers indicates an increased risk of cancer (PERERA & WHYATT, 1994).

We evaluated percentage of aberrant cells, frequency of sister chromatid exchanges per cell and number of micronuclei per 1000 cells.

**Material and methods**

*Subjects and sampling*

Prior to the study, a questionnaire was obtained from each individual for the determination of his health, habits and lifestyle (past and present history, occurrence of cancer in his family, occupational conditions, smoking, drinking, eating habits).

Persons undergoing medical treatment, vaccination or radiography up to 3 month before sampling were not included in the study.

The study was performed on a population of 26 male smelters (group A) and 26 inhabitants living nearby the works up to a distance of 5 km (group B). A control group consisted of 26 inhabitants living in an uncontaminated area in a distance more than 30 km from the works (group C). The characteristics of the persons involved in the study are summarised in Table 1. The mean age of smelters in the exposed group was 39.92 (30-52), in the group B it was 38.23 (31-55) and in the control group C 36.23 (29-52) years. The mean time of employment in the metallurgic plant was 13.3 (6-26) years.

**Exposure data**

The analysis of chromium was made by atomic absorption spectrometry (Varian spectrophotometer AA 30-P) at Hygienic stations in Dolný Kubín and Martin. The mean all-shift concentrations of total Cr in the air of the smelting plant were 0.03-0.19 mg.m$^{-3}$; the values of hexavalent chromium were between 0.019-0.03 mg.m$^{-3}$. The mean concentrations of all chromium in the air nearby the works and in the control area were deeply under the hygienic norm. There, the mean value of chromium was 0.033 μg.m$^{-3}$ (0.01-0.0117).

In the soil, in a distance of 200 m from the works the chromium content was 137 mg.kg$^{-1}$, which is higher than the hygienic norm (100 mg.kg$^{-1}$). The chromium content in the soil from wider distance and from the control area was under the hygienic norm 60.2 mg.kg$^{-1}$ and 46.0 mg.kg$^{-1}$ respectively.

**Chromosomal aberrations (CA)**

Heparinised blood samples were obtained from each person. 0.5 mL of the whole blood was incubated with 5 mL of RPMI medium (SEVAC Praha) supplemented with 20% inactivated foetal calf serum.
Table 2. Frequency of chromosomal aberrations in the exposed and control groups (mean ± SE).

<table>
<thead>
<tr>
<th>Subject</th>
<th>No. of analysed cells</th>
<th>AB.C. (%)</th>
<th>B’</th>
<th>B”</th>
<th>E</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2600</td>
<td>3.76 ± 0.24**</td>
<td>36</td>
<td>35</td>
<td>18</td>
<td>52</td>
</tr>
<tr>
<td>Smokers</td>
<td>1500</td>
<td>3.94 ± 0.27**</td>
<td>33</td>
<td>23</td>
<td>11</td>
<td>37</td>
</tr>
<tr>
<td>Non-smokers</td>
<td>1100</td>
<td>3.53 ± 0.17**</td>
<td>12</td>
<td>12</td>
<td>7</td>
<td>15</td>
</tr>
<tr>
<td>Group B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2600</td>
<td>1.92 ± 0.14</td>
<td>35</td>
<td>12</td>
<td>10</td>
<td>29</td>
</tr>
<tr>
<td>Smokers</td>
<td>1200</td>
<td>2.00 ± 0.18</td>
<td>16</td>
<td>7</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>Non-smokers</td>
<td>1400</td>
<td>1.88 ± 0.11</td>
<td>19</td>
<td>5</td>
<td>5</td>
<td>17</td>
</tr>
<tr>
<td>Control group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2600</td>
<td>1.73 ± 0.19</td>
<td>34</td>
<td>11</td>
<td>1</td>
<td>35</td>
</tr>
<tr>
<td>Smokers</td>
<td>1400</td>
<td>1.78 ± 0.22</td>
<td>20</td>
<td>13</td>
<td>5</td>
<td>18</td>
</tr>
<tr>
<td>Non-smokers</td>
<td>1200</td>
<td>1.66 ± 0.12</td>
<td>13</td>
<td>9</td>
<td>4</td>
<td>18</td>
</tr>
</tbody>
</table>

AB.C. aberrant cells; B’, number of chromatid breaks; B”, number of chromosome breaks; E, number of exchanges; G, number of gaps; **P < 0.001 comparison of group A and control group.

antibiotics and glutamine. Cell division was stimulated by 1% phytohaemaglutinin (Abbott, HA15). The cultures were incubated for 48 hours at 37°C. Two hours before the end of cultivation, Colcemide (Sigma) of final concentration $2 \times 10^{-7}$ mol.dm$^{-3}$ was added. The procedure of blood sample cultivation and slide preparation was done according to the method of Nataraajan & Obe (1980) modified by Bavorová & Očádalová (1989). Dry slides were coded and stained with Giemsa-Romanowski solution for 4 min.

Slides were cytogenetically analysed under optical microscope with 1000× magnification. 100 well-spread metaphases containing 46 centromers were examined per donor. All the basic chromosomal abnormalities, chromatid and chromosome gaps, breaks and exchanges were recorded (ISCN, 1985). Due to controversies in the classification, gaps were not included among aberrant cells. The chromosome damage was expressed as both per cent of aberrant cells and number of breaks per cell.

Sister chromatide exchange (SCE)
Peripheral blood lymphocytes for SCE analysis were cultivated in the same medium as presented above, except that the medium was supplemented with 5-bromodeoxyuridine (BrdU, Sigma, final concentration of 10 μL$^{-1}$). The cultures were incubated in complete darkness for 72 hours. Differential staining of sister chromatids was carried out according to the procedure of Wolff & Perry (1974).

The number of exchanges was evaluated in 50 cells per person. The cytogenetic damage was expressed as number of exchanges per cell.

Micronuclei (Mn)
Cell cultures were set up by adding 0.5 mL of whole blood to 5 mL of RPMI medium as reported above. Cells were grown for a total time of 66 hours and cytochalasin B (Sigma) was added (final concentration 3 μg.mL$^{-1}$) for the last 24 hours. Slide preparation and staining was carried out by a standard method (Fenech, 1993). Number of micronuclei was scored in 1000 binucleated interphase cells with intact cytoplasm in each subject.

Statistical analysis
The results obtained from the exposed and control groups were compared and statistically analysed using Student’s t-test.

Results
The mean values for the different types of chromosomal aberrations are presented in Table 2. The mean number of chromatid as well as chromosomal breaks, and exchanges was significantly ($P < 0.001$) increased in the smelter group in comparison with the control group and the group of inhabitants living in the neighbourhood of the works. Most of the chromosomal aberrations were constituted by chromatid breaks (45 of the total 98 in smelter group and 35 from total 50 in control group).

Significant differences ($P < 0.001$) between smelter and control groups were found in both other cytogenetic parameters, the sister chromatid exchanges per cell and number of micronuclei per 1000 cells. No difference was found between the group of people living in the neighbourhood of the works and control group in any analysed parameter. Mean values of observed frequencies of these cytogenetic parameters are presented in Table 3.

The given cytogenetic parameters were studied separately for smoking and non-smoking individuals in all analysed groups. No significant differences ($P > 0.05$) were observed for percentage
Table 3. Frequency of sister chromatid exchange and micronuclei in exposed and control groups (mean ± SE).

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Number of analysed individuals</th>
<th>SCE/cell</th>
<th>Number of micronuclei/1000 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group A</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>26</td>
<td>6.30 ± 0.22**</td>
<td>10.15 ± 0.28</td>
</tr>
<tr>
<td>Smokers</td>
<td>15</td>
<td>6.32 ± 0.21**</td>
<td>10.58 ± 0.30</td>
</tr>
<tr>
<td>Non-smokers</td>
<td>11</td>
<td>6.25 ± 0.27**</td>
<td>9.97 ± 0.27</td>
</tr>
<tr>
<td><strong>Group B</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>26</td>
<td>4.26 ± 0.14</td>
<td>7.42 ± 0.34</td>
</tr>
<tr>
<td>Smokers</td>
<td>12</td>
<td>4.54 ± 0.12*</td>
<td>7.25 ± 0.31</td>
</tr>
<tr>
<td>Non-smokers</td>
<td>14</td>
<td>3.93 ± 0.15</td>
<td>7.50 ± 0.35</td>
</tr>
<tr>
<td><strong>Control Group</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>26</td>
<td>4.21 ± 0.14</td>
<td>7.19 ± 0.27</td>
</tr>
<tr>
<td>Smokers</td>
<td>14</td>
<td>4.54 ± 0.14*</td>
<td>7.08 ± 0.29</td>
</tr>
<tr>
<td>Non-smokers</td>
<td>12</td>
<td>3.83 ± 0.11</td>
<td>7.28 ± 0.32</td>
</tr>
</tbody>
</table>

**P < 0.001** comparison of group A and control group; *P < 0.05* comparison of smokers and non-smokers within the groups.

of aberrant cells and number of micronuclei between smokers and non-smokers neither in control nor in both other groups.

There were significant differences (P < 0.01) between smoking and non-smoking inhabitants living nearby the works and between smoking and non-smoking persons within the control group as well, in the number of sister chromatid exchanges per cell. We did not find significant difference in this parameter between smokers and non-smokers in the group of the smelters.

**Discussion**

Occupational and experimental exposure to chromium has been widely evaluated in the last decade. According to numerous reports, it is generally accepted that especially hexavalent chromium is a potent mutagen (Majone & Rensi, 1979; De Flora & Wetterhahn, 1989; De Flora et al., 1990; Klein et al., 1992). There is few information about mutagenous effect of environmental exposure to chromium. In our study we used three cytogenetic techniques to evaluate the effect of chromium on 26 smelters as a positive control, 26 inhabitants living nearby the works who are potentially exposed to exhalants emitted by the works, and 26 persons from relatively uncontaminated area as a negative control. We found out that exposure to chromium significantly increases the frequency of chromosomal aberrations, frequency of SCE and frequency of Mn in the occupationally exposed group only, but not in the group living in close surroundings of the works. These results are in accordance with our negative findings of hexavalent chromium in air and soil in the neighbourhood of the works. This minor amount of chromium, which is ingested or inhaled, can be effectively reduced by detoxification mechanisms of human body (De Flora, 2000). Rowbotham et al. (2000) who evaluated environmental exposure to chromium in relation with health effects in British general population concluded, that there is no clear evidence to relate exposure to environmental levels of chromium with adverse health effects either the general UK population or suburbs exposed to chromium around industrialised or contaminated sites. McCarron (2000) who used questionnaire SF 36 for evaluation of self reported health did not find differences between environmentally exposed group and control group. Our cytogenetic findings confirm the previous supposition that low-environmental level of chromium itself does not have negative effect on health.

According to the significant increase of cytogenetic parameters in workers but not in inhabitants living in close surroundings of the works we assume, that increased frequency of bronchopulmonary tumours (Pleško et al., 1997) in given region is probably caused by an increased occurrence of this type of tumours among metallurgists working in the works and therefore occupationally exposed to higher chromium level.

We have to remark, it can be expected that an improved understanding of the relevance of possible long-term accumulation of chromium in the body may facilitate a more complete assessment, in the future, of the bronchopulmonary cancer risks in the general population associated with environmental exposure to chromium.
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References


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