INFLUENCE OF UKRAIN AND STRONTIUM ON THE RAT TOOTH INTERTUBULAR DENTINE.
I. XRF MICROPROBE STUDY

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Abstract

The effects of different doses of Ukrain and/or strontium on the rat teeth intertubular dentine were analysed in cuts perpendicular to the dentinal tubes. To the dentine elemental analysis, scanning electron microscopy (SEM) equipped in XRF (X-ray fluorescence) microprobe was used. Strontium concentration increased from 4.1% in the control group to 5.0% and 6.3% for the groups administered with strontium and strontium together with Ukrain. At the same time, a lowering of calcium concentration was observed. The rat dentine elemental composition was markedly altered after the administration of Ukrain and strontium. It was shown that strontium with Ukrain decreased the demineralisation of the dentine.

Key words: rats, dentine, Ukrain, strontium, XRF microprobe.

The anticancer drug Ukrain (NSC-631570, Nowicky Pharmaceuticals, Austria) has been isolated as semi-synthetic compound from the common weed named greater celandine (Chelidonium majus L.). This plant contains a range of alkaloids, mostly chelidone, better known as benzophenanthridine alkaloid. Over the last decades, many pre-clinical investigations reported selective cytotoxic effects of the drug on tumour cells without adverse side effects on normal cells and tissues. A systematic review of clinical trials has been recently published (3). The molecular mechanism of antineoplastic effects induced by Ukrain is not yet completely understood; however, there are some suggestions that induction of apoptosis may be involved (10, 9, 13). The Lublin Medical University orthopaedic group investigated the influence of Ukrain on rat bone status (4). They found the anabolic effect of Ukrain on bone in ovariectomised rats. However, its action could be slightly osteopenic by affecting biomechanical bone properties. A decrease in bone strength by 13% and also a decrease in bone mineral content were observed (5-7). Up to now, the influence of Ukrain on the teeth was not investigated. Taking into account the effects of Ukrain on the bones, one can expect that also teeth could be similarly affected.

The aim of the study was to evaluate the effect of Ukrain on the mineral part of rat teeth and to estimate if the decrease in mineral content observed earlier for the bones can be compensated by the administration of strontium. If we consider chemical elements from their biological role and human health point of view, strontium belongs to a possibly essential group. It was found that when the deficiency of calcium occurs and bio-accessible magnesium cannot make up the difference, then strontium is preferentially deposited in teeth (2). The teeth have two main calcified parts: the hard enamel and tough dentine. The enamel is highly mineralised; the dentine is of mixed composition: mineral and tissue. In the living organism, the process that forms enamel involves numerous agents, including cells, proteins, enzymes, and inorganic minerals. The dentine was chosen for the study as a part of tooth, which can be easier affected by the administered substances. There is no much choice of the methods to analyse the influence of various compounds or factors on the mineral part of the teeth. To the study of the chemical composition, the best seems to be elemental analysis using a scanning electron microscope (SEM) equipped with XRF (X-ray fluorescence) microprobe (1, 14).

Material and Methods

Forty-two adult rats of an approximate initial weight of 250 g were used. They were kept in separate
cages and randomly allocated to one of six experimental groups. All the groups were given a standard rodent pellet diet ad libitum. The animals from the first group (the control group) received water ad libitum. The experimental animals from other groups also received water without limit, but with different additions. The second group received water supplemented with strontium chloride in a concentration of 7.532 mmol/L. The animals from the third group were given pure water but they were injected additionally with Ukrain (7 mg/kg) once daily. In the fourth group, the dose of Ukrain was twice higher (14 mg/kg). The rats from the groups 5 and 6 received once a day Ukrain (7 and 14 mg/kg, respectively) and were stock up on water with 7.532 mmol/L strontium chloride.

The experiment lasted for six weeks and at the end of the experiment, the animals were decapitated. The full necropsy of the animals was done and individual bones and soft organs were used for various examinations. The extracted teeth after removing the blocks of tissue (in 25% formic acid) and washing in distilled water were finally air-dried at 30°C. The crowns of all the teeth were sectioned latitudinally using a diamond saw. The cut surfaces were not polished. For the microscopic analysis, the intertubular dentine was used.

The chemical composition of intertubular dentine in 25 randomly selected points of the surface for each of six teeth belonging to seven analysed groups was determined by a scanning electron microscope (SEM) equipped with XRF (X-ray fluorescence) microprobe. A microscope LEOSEM1430VP supplied with EDX detector was used (operating conditions of electron microprobe 20 kV, 80 mA beam current). The concentration of C, O, P, Na, Mg, Ca, and Sr on the dentine surface was determined using a standard version of the SEM method. The electron beam penetrated the samples for about 1 mm. Vacuum 10^{-5} Pa was preserved during measurements (8, 11, 12).

Results

The results of the elemental analyses of 42 rat teeth together with standard deviations are summarised in Table 1.

Comparing the calcium and strontium concentrations in control group teeth with those in groups of the animals, which were treated with Ukrain and strontium, the following phenomena could be observed:

- strontium concentration increased from the value of 4.1% for the control group to 5.0%, 5.3%, and 6.3% for the groups 2, 5, and 6, respectively. The results are obvious for animals administered with strontium, but the highest strontium increase was observed in the rats administered with Ukrain together with strontium. When Ukrain was administered alone, the concentration of strontium remained at the same level;
- the lowering of calcium concentration was observed at the same time. Calcium content decreased from 27.6% (control group) to 24.6% (group 2), 23.2% (group 5), and 20.8% (group 6);
- a small decrease in calcium content was also observed in the rats, which were injected only with Ukrain.

The above effects were correlated together and occurred in the teeth of animals provided with water containing strontium but were more distinct in the rats, which were additionally administered with Ukrain. An increase in the dose of Ukrain resulted also in an increase in strontium concentration and decrease in calcium concentration. To conclude, the presence of strontium diminished the demineralisation effect of Ukrain.

In all the analysed samples, the concentration of sodium and magnesium remained at the same levels as in the control group. The changes of percentage concentration of these elements were located in the limit of measure precision.

<table>
<thead>
<tr>
<th>Group</th>
<th>C (%)</th>
<th>O (%)</th>
<th>P (%)</th>
<th>Na (%)</th>
<th>Mg (%)</th>
<th>Ca (%)</th>
<th>Sr (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (control)</td>
<td>14.69±8.74</td>
<td>36.64±4.40</td>
<td>13.53±1.85</td>
<td>1.00±0.30</td>
<td>2.47±0.77</td>
<td>27.58±5.35</td>
<td>4.09±0.50</td>
</tr>
<tr>
<td>Group 2 (Sr)</td>
<td>15.59±8.58</td>
<td>38.73±4.95</td>
<td>12.60±2.36</td>
<td>1.08±0.52</td>
<td>2.41±0.88</td>
<td>24.56±5.97</td>
<td>5.03±0.82</td>
</tr>
<tr>
<td>Group 3 (U)</td>
<td>15.16±4.50</td>
<td>43.54±4.04</td>
<td>11.88±1.64</td>
<td>1.08±0.30</td>
<td>2.40±0.77</td>
<td>21.80±5.03</td>
<td>4.14±0.50</td>
</tr>
<tr>
<td>Group 4 (2xU)</td>
<td>12.52±6.82</td>
<td>39.17±4.23</td>
<td>13.54±1.94</td>
<td>0.96±0.34</td>
<td>2.64±0.84</td>
<td>26.96±5.86</td>
<td>4.21±0.59</td>
</tr>
<tr>
<td>Group 5 (U+Sr)</td>
<td>16.41±7.69</td>
<td>38.92±5.02</td>
<td>12.40±1.86</td>
<td>1.05±0.34</td>
<td>2.66±0.79</td>
<td>23.22±6.35</td>
<td>5.35±0.62</td>
</tr>
<tr>
<td>Group 6 (2xU+Sr)</td>
<td>19.03±8.26</td>
<td>39.69±4.96</td>
<td>11.64±2.01</td>
<td>0.98±0.18</td>
<td>2.56±0.67</td>
<td>20.76±6.38</td>
<td>6.35±0.73</td>
</tr>
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</table>

U – Ukrain.
The measure of carbon, oxygen, and phosphorus concentrations by the SEM microprobe were not as accurate as an analysis of metals, especially for carbon. But two observations could be made: the first, an increase in oxygen concentration for the teeth administered with Ukrain (from 36.6 % to 43.5 %), which was bigger than the increase observed for animals administered only with strontium (36.6% to 38.7 %), and the second, a decrease in phosphorus concentration and at the same time an increase in carbon concentration for the teeth of rats administered with Ukrain or strontium with Ukrain. The latest effect was particularly visible for the group 6 where carbon concentration was as high as 19.0%, compared with 14.7% in control group.

Discussion

In the investigated cross-section of the teeth, the following area could be observed: enamel, dentine enamel junction; intertubular and peritubular dentine, and also peritubular intertubular junction. This relatively less mineral part of the tooth seems to be appropriate for these studies as is more susceptible to substances transported through tubules than enamel. The analysed teeth cross sections were not polished. Polishing is usual practice in electron microscopy, but polished interfaces do not disclose many details, mainly due to the destruction and smearing of soft surface texture. It was shown that diamond saw produced clean cross sections through interfaces (15).

The rat dentine elemental composition was markedly altered after administration of Ukrain and strontium. Strontium concentration increased from 4.1% in control group to 5.3% and 6.3% in the animals administered with strontium and Ukrain. At the same time, the lowering of calcium concentration was observed. Calcium content decreased from 27.6% (control group) to 20.8% (group administered with strontium and Ukrain). A minor decrease in calcium content was also observed in the rats, which were injected only with Ukrain. It can be concluded that increasing the dose of Ukrain results in an increase in strontium concentration and a decrease in calcium concentration. It means that the presence of strontium diminishes the demineralisation effect of Ukrain. The biggest rate of calcium replacement by strontium was observed for the animals administered strontium and Ukrain. It can be explained by the substitution of phosphates for carbonates in the hydroxyapatite.

References

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