EXPERIMENTAL STUDIES ON EFFECT OF SODIUM FLUORIDE AND NITRATE ON BIOCHEMICAL PARAMETERS IN RATS

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Abstract

The aim of the study was to investigate the effects of sodium fluoride (NaF) and nitrate (NO3–), either administered orally alone or in a combination, on the biochemical parameters of rats. Tests on general toxicity were conducted on the basis of methodical recommendations 408 of the Organisation for Economic Co-operation and Development Guideline for Testing of Chemicals: “Subchronic Oral Toxicity – Rodent: 90-d Study”. The following combined effects of the tested chemicals were predominantly observed: additive, synergistic, and antagonistic. Methaemoglobinaemia was noted in groups given NO3– or NaF + NO3– complex.

Key words: rats, sodium fluoride, nitrate, biochemical parameters, combined action.

All organisms are exposed to fluoride from natural and/or anthropogenic sources. Early toxic effects of fluoride in humans are dental and skeletal fluorosis, which are endemic in areas with an elevated exposure to fluoride. Fluoride is also known to cross the cell membranes and to enter the soft tissues. Impairment of the soft-tissue function has been demonstrated in fluoride-intoxicated animals.

Various changes occur after chronic administration of fluoride in the blood, brain, and liver of animals. These include abnormal behaviour patterns, altered neuronal and cerebrovascular integrity, and metabolic lesions. Generation of free radicals, lipid peroxidation, and altered antioxidant defence systems are considered to play an important role in the toxic effects of fluoride (12-14, 16, 17).

The most commonly used medium for identifying fluoride exposure is urine (8, 15). Acute exposure to high doses of fluoride damages renal tissue and causes renal dysfunction. The kidneys are the target organs for fluoride toxicity. On the other hand, pale, granular hepatocytes, characteristic of parenchymal degeneration were observed in mice treated with the compound (0.95 mg/kg). Liver congestion was observed in sheep given a single intragastric dose (9.5 mg/kg) of fluoride (15).

Nitrate is one of the most frequent groundwater pollutants in rural areas. The nitrate in groundwater is primarily from fertilizers, septic systems, etc. Combustion processes can also enhance the nitrate as well as nitrite concentration, due to the emission of nitrogen oxides that can be converted to nitrates and nitrites in the environment. Nitrates and nitrites applied also consist during chemical production and they are used as food preservatives (3, 4, 9).

Nitrate itself is relatively non-toxic to humans. Health problems associated with nitrate occur primarily after nitrate enters the alimentary tract, where bacteria convert the nitrate to nitrite. Nitrite can cause methaemoglobinaemia, an oxygen-deficient condition in blood that can be especially life-threatening to infants under 6 months of age.

Once nitrate has been converted to nitrite in the body, other reactions can occur that can form compounds called N-nitrosamines. There is no direct evidence that these compounds are human carcinogens. Though it is assumed that exposure to these compounds increase the risk of cancer in humans, it is unknown how much of that risk is caused by nitrate-contaminated drinking water.

The aim of this study was to investigate the effect of sodium fluoride and nitrate given orally alone or in combination on the biochemical parameters of rats.
Material and Methods

Sodium fluoride (NaF) and nitrate (NO$_3^-$) (soluble in water) (Fluka, Buchs) were used. Experiments were conducted with 32 Wistar male rats, 6-7 weeks of age and initial body weight 120±20 g. The animals were divided into four equal groups and were kept under standard laboratory conditions. They were cared for in accordance with the law of Republic of Lithuania and the Guide for the Care and Use of Laboratory Animals.

The combined effects of NaF and NO$_3^-$ on the organism was studied by subchronic (90 days) experiments based on methodical recommendations 408 of the Organisation for Economic Co-operation and Development Guideline for Testing of Chemicals: “Subchronic Oral Toxicity – Rodent: 90-d Study” (7).

The doses were chosen as follows: 10.3 mg/kg b.w. of NaF, 238.0 mg/kg b.w. of NO$_3^-$ (as cumulative doses of these substances during 90 d); and 10.3 mg/kg b.w. of NaF + 238.0 mg/kg b.w. of NO$_3^-$. The tested compounds were administered per os by gavage (5 d per week). The control group was given drinking water.

Assessment was made of each animal’s behaviour, the state of skin and eyes, and feed and water consumption. The animals were weighed weekly – from the fifth week – every 2 weeks.

Several days before decapitation the following parameters were examined in urine: overall daily quantity, relative weight, hipuric acid, urea, creatinine, and total protein.

After decapitation of the animals the activities of acetylcholinesterase, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, creatinkinase, and γ-glutamyltransferase in blood; cytochrome P-450 concentration in the liver; and total protein in serum, urine, and liver were determined. Besides, haemoglobin (Hb) levels and percentage of methaemoglobin were measured. The internal organs were autopsied, weighed, and examined pathomorphologically.

Biochemical tests were conducted with clinical biochemical analyser “Humalyzer 2000” (Germany, Tannusstein). Cytochrome P-450 concentration was analysed by spectrophotometer.

The effects of NaF and NO$_3^-$ were characterised by 3 types of the combined action of substances (CAS): additive, antagonistics and synergistic. The additive combined action was equal to the percentile sum of the effect of each individual agent given alone, whereas antagonistic was lower than the sum, the synergistic – greater than the sum. The findings of the tests were processed statistically using Student’s t-test (values are mean ± SE). The results were considered significant when at a level of P<0.05.

Results

The integral indices of the animals’ (behaviour, general appearance, body weight, etc.) showed that the tested compounds (given alone or in combination) induced signs of intoxication. It was noticed that after 6 weeks water consumption was significantly reduced in the treated rats.

In rats exposed to NaF or NaF + NO$_3^-$ an enlarged spleen, thymus, adrenal glands, as well as an increase in absolute and relative weights of the kidneys, testicles and liver were observed. Under the combined effects of these substances, a statistically significant increase in the relative weight of seminal vesicles and epididymis was shown. In group given only NO$_3^-$, a statistically significant increase in relative weight of the spleen, kidneys, and liver was observed. At the end of the experiment, the gain in body weight in all the tested groups was lower than that in the control group.

Investigations of subchronic peroral toxicity showed that the toxic effects of NaF and NO$_3^-$ on individual indices of the functional state of the rats’ organism, manifested differently when the substances were administered alone or in a combination.

Blood biochemical parameters showed the changes in hepatic function of rats treated with NaF and NO$_3^-$ (alone or in combination) (Table 1). Acetylcholinesterase activity significantly decreased (24%) in the group given NaF + NO$_3^-$ in comparison with the control. The inhibition of the acetylcholinesterase activity was correlated with increased activity of transaminases in serum. Naf and NO$_3^-$, as well as the complex of these substances statistically significantly increased alkaline phosphatase activity. The sums (in percentages) of induced effects of single substances were higher than the effect of complex that showed antagonistic combined action of CAS type.

In all the tested groups the increased activity of γ-glutamyltransferase was noted (an antagonistic type of combined effect was evident). It is one of the sensitive parameters of hepatic function. Alanine aminotransferase activity was increased statistically significantly in all the tested groups; however, the effect of the complex of substances was lower in comparison with groups given the compounds separately (antagonistic type of combined effect was manifested). The aspartate aminotransferase activity in groups given only NaF or NO$_3^-$ showed statistically significant difference in comparison with control animals. The increased levels of these enzymes in blood serum can be related to cytotoxic effects on the liver.

Results of our study showed that the tested substances, given alone or in a combination, did not reduce the hepatic detoxication function. The level of hipuric acid did not show statistically significant difference in comparison with control animals. The data showed that the activity of cytochrome P-450 in all the tested groups was similar to that of the control group.

Under the combined effects of NaF + NO$_3^-$ (cumulative doses test), the blood serum total protein level decreased significantly (by 7-14%) versus control. The effects in groups given substances in a combination were greater than that after single administration. The synergistic combined effects of the substances were noted. The total protein level decrease in blood serum is...
related to pancreatic and renal dysfunction. It was confirmed also by a histological examination of organs from the animals exposed to the complex of substances. Under the effect NaF + NO₃⁻, several animals developed kidney degeneration, tubular necrosis, and glomerular inflammation.

Renal function disorders were shown by the changes in total protein in urine. Proteinuria was observed in all the tested groups. The combined effects of the substances was equal to the sum of their isolated effects (an additive a combined type of effect was evident).

Under the exposure of NaF given alone or in combination with NO₃⁻, renal dysfunction was noticed. Urea in serum is one of the sensitive indices of renal function. In all the tested groups, the serum nitrogen level was increased (urea 58%, 41%, 56%; P<0.001, P<0.001, P<0.001, respectively). Antagonistic combined effects of the substances were noted. Increased creatinine levels, as well as decreased urine nitrogen levels in all the treated groups and reduced creatinine in urea were observed.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>NaF</th>
<th>NO₃⁻</th>
<th>NaF + NO₃⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylcholinesterase, U/L</td>
<td>247.8 ±17.0</td>
<td>234.0 ±12.0</td>
<td>246.0 ±12.0</td>
<td>189.0 ±6.0**</td>
</tr>
<tr>
<td>Alkaline phosphatase, U/L</td>
<td>298.0 ±10.0</td>
<td>430.0 ±24.0***</td>
<td>406.0 ±18.0***</td>
<td>486.5 ±22.0***</td>
</tr>
<tr>
<td>Alanine aminotransferase, U/L</td>
<td>77.0 ±4.0</td>
<td>98.7 ±5.2**</td>
<td>105.0 ±4.2***</td>
<td>110.0 ±12.0*</td>
</tr>
<tr>
<td>Aspartate aminotransferase, U/L</td>
<td>226.0 ±13.0</td>
<td>282.0 ±19.3*</td>
<td>268.0 ±8.3**</td>
<td>248.0 ±10.7</td>
</tr>
<tr>
<td>γ-glutamyltransaminase, U/L</td>
<td>75.6 ±2.6</td>
<td>99.0 ±3.6***</td>
<td>94.6 ±4.3**</td>
<td>86.8 ±4.2*</td>
</tr>
<tr>
<td>Creatinkinase, U/L</td>
<td>88.8 ±7.8</td>
<td>195.0 ±12.0***</td>
<td>101.4 ±9.2</td>
<td>109.0 ±3.9*</td>
</tr>
<tr>
<td>Cytochrome P-450, mmol/g in liver</td>
<td>48.9 ±2.0</td>
<td>49.5 ±2.4</td>
<td>48.1 ±1.9</td>
<td>51.7 ±2.8</td>
</tr>
<tr>
<td>Total protein in serum, g/L</td>
<td>66.1 ±0.7</td>
<td>65.6 ±0.7</td>
<td>61.3 ±1.8*</td>
<td>56.8 ±0.9***</td>
</tr>
<tr>
<td>Total protein in urine, mmol/L</td>
<td>0.0</td>
<td>1.9 ±0.2***</td>
<td>0.9 ±0.4*</td>
<td>2.9 ±0.3***</td>
</tr>
<tr>
<td>Total protein in liver, mg/g</td>
<td>191.0 ±23.8</td>
<td>189.3 ±15.2</td>
<td>185.8 ±17.9</td>
<td>200.0 ±16.4</td>
</tr>
<tr>
<td>Hb, g/L</td>
<td>141.0 ±4.0</td>
<td>133.0 ±4.0</td>
<td>130.0 ±3.0*</td>
<td>135.0 ±5.0</td>
</tr>
<tr>
<td>Methaemoglobin, %</td>
<td>0.5 ±0.1</td>
<td>0.7 ±0.1</td>
<td>1.2 ±0.1***</td>
<td>1.2 ±0.1***</td>
</tr>
<tr>
<td>Creatinine, µmol/L /serum</td>
<td>43.8 ±1.3</td>
<td>56.6 ±1.7***</td>
<td>50.5 ±4.4</td>
<td>53.8 ±2.8***</td>
</tr>
<tr>
<td>Creatinine, mmol/L in urine</td>
<td>4.6 ±0.2</td>
<td>3.1 ±0.3***</td>
<td>2.5 ±0.1***</td>
<td>2.9 ±0.3**</td>
</tr>
<tr>
<td>Urea in serum, mmol/L</td>
<td>4.1 ±0.2</td>
<td>6.5 ±0.4***</td>
<td>5.8 ±0.3**</td>
<td>6.4 ±0.2***</td>
</tr>
<tr>
<td>Urea in urine, mmol/L</td>
<td>551.8 ±56.2</td>
<td>374.1 ±42.9*</td>
<td>461.8 ±49.3</td>
<td>361.2 ±64.7*</td>
</tr>
<tr>
<td>Hipuric acid in urine, mg/mL</td>
<td>24.7 ±2.7</td>
<td>21.0 ±2.0</td>
<td>19.8 ±2.4</td>
<td>22.4 ±1.4</td>
</tr>
</tbody>
</table>

*P<0.05, **P<0.01, ***P<0.001; ±SD
Discussion

Fluoride and nitrate are ubiquitous in the environment. The main problem is that the man in his natural environment is exposed not to isolated substances but to the complex of these substances. While in conventional toxicity all the attention is paid to one chemical, there are less data on the combined effect of various substances. For instance, Polish scientists (5) revealed that oxidative enzyme activity of α-glycerophosphate dehydrogenase and succinate dehydrogenase increased with sodium selenite and decreased with sodium fluoride and with both compounds together. Each of the compounds caused an increase in the activity of acid phosphatase, while the combination of the substances caused a decrease in the activity of this enzyme.

Epidemiological and clinical investigations have proven methaemoglobinaemia to be the major manifestation of the toxic effects of nitrates (4). The results of our study showed that NO$_3^-$ at the dose of 238 mg/kg b.w. increased by 126% methaemoglobin level (2.3 times, $P<0.001$), whereas under the combined NaF + NO$_3^-$ effect the increase was by 134% (2.4 times, $P<0.001$) higher; the antagonistic combined type of effect was evident.

According to the literature, fluoride is a well known inhibitor of numerous enzymes (1, 5, 6, 10). It is known that sodium fluoride administration strongly affects urinary biochemical indices (2, 8, 11). The results of the present study demonstrated that sodium fluoride and nitrate caused severe disturbance of kidney and liver function. The complex of tested substances inhibited acetylcholinesterase activity (24%) in comparison with the control group. Increase in the activity of the alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, γ-glutamyltranspeptidase and creatinkinase were observed in all the tested groups. Our data showed that long term exposure of adult rats to sodium fluoride and nitrate had no effect on the cytochrome P-450 in the liver.

The kidneys are the target organs for fluoride toxicity (2, 8, 11). The substances given alone or in combination induced renal dysfunction. The level of total protein increased in urine and declined in serum, as well as proteinuria, oliguria, hypostenuria, and histological examination showed renal dysfunction.

In conclusion, according to the blood biochemical indices, the following combined effects of the tested chemicals were predominantly observed: additive, synergistic, and antagonistic.

References