Influence of exogenous environmental factors on the accumulation of heavy metals

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Abstract. The aim of the present study was to investigate the influence of unbalanced diet on the content of trace elements in hair and liver tissue of laboratory animals. The study was conducted on male Wistar rats being two months of age (N = 20, weight=180g). The rats of experimental group consumed a semi-synthetic diet consisting of basic diet (50 %), fast food products (50 %), carbonated soft sweet drinks and water. Biochemical parameters (ALT, AST, total protein, total bilirubin, urea, creatinine, cholesterol) and the activities of antioxidant enzymes (Cu-Zn SOD, GPx) were assessed. The content of Al, Cd, Pb, Sr in hair and liver tissue was determined by atomic emission and mass spectrometry with inductively coupled argon plasma. A significant increase in the Al content in animals’ hair of the experimental group of 1.7 times was established. A positive correlation between Al in the liver tissue and Al in animal hair (r = 0.809, p <0.05) was found. A significant increase of 5 and 2 times in ALT and AST was found, respectively. In addition, a negative correlation was found between Al liver and the activity of plasma GPx (r =–0.903, p<0.05).

1. Introduction

The sources of the influence of toxic substances on the body are very diverse – air, water, food, industrial toxicants, toxicants used in everyday life, etc. Currently, the question of the effect of various doses of toxicants on the human and animal body is being actively studied.

Food safety is a growing consumer concern and requires manufacturers to ensure that there are no potentially hazardous compounds in their products. Food safety is becoming increasingly important in terms of changing food habits and globalizing food supply [1]. Food can be contaminated with various toxicants throughout the food chain. Along with a decrease in the quality of food products, deviations from modern principles of healthy nutrition are observed towards a deficiency of macro- and micronutrients, vitamins, minerals, which also negatively affects the health of the population.

The most common heavy metals contaminating foods throughout the food chain are aluminum [2–4], cadmium [5–7], lead [8–10], arsenic [11, 12].

To assess the toxic load on the body, a variety of biosubstrates (hair, nails, blood, urine) are examined. In experimental studies, the content of toxic substances in various organs of laboratory animals is studied. It should be noted that at present, preference is given to non-invasive research methods (hair analysis), which fully reflect the influence of toxic substances on the human and animal body [13].
2. Materials and methods

2.1. Animals
The study was conducted on male Wistar rats from two months of age (N = 20, weight=180±10.2 g). They were maintained in controlled environment (12:12 h light/dark cycle). All procedures used in the study were performed in accordance with the protocol approved by the Institutional Animal Care and Use Committee of the Federal Research Center for Biological Systems and Agrotechnologies of the Russian Academy of Sciences, Orenburg, Russian Federation and complied with Directive of the European Parliament and the Council of the European Union 2010/63 / EU.

2.2. Experimental design
During the accounting period (8 weeks), the animals were divided into 2 groups. The rats of experimental group consumed a semi-synthetic diet consisting of basic diet (50 %), fast food products (50 %), carbonated soft sweet drinks and water; the second group was a control one, and received basic diet and water without restrictions. The basic diet was compiled in accordance with the recommendations of the Institute of Nutrition of the Russian Academy of Sciences and contained corn starch (58 g), casein (25 g), unrefined sunflower oil and lard, 4 % salt mixture, 1 % mixture of vitamins, 2 % microcrystalline cellulose. The composition of the diets was the same in terms of the quantitative content of protein, fat and carbohydrates. At the end of the treatment period rats were euthanized under deep anesthesia. Hair, blood and liver tissue were taken for further investigation.

2.3. Analytical procedures
The content of Al, Cd, Pb, Sr in hair and liver tissue was determined in the laboratory of the ANO "Center for Biotic Medicine" (registration number in the state register – Ross.Ru 0001. 513118 dated May 29, 2003; Registration Certificate of ISO 9001: 2000, Number 4017-5.04.06) using atomic emission and mass spectrometry with inductively coupled argon plasma (Optima 2000 DV and ELAN 9000 (Perkin Elmer, USA).

Biochemical parameters (alanine aminotransferase (ALT), aspartate aminotransferase (AST) total protein, total bilirubin, urea, creatinine and cholesterol) were determined spectrophotometrically using commercial kits by biochemical analyzer (Clima MC-15 A / O Unimed). The activities of antioxidant enzymes such as CuZn-superoxide dismutase (Cu-Zn SOD) and glutathione peroxidase (GPx) were measured in erythrocyte lysate by spectrophotometric method.

2.4. Statistical analysis
Mathematical processing of data was carried out using the program "Statistica 10.0". Parameters of descriptive statistics for quantitative indicators are given in the form of a median (Me) and interquartile latitude (25th, 75th percentile – Q1; Q3). Since n does not exceed 30, the Mann-Whitney U test was used to estimate the significance of the similarity (difference) of two independent samples. The significance level was considered reliable at p <0.05. Correlation analysis was carried out using the Spearman correlation coefficient.

3. Results and discussion

3.1. Effects of treatments on body weight of laboratory animals
Body weight of rats was similar between groups at the beginning and end of experiment (Fig. 1). The water consumption in the experimental group was higher, most likely due to the sweet carbonated drinks in the diet.

3.2. Effects of treatments on biochemical blood parameters of laboratory animals
Comparison of biochemical blood parameters revealed a 1.14-fold decrease in the total protein content in the experimental group compared to that of the control group and a 1.2-fold increase in the urea level; however, these changes were not significant (Table 1).
Figure 1. Animals’ body weight during experimental period, g

Table 1. Biochemical blood parameters of laboratory animals

| Indices                  | Experimental group | Control group | MWU p   |
|--------------------------|--------------------|---------------|---------|
| Total protein g/l        | 74.3 (65.1–85.7)   | 84.9 (78–87)  | 0.364   |
| ALT U/l                  | 106.0 (100–119.2)  | 21.5 (20.1–31)| 0.000*  |
| AST U/l                  | 307.0 (249.2–352)  | 144.3 (114–181)| 0.007*  |
| Total Bilirubin µmol/l   | 4.1 (3.1–4.9)      | 6.2 (5.9–7.1) | 0.003*  |
| Urea mmol/l              | 8.5 (6.8–10.1)     | 7.2 (5.2–9.3) | 0.289   |
| Creatinine mmol/l        | 79.0 (74.4–86.7)   | 82.3 (81.7–88.3)| 0.053   |
| Cholesterol mmol/l       | 2.1 (1.95–2.25)    | 1.4 (1.1–1.9) | 0.002*  |

Data presented as median (25–75); MWU p=p values as assessed by Mann-Whitney U-test; * – difference significant at p<0.05

Cholesterol was 1.5 times (p = 0.002) higher in the experimental group. The level of ALT and AST was significantly higher in animals of the experimental group. The increased activity of these enzymes against the background of normal bilirubin may indicate pancreas dysfunction. Thus, food stress has affected the state of protein and fat metabolism in the body. Numerous studies confirm the influence of the diet on the trace element composition of various tissues of the human and animal body [14]. In our work, no significant changes in heavy metals in liver tissue were detected; however, there was a tendency to accumulation of aluminum in animals of the experimental group (Table 2).

3.3. Effects of treatments on content of toxic elements in hair and liver of laboratory animals

Changes of trace elements in animals’ hair were more pronounced. A number of authors considered hair as the most informative biosubstrate of changes in elemental status [15].

Table 2. Content of toxic elements in hair and liver of laboratory animals, Me (Q1-Q3)

| Elements | Experimental group | Control group | MWU p   |
|----------|--------------------|---------------|---------|
| **Liver**|                    |               |         |
| Al       | 6.01 (2.5-8.7)     | 3.2 (2.1 – 4.2)| 0.173   |
| Cd       | 0.04 (0.03-0.22)   | 0.0375 (0.03 – 0.04)| 0.727   |
| Pb       | 0.13 (0.02-0.24)   | 0.33 (0.21 – 0.45)| 0.200   |
| Sr       | 1.73 (1.08 – 2.12) | 2.56 (2.43 – 2.78)| 0.643   |
| **Hair** |                    |               |         |
| Al       | 3.95 (2.9 –6.9)    | 2.27 (1.9 -4.14)| 0.017*  |
| Cd       | 0.032 (0.003 – 0.057)| 0.032 (0.007- 0.041)| 0.594   |
| Pb       | 0.141 (0.078 – 0.180)| 0.136 (0.104 – 0.220)| 0.493   |
| Sr       | 1.340 (1.020 – 1.820)| 0.690 (0.580 – 1.790)| 0.732   |

Data presented as median (25–75); MWU p=p values as assessed by Mann-Whitney U-test; * – difference significant at p<0.05
A significant increase in the aluminum content by a factor of 1.7 was found in the hair of animals of the experimental group. Cd, Pb and Sr content in the experimental group did not differ from the control one. Interelement correlations, as indicators of metabolic disorders, are widely studied in animals and humans in different tissues. In present paper, a correlation was found between the Al content in the liver tissue and Al in hair ($r = 0.809$, $p < 0.05$) (Fig. 2).

![Figure 2. Correlation between Al hair and Al liver content](image)

### 3.4. Effects of treatments on the activities of antioxidant enzymes of laboratory animals

Heavy metals can exhibit toxic effects, including through oxidant imbalance. For example, in many works, the pro-oxidant activity of aluminum was noted. Al could enlarge oxidative stress and also interfere with the function of glutathione peroxidase (GPx). Antioxidant enzyme GPx catalyzes the reduction of lipid hydroperoxides and the reduction of hydrogen peroxide to water using glutathione as a substrate [16].

| Indices   | Experimental group | Control group | $p$        |
|-----------|--------------------|---------------|------------|
| GPX U/ml  | 156 (124–245)      | 240.0 (229.0–251.0) | 0.075      |
| SOD U/ml  | 443 (401–499)      | 491.0 (352.0–498.0) | 0.970      |

Data are presented as median (25–75); $p$ values as assessed by Mann-Whitney U-test; difference significant at $p<0.05$

![Figure 3. Correlation between Al hair content and plasma GPx](image)
For indicators of the antioxidant system, no significant intergroup differences were obtained. However, an inverse correlation was found between the aluminum content in the liver tissue and the level of plasma GPX activity ($r = -0.903$, $p < 0.05$). According to the results of many studies, an increase in the toxic load of heavy metals, including aluminum, was accompanied by a decrease in the activity of glutathione peroxidase and a change in the activity of SOD [17, 18].

Increase in toxic elements in tissues can occur against the background of trace element imbalance caused by an unbalanced diet. Additionally, toxic metals, including aluminum, can come from food, despite the established maximum permissible concentrations [19, 3, 4].

4. Conclusion
The paper studied the features of the accumulation of toxic elements in hair and liver tissue of laboratory animals on the background of an unbalanced diet (fast food). Imbalance of chemical elements was less pronounced in liver tissue compared to that of the hair. A significant increase in the Al content in animal hair of the experimental group of 1.7 times was established. Positive correlation between Al in the liver tissue and Al in animal hair ($r = 0.809$, $p < 0.05$) was found. A significant increase of 5 and 2 times in ALT and AST was found, respectively. In addition, a negative correlation was found between Al liver and the activity of plasma GPx ($r = -0.903$, $p < 0.05$). Thus, the data obtained in our study were a clear demonstration of the adaptive changes in the body in response to a change in diet, which can be characterized as food stress.

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