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ARSENIC CONTENTS IN RATS' FUR AS AN INDICATOR OF EXPOSURE TO ARSENIC. PRELIMINARY STUDIES

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ABSTRACT

Background. Since arsenic compounds have an affinity to thiol groups their greatest amounts can then be found in the tissues containing sulphur - rich proteins, like *beta*- keratin in skin, hair and nails. Accumulation of arsenic also depends on the macronutrient content in daily food ration. The deficiency and excess of both the protein and fat may contribute to a higher content of arsenic in the organism, including hair in human or fur in animals.

Objective. Hair and fur is a good indicator of population exposure to many toxic substances, including arsenic. The degree of arsenic accumulation may depend on the diet and nutritional status. The aim of this study was to determine the effect of protein and fat in diet on the accumulation of arsenic in rats' fur.

Material and Methods. A total number of 70 male Buffalo rats (body weight 200 - 220 g, age - 6 weeks) were divided into 10 groups. Rats were housed in plastic cages (4 per cage) in a 12h light/dark cycle for 6 weeks. The diets of different protein and fat contents ware administered to the animals. Five of ten groups of rats received throughout the whole period 10 ppm sodium arsenite dissolved in distilled drinking water (about 250 µg As/animal/day). The arsenic were determined with the method of atomic adsorption spectrometry in conjunction with a graphite-furnace atomize using a Varian AA240FS apparatus.

Results. The highest arsenic concentrations were found in fur of rats which were given low protein diet and water with arsenic. The lowest arsenic contents were found in fur of rats, which were given control diet and high protein diet with arsenic in water.

Conclusions. Balanced control diet or high protein diet protected organism from arsenic accumulation, only small increase of arsenic content in rats' fur, compared to the control group, was observed.

Key words: *arsenic, rats' fur, protein and fat in diet, exposure to arsenic*

STRESZCZENIE

Wprowadzenie. Związki arsenu wykazują powinowactwo do grup tiolowych białek, dlatego też najwyższą kumulację tego pierwiastka obserwuje się w tkankach zawierających białka bogate w siarkę takich jak skóra, włosy i paznokcie. Kumulacja tego pierwiastka zależy również od zawartości makroskładników diety w całodziennej racji pokarmowej, niedobór i nadmiar zarówno białek jak i tłuszczów przyczyniać się może do zwiększonej zawartości arsenu w organizmie, w tym również włosach u ludzi, czy sierści u zwierząt.

Cel. Włosy i sierść zwierząt są dobrym wskaźnikiem ekspozycji populacji na wiele substancji toksycznych, w tym arsenu. Stopień kumulacji tego pierwiastka może być natomiast zależny od sposobu żywienia i stanu odżywienia. Celem niniejszej pracy było zbadanie wpływu zawartości białka i tłuszczu w diecie na akumulację arsenu w sierści szczurów doświadczalnych.

Materiał i metody. 70 samców szczurów Buffalo (waga ciała 200 - 220 g, wiek 6 tygodni) - podzielono na 10 grup doświadczalnych. Szczury umieszczono w klatkach z tworzywa sztucznego (4 zwierzęta na klatkę) i przetrzymywano przez 6 tygodni w 12-godzinnym cyklu dzień/noc Zwierzętom podawano pasze z niedoborem lub nadmiarem białka lub tłuszczu. Pięć z dziesięciu grup szczurów otrzymywała przez cały okres 10 ppm arseninu sodu rozpuszczonego w destylowanej wodzie pitnej (około 250 µg As/osobnika/dzień). Arsen oznaczono metodą absorpcyjnej spektrometrii atomowej z kuwetą grafitową przy użyciu aparatu Varian AA240FS .

Wyniki. Najwyższe stężenia arsenu stwierdzono w sierści szczurów, którym podawano dietę niskobiałkową i wodę z arsenem. Najniższą zawartość arsenu stwierdzono w sierści szczurów, które karmiono dietą kontrolną lub wysokobiałkową, zatruwanych arsenem.

Wnioski. Zrównoważona dieta kontrola lub wysokobiałkowa wykazywała działanie ochronne przeciwko kumulacji arsenu. W porównaniu z grupą kontrolną obserwowano tylko niewielki wzrost zawartości arsenu w sierści szczurów.

Słowa kluczowe: *arsen, sierść szczurów, białkowa i tłuszczowa dieta szczurów, narażenie szczurów na arsen*

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INTRODUCTION

Arsenic is widely distributed in the environment and is one of the most important global environmental toxicants. The main source of air and soil contamination with arsenic compounds is coal and oil mining industry as well as mining and metallurgy of nonferrous metals. The cases of arsenism due to chronic exposure were observed among inhabitants of regions where such industry is well developed. Short exposure results in acute effects characterized by vomiting, abdominal colic, and diarrhea. In rare cases, these symptoms may lead to vascular shock and death [16]. The long term, regular exposure to arsenic compounds from both food and inhalation manifests in skin lesion and disorders in functioning of cardiovascular, neural and respiratory systems. Investigations prove the negative arsenic impact on reproductive abilities in both sexes [11]. It causes gonads atrophy and occurrence tissue disease. There are extensive documentations that among women consuming water containing arsenic increase fetal, neonatal and postnatal mortalities, and elevations in low birth weights, spontaneous abortions, still-birth, pre-eclampsia and congenital malformations [1].

Arsenic is cumulated mostly in liver and kidneys, but after 24 hours little remains in these organs. Arsenic compounds have an affinity to thiol groups and the greatest amounts can then be found in the tissues containing sulphur rich proteins and largely in the form of sulphur rich β-keratin like skin, hair and nails. The chemical analysis of hair or fur samples from populations has been used to monitor excessive exposure of that population to a number of toxic substances, including arsenic. They can be collected in large quantities from a living organism, and sample collection is easy [6].

The concentration of arsenic in hairs ranging from 0.08 – 0.25 µg/g for people from areas not contaminated with arsenic to more than 9 µg/g for people ingesting drinking water containing arsenic regularly [10,14]. *Liebscher* and *Smith* reported a range of 0.02 to 8.17 µg/g in 1250 hair samples from persons living in the industrial city of Glasgow, Scotland [9]. *Gautam* et al. [4] reported concentrations of arsenic in hair collected from arsenic affected areas of West Bengal, India in range 0.17–14.39 µg/g.

Hindmarsh et al. [6] showed approximate interpretation of hair arsenic levels. They propose as a normal for people, content of arsenic in hair less than 1 µg/g dry weight. Characterized chronic poisoning ≥ 10 µg/g (can be less), for lethal 45 µg/g (can be lower, also higher levels have been reported in subjects who survived) and as an external contamination up to many thousands µg/g.

Most of people in the world are characterized by not proper nutrition style. In developing countries usually occurs malnutrition. In developed countries diet is usually high energetic, there exceeded proteins and fats, especially animal fats, in diet occurs. Anomalous nutrition can lead to higher organism susceptibility for all toxic trace elements including arsenic. Insufficient proteins and polyunsaturated fatty acid in diet cause enzymes' activity reduction, it cause lower activity of cytochrome P-450 [17]. In the metabolism of arsenicals, S-adenosyl-L-methionine (SAM) and reduced glutathione (GSH) are requisites as a methyl group donor and a reducing agent, respectively [5].

Protein – energetic inadequate intake occurred in developing countries can lead to lower organism ability to arsenic detoxification. In developed countries, in spite of arsenic content in foodstuff is lower, higher consumption can cause excessive arsenic intake.

The aim of this study was to examine the role of protein and fat content in diet on the accumulation of arsenic in rats' fur.

MATERIAL AND METHODS

A total number of 70 male Buffalo rats (body weight 200 - 220 g, age - 6 weeks) were randomly divided into ten groups (7 animals per each group): control diet (I, I+As), low fat diet (II, II+As), high fat diet (III, III+As), low protein diet (IV, IV+As), high protein diet (V, V+As) with 10 µg As/ml or without arsenic in drinking water. Rats were housed in plastic cage (4 per cage) in a 12 h light/dark cycle. Animals were acclimated to laboratory conditions for 1 week prior to the start of this study. Rats received half – synthetic diet AIN – 93 recommended for laboratory rodents. Standard diet contents 14% protein and 4% fat, high protein diet contents 18 % proteins and low protein diet 10%. High fat diet contents 6% of fat and low fat 2%. Five of ten groups of rats received throughout the whole period 10 ppm sodium arsenite dissolved in distilled drinking water. Average water consumption was 25 ml/animal/day. Arsenic intake amounted 250 µg/animal/day. After six weeks fur samples were collected from all body parts as near as possible to the skin and stored in polyethylene bags.

To determine contents of arsenic 0.5 g samples were weighed and mineralized with 5 ml 65% nitric acid and 1 ml of 30% H_2O_2. The elements were determined with the method of atomic adsorption spectrometry in conjunction with a graphite-furnace atomize using a Varian AA240FS apparatus. Arsenic was determined at the 193.7 nm wavelength. The average recovery of arsenic reached about 98% and accuracy about 3%.

To determine significant differences between median arsenic content depending on the protein and fat content in diet results were analyzed using the non-parametric *Kruskal's – Wallis* test.

RESULTS AND DUSCUSSION

Table 1 shows median arsenic concentration in rats' fur depended on diet. Arsenic content in fur of not exposed rats was low (7.0 – 15.0 µg/g). Exposure to arsenic caused accumulation of this element in the fur. The largest increase arsenic content in rats' fur presented as percent of control value for each diet (Figure 1) was observed in low fat (1020%) and low protein diet (602%). The lowest increase arsenic content was observed in control group.

water is used as drinkable water. In Argentina, Bangladesh, Chile, China, India, Mexico, Romania, Taiwan and Vietnam determined amount of arsenic in water ranged within 1 - 5300 µg/L, which means that allowable contents specified by FAO/WHO 10 - 50 µg/L experts is exceeded more than one hundred times. It is estimated that even 10% peoples living in Bangladesh drink water containing over 500 µg As/L [8]. Between occupants 37 from 52 regions of this country there were skin changes observed. In 90% hair samples, 98% nail samples and 95% urine samples arsenic content is much higher than values known as a natural [7]. The great amounts of arsenic descend also from rice. The common inhabitant of developing countries eats 400 - 650 g of rice weekly. The average contamination of arsenic in rice is 0.57 – 0.69 mg/kg, which means the arsenic intake at the level 30% – 45% PTWI (only from rice) [13].

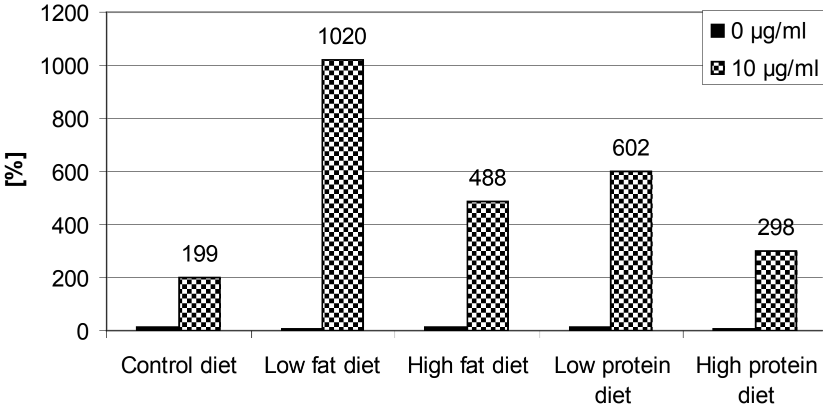

Figure 1. Increase of arsenic in rats' fur presentet as percent [%] of control value for each diet

Compared arsenic accumulation in exposed rats, depending on the diet, it was shown that all modifications of diet, despite high protein diet, caused significant increase of arsenic content compared to the control group (Table 1).

The highest arsenic contents were found in fur of rats, which were given low fat diet (75.1 µg/g), and low protein diet (72.8 µg/g) poisoned with arsenic. This is alarming because most of peoples, who drink water affected with arsenic, suffer with malnutrition and protein deficient. The biggest amounts of arsenic in the daily rations appear among peoples living in developing countries coming from potable water, especially in regions where ground

Presented studies showed that balanced diet with standard protein and fat content can protect organism from arsenic accumulation. Also exceeded content of protein in diet caused only small increase of arsenic content in rats' fur. It might be caused by high content of cystheine and methionine in diet, cystheine is necessary to glutathione synthesis. An increased glutathione concentration could protect the organ from arsenic – inducted lipid peroxidation. Dietary protein content modifies the process of glutathione metabolism and the status of the antioxidant system [9].

The similar effect protecting rats' organism from arsenic toxic action by increasing one of diet compounds

Table 1. Arsenic content in rats' fur [µg/g]

Arsenic content in drinking wateR	Arsenic content in fur [µg As/g]														
	Control diet			Low fat diet			High fat diet			Low protein diet			High protein diet		
	n=7			n=7			n=7			n=7			n=7		
	Me	±	Q	Me	±	Q	Me	±	Q	Me	±	Q	Me	±	Q
0 µg/mL	15.0	±	32.0	7.36	±	9.31	11.1	±	10.3	12.1	±	8.3	7.02	±	10.4
10 µg/mL	29.9^{ABC}	±	30.0	75.1^{A}	±	99.7	54.2^{B}	±	2.5	72.8^{CD}	±	20.2	20.9^{CD}	±	5.3

Me – median value, Q - quartile deviation, ABCD –significant differences p< 0,005 observed *Chattopadhyay* et al. [2]. Added L–ascorbic acid (25 mg/100 g body weight) to diet caused increase FSH, LH and estradiol level up to the level noticed in control group, while in group, which were given arsenic without L–ascorbic acid those levels were twice lower. *Nandi* et al. [12] shown that adding cystheine or ascorbic acid improve biochemical indexes in rats' liver and kidneys. Also *Garcia-Chavez* et al. [3] shown that added *alpha*–tocoferol (125 mg/kg body weight) protected brain from arsenic toxity. Similar observation noticed *Shila* et al. [15] with added *alpha*-lipolic acid.

CONCLUSIONS

1. The accumulation of arsenic in fur was the lowest in high protein diet rats
2. In fur of rats on low fat diet the increase of arsenic were the greatest
3. Further studies are required to define the suitability of fur as an indicator of environmental and food pollution.

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