THE IMPACT OF A BOVINE PROTEINS REACH DIET ON CADMIUM, ZINC AND COPPER CONCENTRATION IN LIVER, KIDNEY AND FECES OF CADMIUM INTOXICATED RATS

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Cadmium is a highly toxic even at low concentrations to animals which has no biological functions. The purpose of this study was to assess the effect of increased amounts of bovine milk casein and whey protein in the diet of rats intoxicated with Cd on mineral homeostasis. Twenty four mature female Wistar rats were divided into four equal groups. Two control groups fed a basal diet free of casein and whey protein: group I (+ve control) administered additionally with $CdCl_2$ in drinking water (19.4mmol/l), group II (-ve control) without $CdCl_2$ in drinking water. The rats from group III and group IV received a basal diet during the first 5 weeks, while during the consecutive 5 weeks received casein and whey protein rich diet (5.6%+5.8%, respectively) and group III was administered additionally with CdCl2 in drinking water at the same dose as group I. The experiment lasted 10 weeks. At the end of experiment, cadmium concentration in liver, kidney and feces was the lowest the –ve group, while their content increased significantly in the +ve control rats compared to rats of –ve control and both experimental groups. Zinc concentration in the liver was the lowest in the +ve control treated rats and did not differed from that of the -ve control treatment. Significantly higher concentrations of Zn were found in the casein+whey treated rats indifferently of the treatment with Cd. The Zn concentration in kidneys did not differed between treatments while significantly higher concentration of Zn was found only in the feces of the –ve control rats. The Cu concentration was the significantly lower in the liver of the –ve treatment rats, while in the kidney of the –ve control rats the Cu concentration was significantly higher when compared to other treatments. Fecal Cu concentration was significantly higher in the rats of the –ve control treatment and differed from other groups, while the lowest Cu concentration was found in the Cas+WP+Cd and differed significantly from that of the Cas+WP and –ve control treated rats. This study showed that the administration of $CdCl_2$ in drinking water effectively increased the level of Cd in female rats kidney and liver and casein+whey proteins diet decrease the Cd concentration in liver and kidney and increase zinc concentration in liver.

Keywords: HEAVY METALS, CONTAMINATION, BOVINE PROTEINS, RAT.

Cadmium (Cd) is a mineral element, which cannot be broken down in to less toxic substances in the environment, and is highly toxic even at low concentrations to animals and humans, and has no biological functions [6]. It is ranked the 7th toxicant in the Priority List of Hazardous Substances of the Agency for Toxic Substances and Disease Registry (ATSDR, 2007). Cd is arising from earth's crust associated with lead, zinc and copper. Currently, this metal has become an environmental and public health problem due to its constant release by industrial activity, batteries, pigments, consumer

electronics, quantum dots, and smoking [15]. This metal enters the food chain through contaminated air, water, and soils. Hence, general population is exposed to Cd by contaminated water and food [17]. Because of the biologic half-life of cadmium is long, prolonged low level exposure leads to excessive accumulation in certain tissues, especially in the kidney, so the most dangerous characteristic of cadmium is that it accumulates throughout a lifetime [2].

Acute Cd exposure produces toxicities to the lung, liver, testes, and brain, while chronic exposure to Cd often leads to renal dysfunction, anemia, osteoporosis, and bone fractures [9, 10]. Cd is a potent carcinogen in a number of tissues of rodents and classified as a human carcinogen [20].

Cd poisoning mechanism is dependent on organism mineral inflow, ingested dose, metal chemical form, during of exposure to mineral element, animal species and age [19]. The basic mechanism of toxicity of cadmium is the induction of oxidative stress and lipid peroxidation of cell membranes.

The treatment strategies for Cd toxicity include chelating and antioxidant therapies. Until now in treating Cd intoxication, chelating compounds have been used, which burdened with numerous undesirable symptoms [14]. For this reason, there is the need to find natural compounds that help in protection against cadmium induced toxicity with fewer or no side effects.

Among the milk protein products, whey proteins and casein products are now produced in large quantities as protein ingredients for food industry which provide specific health benefits beyond its basic nutritional value. Whey's curative properties had been known for prior century's. Hippocrates (466-377 BC), the father of medicine, recommended whey to his patients [12]. The bioactive peptides which released from whey proteins and caseins during digestion possess very important biological functionalities, including antioxidative, anticytotoxic, and mineral - carrying activities [11].

The aim of the study was to evaluate the impact of increased amounts of bovine milk casein and whey protein in the diet of rats intoxicated with Cd on mineral homeostasis.

Materials and methods.

Experiments. The study was performed on 24 mature female Wistar rats in standard laboratory conditions on a 12/12 dark/light cycle and lasted for 10 weeks After the adaptation period rats were randomly divided into four equal groups. Group I (+ve control) was administered $CdCl_2$ (Sigma-Aldrich, Germany) in drinking water (19,4 mmol/l), group II (-ve control) was administered drinking water without $CdCl_2$, these two groups were fed a basal diet free of casein (Cas) (KASKA DIARY®, Poland) and whey protein (WP) (Spomlek, Poland) for ten weeks. The rats from group III and group IV received a basal diet during the first 5 weeks, while during the consecutive 5 weeks received casein and whey protein rich diet (5.6%+5.8%, respectively), but only group III gr. got $CdCl_2$ in water. Rats had free access to water and feed and were killed by a CO_2 overdose at the end of the experiments. Internal organs were isolated, rinsed in physiological solution and dried. Fecal samples were collected from the rectum and placed in 2 ml Eppendorf tubes. All samples were frozen in liquid nitrogen and kept for further analyses at $^{-}20$ °C.

Elements analysis. For mineral analysis the desired amount of the samples (≈0.5 g) was weighed and dried for 1 hour in a porcelain melting pot on a hot plate (100 °C) and submitted to dry mineralisation at 440 ºC. The mineralized samples were dissolved in 1 molar solution of ultrapure nitric acid (Sigma-Aldrich, Germany) and the concentration of Cd, Cu and Zn were analyzed by graphite furnace atomic absorption spectroscopy (GFAAS) using a SpectrAA 220Z (Varian, Australia) with a Zeeman corrector equipped with a GTA 100z electro thermal atomizer and a Varian PSD 100 auto sampler. For the graphite furnace measurements, argon was used as the inert gas. Additionally, pyrolytic coated graphite tubes with a platform were used. Hallow cathode lamps were used as sources for each of the elements investigated. Only optimal instrumental parameters, as suggested by the

instrumental procedures, were used during analysis by GFAAS. A palladium solution (500 μg/mL) was used as a chemical modifier for the analysis of cadmium. The atomic absorption signal was indicated by the peak area seen in the calibration curve.

Statistics. Data were collected and submitted to statistical analysis using the IBM SPSS Statistics software. Analysis of variance and post-hoc Tukey test were performed and the significance of the differences between the variables were settled for $P<0.05$.

Results and discussion. The concentrations of cadmium, zinc and cooper of control and intoxicated rats fed a standard diet and fed a casein and whey protein diet are displayed in table 1.

Cadmium concentration in liver, kidney and faces was the lowest in samples from –ve control and the casein+whey treated groups of rats. Cd concentrations in kidney, liver and faces increased significantly in the +ve control rats compared to rats of –ve control and casein+whey protein groups. The concentration of Cd in samples from rats treated with Cd in drinking water and fed a casein and whey protein diet (casein+whey+Cd group) was intermediate and only Cd concentration in kidney was significantly higher when compared to negative control and Cas+WP treated rats (table).

Zinc concentration in the liver was the lowest in the +ve control treated rats and did not differed from that of the –ve control treatment. Significantly higher concentrations of Zn were found in the Cas+Whey treated rats indifferently of the treatment with Cd. The Zn concentration in kidneys did not differed between treatments. Significantly higher concentration of Zn was found in the faces of the –ve control treated rats compared to other treatments (table).

The Cu concentration was the lowest in the liver of the –ve treatment rats and exhibited a significant increase in the liver of rats in other treatments. Significantly higher Cu concentration compared to other treatments was found in the kidney of the –ve control rats. Fecal Cu concentration was significantly higher in the rats of the –ve control treatment and differed from other groups, while the lowest Cu concentration was found in the Cas+WP+Cd and differed significantly from that of the Cas+WP and –ve control treated rats (table).

Table

Cadmium, zinc and cooper concentration in liver, kidney and faces of control and cadmium intoxicated rats with $CdCl_2$ fed a standard or casein+whey enriched diet

Sample	Treatment			
	Gr. I (+ve control)	Gr. II (-ve control)	Gr. III (Cas+WP+Cd)	Gr. IV (Cas+WP)
Cadmium (mg/kg)				
Liver	0.11 ± 0.06 a	0.008±0.005 b	0.07±0.015 ab	0.006 ±0.001 b
Kidney	0.17 ±0.07 a	0.013 ±0.002 b	0.12 ±0.031 a	0.011 ± 0.001 b
Feces	0.57±0.19 a	0.062± 0.013 b	0.18 ±0.053 ab	0.088 ±0.058 b
Zinc (mg/kg)				
Liver	26.1 ± 1.14 a	27.7 ± 0.63 ab	30.9 ± 0.96 b	30.8 ± 1.33 b
Kidney	27.9 ± 1.92 a	24.6 ±1.38 a	28.3 ± 0.94 a	25.8 ± 1.38 a
Feces	254.9 ± 21.4 a	357.4 ± 32.7 b	226.8 ± 26.8 a	265.4 ± 23.1 a
Cooper (mg/kg)				
Liver	16.4 ± 1.1 a	6.6 ± 0.3 b	13.6 ± 2.1 a	16.9 ± 1.4 a
Kidney	20.9 ± 2.8 a	36.2 ± 5.0 b	16.0 ±1.5 a	17.7 ± 2.7 a
Feces	35.2 ± 3.3 ab	66.2 ± 4.0 c	24.7 ± 5.1 a	45.4 ± 6.5 b

Data represent Mean ± SD, a – different lower case letter means statistical significance at $P < 0.05$.

The dose of cadmium used in this experiment was setted to be close to the dose causing the lowest observed adverse effect LOAEL, which according to the "Toxicological Profile for Cadmium -

Agency for Toxic Substances" http://www.atsdr.cdc.gov/toxprofiles [1] amounts 4-5 mg/kg b. w. for sub chronic oral exposure of Wistar rats. In our studies it is clearly shown that the water consumption containing 19.4 mmol/l asserts close to the LOAEL dose. This dose was able to increase the Cd level in liver and kidney about ten fold in the Cd treated rats compared to that of the treatments without $CdCl_2$ in water.

Absorbed cadmium is eliminated from the body primarily in urine. The rate of cadmium excretion is low and accumulation in the body can be significant, probably because cadmium remains tightly bound to metallothionein, which is almost completely reabsorbed in the renal tubules. Both kidney and liver are cadmium stores; 50–85 % of the body burden is stored in kidney and liver, 30–60 % being stored in the kidney alone. Tissue cadmium concentrations increase with age, but, concentration in blood reflects recent exposure.

There is sufficient evidence that cadmium compounds, such as cadmium chloride, are carcinogenic in animals. Increased rates of testicular, prostate, and lung cancer in animals have been described [16]. It was hypothesized that Cd uptake involves competition with essential elements like Ca or Zn for specific transport systems [8]. Due to their similarities to essential metals, toxic metals are transported and eliminated through many common cellular mechanisms by "molecular mimicry" [4]. As a result, there exist toxico-kinetic and toxico-dynamic interactions among toxic and essential metals [21], iron inhibits lead and cadmium intestinal uptake due to shared absorption mechanisms [3], conversely, toxic metals may inhibit essential element absorption [18]. Cadmium and zinc are also known to have a variety of interactions due to the metal-binding protein metallothionein [5].

Growing evidence shows that the intake of milk antioxidants including antioxidant peptides or proteins and others has a positive effect, as they can protect the body against the radical-induced injury [22]. Milk proteins can be digested by proteases or fermented by edible microorganisms to produce protein hydrolysates, which are more active than the parent proteins. Many of these hydrolysates or the purified peptides were found to have antioxidant properties, and thus received increasing attention in the recent years [7]. Among the milk protein products, whey proteins and casein products are now produced in large quantities as protein ingredients for food industry which provide specific health benefits beyond its basic nutritional value. A lot of researches have been carried out on casein bioactive hydrolysates. It's well understood that peptides are not active within the parent protein but, they can be released and activated following enzymatic hydrolysis [13].

C O N C L U S I O N

1. The administration of $CdCl_2$ in drinking water effectively increased the level of Cd in female rats kidney and liver.
2. Casein+whey proteins diet decrease the Cd concentration in liver and kidney and increase zinc concentration in liver.

ВПЛИВ РАЦІОНУ, ЗБАГАЧЕНОГО БИЧАЧИМИ ПРОТЕЇНАМИ, НА КОНЦЕНТРАЦІЮ КАДМІЮ, ЦИНКУ ТА МІДІ В ПЕЧІНЦІ, НИРКАХ І ФЕКАЛІЯХ ЩУРІВ, ІНТОКСИКОВАНИХ КАДМІЄМ

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А Н О Т А Ц І Я

Кадмій дуже токсичний навіть у низьких концентраціях у тварин, в яких не виконує жодних біологічних функцій. Метою даного дослідження було оцінити ефект збільшення кількості бичачого молочного казеїну та сироваткового білка в раціоні щурів, інтоксикованих кадмієм, на мінеральний гомеостаз. Двадцять чотири зрілі самки щурів Вістар були розділені на чотири рівні групи. Дві контрольні групи годували стандартним раціоном без казеїну та сироваткового протеїну: групі I (+ ve контроль) вводили додатково $CdCl_2$ з питною водою (19,4ммоль / л), групі II (-ve контроль) – питну воду без $CdCl_2$. Щури з групи III та групи IV отримували стандартний раціон протягом перших 5 тижнів, а протягом наступних 5 тижнів отримували раціон з казеїном та сироватковим білком (5,6% + 5,8%, відповідно), III групі вводили $CdCl_2$ додатково з питною водою в тій же дозі, що і групі I. Експеримент тривав 10 тижнів. Наприкінці експерименту концентрація кадмію в печінці, нирках та фекаліях була найнижчою у групі -ve, тоді як їх вміст значно підвищився у + ve щурів контрольної групи порівняно з щурами -ve контролю і обох експериментальних групах. Концентрація цинку в печінці була найменшою у щурів + ve контрольної групи, і не відрізнялася від концентрації у тварин -ve контрольної групи. Значно вищі концентрації Zn були виявлені в щурів, що споживали казеїн + сироватковий білок, незалежно від вмісту в раціоні Cd. Концентрація Zn в нирках не відрізнялася у дослідних групах, тоді як значно вища концентрація Zn була виявлена лише у фекаліях контрольних щурів. Концентрація Cu була значно нижчою в печінці щурів -ve групи, тоді як у нирках щурів -ve контрольної групи концентрація Cu була значно вищою порівняно з іншими речовинами. Фекальна концентрація Cu була значно вищою у щурів -ve контрольної групи та відрізнялася від інших груп, тоді як найнижча концентрація Cu була виявлена в щурів, що споживали казеїн + сироватковий білок + Cd і значно відрізнялася від концентрації в тварин з раціоном казеїн + сироватковий білок та щурів -ve контрольної групи. Це дослідження показало, що введення $CdCl_2$ у питну воду ефективно підвищувало рівень Cd у нирках та печінці самок щурів і раціон казеїн + сироваткові білки знижує концентрацію Cd у печінці та нирках та збільшує концентрацію цинку в печінці.

Ключові слова: ВАЖКІ МЕТАЛИ; ЗАБРУДНЕННЯ; БИЧАЧІ ПРОТЕЇНИ; ЩУРИ.

ВЛИЯНИЕ РАЦИОНА, ОБОГАЩЕННОГО БЫЧЬИМИ ПРОТЕИНАМИ, НА КОНЦЕНТРАЦИЮ КАДМИЯ, ЦИНКА И МЕДИ В ПЕЧЕНИ, ПОЧКАХ И ФЕКАЛИЯХ КРЫС, ИНТОКСИЦИРОВАННЫХ КАДМИЕМ

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А Н Н О Т А Ц И Я

Кадмий очень токсичен даже в низких концентрациях у животных, в которых не исполняет биологических функций. Целью данного исследования было оценить эффект увеличения количества бычьего молочного казеина и сывороточного белка в рационе крыс, интоксицированных кадмием, на минеральный гомеостаз. Двадцать четыре зрелые самки крыс Вистар были разделены на четыре равные группы. Две контрольные группы кормили стандартным рационом без казеина и сывороточного протеина: группе I (+ ve контроль) вводили дополнительно CdCl2 с питьевой водой (19,4ммоль / л), группе II (-ve контроль) - питьевую воду без CdCl2. Крысы из группы III и группы IV получали стандартный рацион в течение первых 5 недель, а в течение следующих 5 недель получали рацион с казеином и сывороточным белком (5,6% + 5,8% соответственно), III группе вводили CdCl2 дополнительно питьевой водой в той же дозе, что и группе I. Эксперимент продолжался 10 недель. В конце эксперимента концентрация кадмия в печени, почках и фекалиях была самой низкой в группе -ve, тогда как их содержание значительно повысился в + ve крыс контрольной группы по сравнению с крысами -ve контроля и обеих экспериментальных группах. Концентрация цинка в печени была наименьшей у крыс + ve контрольной группы, и не отличалась от концентрации у животных -ve контрольной группы. Значительно выше концентрации Zn были обнаружены у крыс, потреблявших казеин + сывороточный белок, независимо от содержания в рационе Cd. Концентрация Zn в почках не отличалась в опытных группах, тогда как значительно выше концентрация Zn была обнаружена только в фекалиях контрольных крыс. Концентрация Cu была значительно ниже в печени крыс -ve группы, тогда как в почках крыс -ve контрольной группы концентрация Cu была значительно выше по сравнению с другими веществами. Фекальная концентрация Cu была значительно выше у крыс -ve контрольной группы и отличалась от других групп, тогда как самая низкая концентрация Cu была обнаружена у крыс, потреблявших казеин + сывороточный белок + Cd и значительно отличалась от концентрации у животных с рационом казеин + сывороточный белок и крыс -ve контрольной группы. Это исследование показало, что введение CdCl2 в питьевую воду эффективно повышало уровень Cd в почках и печени самок крыс и рацион казеин + сывороточные белки снижает концентрацию Cd в печени и почках и увеличивает концентрацию цинка в печени.

Ключевые слова: ТЯЖЕЛЫЕ МЕТАЛЛЫ, ЗАГРЯЗНЕНИЕ, БЫЧЬИ ПРОТЕИНЫ, КРЫСЫ.

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