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Tannic Acid Influence on Lead and Cadmium Accumulation in the Hearts and Lungs of Rats

Wpływ kwasu taninowego na akumulację ołowiu i kadmu w sercach i płucach szczurów

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article; **G** – other

Abstract

Background. The presence of heavy metals in food products has become a global problem. In order to reduce the absorption of heavy metals from food we should consider substances which bind these toxic metals and are generally available and easy to apply, such as tannins.
Objectives. The study aimed at verifying if oral administration of tannic acid could reduce the accumulation of lead and cadmium in the heart and lungs of rats subjected to a continuous exposure of toxic metals in low doses.
Material and Methods. Adolescent and adult male Wistar rats were given tannic acid (2% solution) or distilled water containing 0, 50, 100 mg Pb (as $(CH_3COO)_2Pb$)/L or 0, 7, 14 mg Cd (as $CdCl_2$)/L, for 6 or 12 weeks.
Results. Administering a 2% solution of tannic acid alternately with Pb or Cd to the rats was the effective method of reducing lead and cadmium content in the rats' heart and lungs.
Conclusions. The obtained results may be referred to people. It is necessary to conduct further research in order to confirm the hypothesis that tannic acid, present in numerous food products and primarily in drinks (wine, tea and coffee), used in the human diet, may reduce the accumulation of lead and cadmium in the tissues and thus weaken their toxicity, which is important regarding our common exposure to heavy metals found in food (**Adv Clin Exp Med 2013, 22, 5, 615–620**).

Key words: lead, cadmium, tannic acid, accumulation, hearts, lungs, rats.

Streszczenie

Wprowadzenie. Obecność metali ciężkich w żywności jest problemem globalnym. Aby ograniczyć wchłanianie metali ciężkich z żywności, należy brać pod uwagę substancje wiążące te toksyczne metale, które przy okazji będą łatwe w użyciu i ogólnie dostępne, np. kwas taninowy.
Cel pracy. Sprawdzenie, czy doustne podawanie kwasu taninowego spowoduje zmniejszenie akumulacji kadmu i ołowiu w sercu oraz płucach szczurów wystawionych na stałą ekspozycję tych metali toksycznych w małych dawkach.
Materiał i metody. Rosnące i dorosłe samce szczurów Wistar otrzymywały 2% roztwór kwasu taninowego lub wodę destylowaną zawierającą 0, 50, 100 mg Pb (w postaci $(CH_3COO)_2Pb$)/L albo 0, 7, 14 mg Cd ($CdCl_2$)/L przez 6 lub 12 tygodni.
Wyniki. Zastosowanie 2% roztworu kwasu taninowego naprzemiennie z Pb lub Cd okazało się skutecznym sposobem ograniczenia koncentracji tych metali w sercach i płucach szczurów.
Wnioski. Uzyskane wyniki można odnieść do ludzi. Należy prowadzić dalsze badania mające na celu potwierdzenie, czy stosowanie w diecie człowieka kwasu taninowego, występującego w wielu pokarmach, a przede wszystkim w napojach (wino, kawa, herbata), może ograniczyć kumulowanie ołowiu i kadmu w tkankach i tym samym osłabić ich toksyczne działanie. Jest to ważne z uwagi na powszechne narażenie ludzi na metale ciężkie, których podstawowym źródłem jest żywność (**Adv Clin Exp Med 2013, 22, 5, 615–620**).

Słowa kluczowe: ołów, kadm, kwas taninowy, akumulacja, serca, płuca, szczury.

Lead and cadmium are toxic elements whose occurrence in the environment is related to anthropogenic activity [1]. The high level of heavy metals in the environment is reflected in their amount found in plant and animal organisms which is then used to make food for people. The circulation of heavy metals in the environment is connected with the food chain including the soil – the plant – the animal – man. The transition of metals to a higher link results in a cumulative increase of their content [2]. Cadmium and lead are characterized by high accumulation ability; they are easily absorbed from the digestive tract and easily permeate biological barriers [3, 4]. The half-life of cadmium in soft tissues is 5–30 years [1], whereas for lead this time amounts to ca. 30 days [5]. These elements administered orally are deposited, among others, in the heart muscle [6] and in the lungs [7].

Lead and cadmium in large doses reveal toxic activity. Studies on the toxicity of heavy metals were performed in most cases on rats [8, 9], but also on hamsters [10] and mice [11]. In those studies the doses [12], the time of exposure to the element (from a few hours to several months) [13–15] and the form of administration were all different (mainly with drinking water, by inhalation or with food) [16]. However, what is especially dangerous to the human organism, is its long exposure to the effects of these xenobiotics. Acute intoxication with cadmium and lead occurs infrequently, and it is their chronic toxicity that is more common. In order to reduce the absorption of heavy metals from food, it is necessary to consider substances which bind these elements and are at the same time simple to use and generally accessible. Tannic acid in *in vitro* conditions is a very efficient chelator for lead and cadmium [17], so it may be assumed that it will reduce the absorption of these metals into the animal's tissues. Studies performed by Kim et al. [18] on mice showed that the use of tannic acid led to decreasing the accumulation of cadmium in the organism when the authors used very large doses of the element (20 mg/kg/day). Easily available food products containing tannic acid include tea and coffee [19].

The present study aimed at verifying if the oral administration of a 2% solution of tannic acid would lower the accumulation of lead and cadmium in the heart and the lungs of rats exposed to constant activity of these toxic metals in small doses. Since toxicity depends on its degree of distribution, uptake and metabolism, we investigated the differential accumulation of cadmium and lead in organs of adolescent and adult rats.

Material and Methods

Animals and Diet

The experiment was performed with the acceptance of the II Ethical Committee at the University of Life Sciences in Lublin, No. 18/2010. Principles embodied in the declaration of Helsinki were adhered to and all animal protocols conformed to the Guiding Principles for Research Involving Animals. The rats were allowed a period of one week to stabilize with their new environment. The proper experiment took 12 weeks (the first period of weeks 1–6 and the second period of weeks 7–12). Male Wistar rats (30 growing, weighting 169.3 ± ± 14.7 g at 35 days of age, and 30 adult, weighting 349.8 ± 17.4 g at 84 days of age) were assigned to five experimental groups, each group including 12 rats (6 adolescent and 6 adult). The rats were given distilled water or drinking fluids *ad libitum* and were fed a standard chow *ad libitum* with 160 g protein, 28 g fat, 50 g crude fibre, 70 g crude ash in 1 kg of chow, metabolizable energy of the diet is 11 MJ. Lead, cadmium and tannic acid concentration in chow was determined in our laboratory: Pb and Cd by means of the AAS technique in the Varian Spectr AA 880 apparatus, whereas tannic acid by Tyczkowska`s method [20]. Diet contained 54.24 μg of Pb, 5.12 μg of Cd and 3.1 g of tannic acid in 1 kg. The animals were individually housed in polypropylene cages in an environmentally controlled clean air room, with a temperature of ca. 21°C, a relative humidity of ca. 55% and a 12 h light/12 h dark cycle.

Experimental Design

Tannic acid (TA), cadmium chloride and lead acetate were produced by POCH SA (Poland). The rats were given heavy metals or tannic acid as follow: 50 mg Pb per 1 L of distilled water (group Pb); 100 mg Pb per 1 L of distilled water or 2% water-based TA solution (alternately every 7 days) (group Pb/TA); 7 mg Cd per 1 L of distilled water (group Cd); 14 mg Cd per 1 L of distilled water or 2% water-based TA solution (alternately every 7 days) (group Cd/TA). Table 1 presents the methodical system of the experiment and mean consumption of tannic acid (2% solution), lead (as $(CH_3COO)_2Pb$) and cadmium (as $CdCl_2$) from drinking fluids. Based on drinking fluids consumption per animal per week, adolescent rats received 8.28 mg Pb (group Pb), 8.08 mg Pb + 55.97 mL of 2% TA solution (group Pb/TA), 1.15 mg Cd (group Cd) or 1.16 mg Cd + 58.12 mL of 2% TA solution (group Cd/TA). Adult rats received 9.96 mg Pb, 9.66 mg Pb + 48.18 mL of 2% TA solution,

Table 1. Experimental design and mean consumption of tannic acid (TA), cadmium and lead from drinking fluids

Tabela 1. Układ doświadczenia oraz średnie pobranie kwasu taninowego (TA), ołowiu i kadmu z napojami

Experimental groups		Treatment	Consumption of tannic acid (2% solution), mL/week		Consumption of Cd and/or Pb, mg/week		Consumption of Cd and/or Pb, mg/kg BW/day	
			adolescent rats	adult rats	adolescent rats	adult rats	adolescent rats	adult rats
I – control	C	distilled water	–	–	–	–	–	–
II	Pb	50 mg Pb/L distilled water *	–	–	8.28 Pb	9.96 Pb	3.26 Pb	3.37 Pb
III	Pb/TA	100 mg Pb/L distilled water * 2% water-based TA solution (alternately every 7 days)	55.97	48.18	8.08 Pb	9.66 Pb	3.38 Pb	3.24 Pb
IV	Cd	7 mg Cd/L distilled water **	–	–	1.15 Cd	1.15 Cd	0.43 Cd	0.38 Cd
V	Cd/TA	14 mg Cd/L distilled water ** 2% water-based TA solution (alternately every 7 days)	58.12	46.92	1.16 Cd	1.02 Cd	0.49 Cd	0.35 Cd

* as $(CH_3COO)_2Pb$; ** as $CdCl_2$; BW – body weight.
* w postaci $(CH_3COO)_2Pb$; ** w postaci $CdCl_2$; BW – masa ciała.

1.15 mg Cd or 1.02 mg Cd + 46.92 mL of 2% TA solution, respectively. Rats were given lead at about 3.3 mg/kg body weight/day and cadmium in quantities not exceeding 0.5 mg/kg body weigh/day (Tab. 1). At the end of the experimental periods (6 or 12 weeks), animals were put down in CO_2, and they died as a result of their spinal cord being broken. There were six animals in each group (3 adolescent and 3 adult). Hearts (without blood) and lungs were immediately extricated and dissected in iced saline. The tissue samples were put in separate plastic containers.

Chemical Analysis

Portions of hearts and lungs (ca. 3 g) were dried first at a temperature of 103°C for ca. 48 hours, and mineralised in a muffle furnace at 450°C for 16 h. After a complete mineralisation the burnt samples were dissolved in a 10 mL of 0.1 N HNO_3 and transferred into flasks. The content of cadmium and lead was determined by GF AAS technique in the Varian Spectr AA 880 apparatus, including atomisation in a graphite furnace and using the Zeeman background correction. Argon was used as the pure gas. The content of lead was determined at a wavelength $\lambda = 217.0$ nm (LOD 0.011 µg/kg) and cadmium at $\lambda = 228.8$ nm (LOD 0.0001 µg/kg). The deviation of duplicate measurement was below 5%. Quality control of analytical measurements was performed using blank samples and certified reference material (CRM-185R – bovine liver). Mean recovery rate of Pb was 95% and that for Cd was 96%. The spectrometer was calibrated according to the Merck (Germany) standard containing 50 µg/L Pb and 2.5 µg/L Cd. The analyses were performed in three replications.

Statistical Analysis

Data analysis was done by STATISTICA 6.0 computer program. Statistically significant differences were established at the level of $p < 0.05$. The significance of differences between the mean values in the groups was estimated with the use of Duncan's tests.

Results

The Content of Lead in the Rats' Heart and Lungs

In both experimental periods the application of tannic acid (Pb/TA) in adolescent and adult rats resulted in depositing smaller amounts of lead in the studied tissues, compared to group Pb (Fig. 1). The animals' age affected lead concentration in their heart and lungs: the heart of the adolescent rats accumulated more Pb than in the

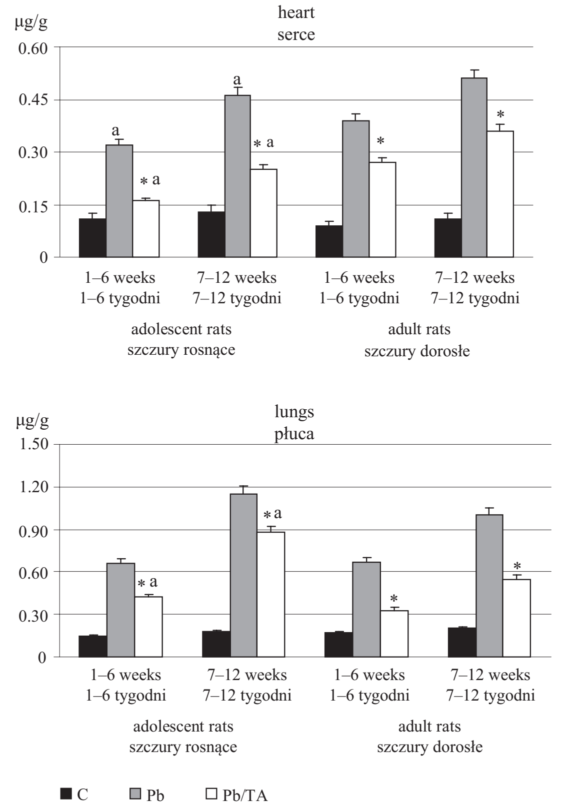

Fig. 1. The distribution of Pb in the hearts and lungs (for 6 and 12 weeks of exposure), mean ± SD, * significant versus Pb ($p < 0.05$); a – significant versus adult rats ($p < 0.05$)

Ryc. 1. Dystrybucja Pb w sercach i płucach (po 6 i 12 tygodniach ekspozycji), wartość średnia ± SD, * istotne wobec Pb ($p < 0,05$); a – istotne wobec szczurów dorosłych ($p < 0,05$)

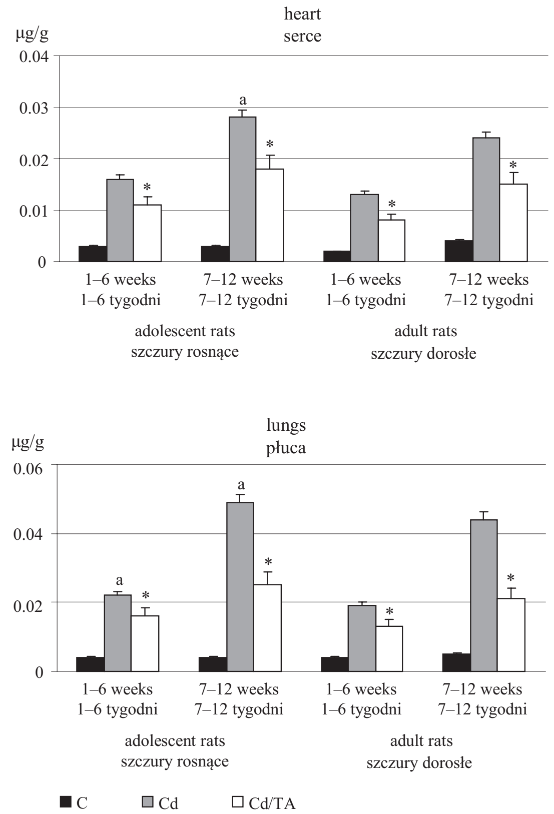

Fig. 2. The distribution of Cd in the hearts and lungs (for 6 and 12 weeks of exposure), mean ± SD, * significant versus Cd ($p < 0.05$); a – significant versus adult rats ($p < 0.05$)

Ryc. 2. Dystrybucja Cd w sercach i płucach (po 6 i 12 tygodniach ekspozycji), wartość rednia ± SD, * istotne wobec Cd ($p < 0,05$); a – istotne wobec szczurów dorosłych ($p < 0,05$)

adult animals. The lungs of both young and adult rats receiving only a solution of lead (group Pb) did not reveal statistically significant ($p = 0.245$) differences in the content of Pb. At the same time it was observed that the lungs of young rats from Pb/TA group contained significantly ($p = 0.012$) more Pb than in the adult rats. It was observed that the lungs accumulated much more Pb than the heart.

The Content of Cadmium in the Rats' Heart and Lungs

The hearts and lungs of the young rats accumulated significantly more Cd than in the adult animals (Fig. 2). Irrespective of the rats' age, it was noted that using TA in the form of a 2% water-based solution administered alternately with a cadmium solution (group Cd/TA) resulted in a statistically significant ($p = 0.009$) reduction of Cd in the heart and lungs, compared to group Cd. The rats' lungs accumulated more cadmium than their hearts.

Discussion

The study aimed at verifying if oral administration of tannic acid could reduce the accumulation of lead and cadmium in the heart of rats subjected to a continuous exposure of toxic metals in low doses (about 3 mg Pb/kg body weight/day and cadmium in quantities not exceeding 0.5 mg/kg body weight/day). So far there has been hardly any comparative data regarding this problem in rats, which are model animal substitutes for humans.

The author's own studies revealed that administering a 2% solution of tannic acid alternately with Pb or Cd to the rats was the most effective method of reducing lead and cadmium content in the animals' heart and lungs. Polyphenol compounds, including tannic acid, have an ability to chelate metallic elements [21]. Tannic acid *in vitro* is a very effective chelator for lead and cadmium [17]. The author's own studies suggest that tannic acid may also chelate Pb and Cd inside the animal's organism. Kim et al. [18] performed research which aimed at determining the effect of tannic acid on the absorption of cadmium in mice. The authors used tannic acid amounting to 0.5 mg/mL, 1 mg/mL and 2 mg/mL with a simultaneous administration of cadmium in a very large dose of 20 mg/kg/day. Both tannic acid and Cd were given orally. The administration of tannic acid resulted in reducing Cd accumulation in the organism of the mice and in the analyzed organs.

The author's own studies showed that the lungs of the rats contained much more Pb and Cd than their heart. Nwokocha et al. [22] observed that the lungs of the rats given water containing 100 ppm of lead acetate revealed significantly more Pb than their heart. Studies performed by Brzóska et al. [23] showed that intoxicating rats with cadmium administered orally at different levels of concentration led to depositing higher amounts of Cd in the lungs than in the heart. It should be noted that the difference increased along with a higher supply of Cd. Lead and cadmium show their toxic effects via depletion of glutathione and bonding to sulphydryl groups of proteins [24]. Proteins, such as metallothioneins and amino acids, are examples of biomolecules, which are able to bind toxic metals in biological matrices. Metallothioneins are induced on exposure to heavy metals. A positive correlation has been observed between Cd content and metallothionein levels in the brain and lungs of monkeys [25]. The synthesis of metallothioneins in the organs of rats drinking water containing 50 ppm Cd was higher in the lungs than in the heart [26]. Moreover, such a higher concentration of Cd and Pb in the lungs can be accounted for by their better blood supply, since as much as 5000 mL of blood per one minute flows through a man's lungs [27], compared to 250 mL of blood in the heart [28].

In the present study, the hearts and lungs of the young rats accumulated significantly ($p < 0.05$) more Cd and Pb than in the adult animals. Satarug et al. [29] found that cadmium absorption and accumulation depend, among others, on animals' age: young individuals tend to absorb more of Cd. Experimental animals studies show that adult animals absorb less toxic metals than young animals [30]. Studies performed by Horiguchi et al. [31] suggest a higher accumulation of Cd in young female Japanese. Oral absorption in children seems to be higher than in adults. Human infants may absorb as much as 50% of Pb from diet, compared with 10% for adults [32]. Because of the not fully developed liver and/or kidneys in the adolescent individuals, the excretion processes are generally poorer than in adults [33, 34]. The faster distribution in both infants and children and a slower elimination are probably the cause. Young animals and infants have a low body weight and lower immunity [35]. Moreover, the activity of most digestive enzymes in young is much lower than in adults, the gastric pH is higher, and the intestinal epithelium is not fully developed [36]. This explains their enhanced susceptibility to the potential effects of lead.

The rats are relevant models for humans. The obtained results may be referred to people. It is necessary to conduct further research in order to confirm the hypothesis that tannic acid, present in numerous food products and primarily in drinks (wine, tea and coffee), used in human diet may reduce the accumulation of cadmium and lead in the tissues and thus weaken their toxicity, which is important regarding our common exposure to heavy metals found in food.

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