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Alteration in bone geometric and mechanical properties, histomorphometrical parameters of trabecular bone, articular cartilage, and growth plate in adolescent rats after chronic co-exposure to cadmium and lead in the case of supplementation with green, black, red and white tea

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ABSTRACT

Adolescent male Wistar rats were used to check whether regular consumption of black, red, white, or green tea would have a protective effect on femur development during 12-week exposure to Cd and Pb (7 mg Cd and 50 mg Pb in 1 kg of the diet). The animals were randomly divided (n = 12) into a positive control (without Cd, Pb and teas), a negative control group (Cd and Pb), and groups supplemented additionally with green (GT), black (BT), red (RT), and white tea (WT). Heavy metals reduced the geometric and densitometric parameters and the total thickness of articular cartilage irrespective of tea administration and influenced mechanical endurance, growth plate thickness, and trabecular histomorphometry depending on the tea type. It is difficult to indicate which tea has the best protective effects on bone and hyaline cartilage against heavy metal action.

1. Introduction

Besides water, tea is the most frequently consumed beverage in the world (Wu and Wei, 2002). Each type of tea has its own characteristics. Black and green teas are the most frequently drunk varieties with different taste provided by polyphenols (Hilal and Engelhardt, 2007). The presence of antioxidants in tea, mainly polyphenols, determines its healthy properties and classifies it as a functional food product (Wu and Wei, 2002). Among polyphenols, tannic acid is one of the strongest antioxidant naturally occurring in plant products (Gülçin et al., 2010). Tannins are used in medicine primarily due to their strong properties, including the possibility of binding toxic metals as well as inhibition of absorption and intensification of excretion thereof from the body (Kim and Rhee, 1994; Khalaf et al., 2012). This is especially important in the case of the heavy metal contamination of the human environment, including food, which is a global issue (EFSA, 2012a,b).

Cadmium (Cd) and lead (Pb), i.e. toxic metals with a biological half-life of 10–30 years within the organ system, occur commonly in the human environment (Winiarska-Mieczan, 2013). Lead is widely distributed in nature (soil, water, food, and air) as a contaminant, because metallic lead and its oxides are extensively used in industry. Lead can accumulate in the human body when taken up

Abbreviations: A, the cross-section area; BMD, bone mineral density; BT, black tea; BV, the bone volume; BV/TV%, the relative bone volume; Ca, calcium; Cd, cadmium; CI, cortical index; CRT B A, cortical bone area; CRT B CNT, cortical bone mineral content; CRT B DEN, cortical volumetric bone mineral density; GT, green tea; IGF-I, insulin-like growth factor I; MRWT, mean relative wall thickness; Pb, lead; pQCT, peripheral quantitative computed tomography; RT, red tea; TA, tannic acid; TV, tissue volume; Tb.Th, trabecular thickness; Tb.Sp, trabecular separation; TOT B A, total bone area; TOT B CNT, total bone mineral content; TOT B DEN, total bone mineral density; TRAB A, trabecular bone area; TRAB B CNT, trabecular bone mineral content; TRAB B DEN, trabecular volumetric bone mineral density; WT, white tea; WHO, World Health Organization.

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mainly from drinking water, cigarette smoke, and via oral exposition with food, where it is typically present in an inorganic form as salts, oxides, and sulfides. After absorption from the gastrointestinal tract, it is transported first to the liver, where it accumulates, next to kidney, heart, and brain, and later to the muscle or bone tissue from which it can be released. Lead in bone has the ability to substitute calcium ions, accounting for accumulation in this tissue of 95% of total lead contained in the organism.

Cadmium is also commonly present in nature. Due to weathering, this element is released to the environment (Hogervorst et al., 2007). Like lead, cadmium gets into the environment mainly as a result of human activities. Moreover, vegetables, cereals, and fruits contain the largest quantities of Cd since it is rapidly absorbed by plants from soil. In humans, the rate of Cd absorption from the small intestine is approx. 5% of the amount present in the diet. Cadmium accumulates gradually in the human body, where it gives rise to a number of adverse health effects, especially nephrotoxicity and bone damage (WHO, 1992; Staessen et al., 1999; Chen et al., 2011).

Based on our results, it is clear that long-term exposure of adult rats to Cd and Pb levels comparable to those encountered in either occupational or environmental exposures of humans causes a toxic effect, especially in the liver, and can change the structure of intestinal mucosa, irrespective of tea administration (Tomaszewska et al., 2015a,b).

Additionally, the lack of knowledge of the influence of tea on the mineralization and functional adaptation of the vertebrate skeleton exposed to heavy metals during development motivated us to determine the influence of different tea extracts on bone tissue properties in rats co-exposed to Cd and Pb.

2. Materials and methods

2.1. Ethics statement

The experimental procedures used throughout this study were approved by the Local Ethics Committee on Animal Experimentation of the University of Life Sciences of Lublin, Poland. All experiments complied with the Guiding Principles for Research Involving Animals. All efforts were made to minimize the number of animals used as well as their suffering.

2.2. Animals and basal diet

Adolescent 6-week-old male Wistar rats (n = 72) weighing 205.6 ± 12.1 g were used. All the animals were weighed and observed in individual polypropylene cages (the dimensions of 380 × 200 × 590 mm) and acclimated in the laboratory for 7 days. The rats were kept in a room at a temperature of 21 ± 3 °C, 55 ± 5% humidity, and a 12 h: 12 h day/night cycle. The rats had free access to water. The animals were randomly divided into a positive control group (the PC group; not treated with Cd, Pb, and teas; n = 12), the Cd + Pb group as a negative control (the NC group; co-exposed to Cd and Pb; n = 12), and four groups subjected to the supplementation with the different tea extracts as well as Cd and Pb – green tea (GT; n = 12), black tea (BT; n = 12), red tea (RT; n = 12), and white tea (WT; n = 12) (Fig. 1). The rats from the PC group were fed ad libitum with common laboratory animal feed, whereas the rats co-exposed to Cd and Pb were fed with common laboratory animal feed mixed with 7 mg Cd/kg and 50 mg Pb/kg. Standard solutions of Cd (1000 mg Cd as $CdCl_2$/1 l H_20) and Pb (1000 mg Pb as $Pb(NO_3)_2$/1 l H_20) were purchased from Merck (Germany). The rats were weighted every 7 days. The weekly intake of feed provided the basis for the calculation of the average weekly supply of Cd and Pb (Table 1). Fresh feed was provided every 7 days. The feed was prepared in the laboratory by adding water-based solutions of Cd (as $CdCl_2$) and Pb (as $(CH_3COO)_2Pb$) to ground standard feed, which was later carefully mixed and granulated by mechanical methods (water was deionized in the Hydrolab (Poland, Gdańsk) apparatus.). The level of the metals supplied in the feed (7 mg for Cd/kg and 50 mg for Pb/kg) was calculated to ensure that the daily supply of Cd and Pb did not exceed the environmental exposure of humans (Winiarska-Mieczan, 2013; Tomaszewska et al., 2015a,b).

After 12 weeks the rats were starved for 24 h, weighted and euthanized by CO_2 inhalation. Immediately after euthanasia, blood was sampled directly from the rats' heart into 6 ml Vacutest vacuum tubes with heparin-Li as a coagulant. Left femora were isolated.

2.3. Preparation of tea solutions

The teas used in the experiment were purchased from a commercial source. The infusions of black tea (India, Lipton), green tea (China, Lipton), red tea (China, Lipton), and white tea (China, Lipton) were prepared in the manner described previously (Winiarska-Mieczan, 2013; Tomaszewska et al., 2015a,b). Fresh drinking solutions were provided every two days.

2.4. Determination of the content of Cd and Pb in bone

Bone samples (approx. 3 g) were dried at a temperature of 103 °C for 24 h. Afterwards, they were subjected to combined mineralization in a muffle furnace: the samples were dry-mineralized at 450 °C for 12 h. The resulting ash was mixed with 2 ml of hydrogen peroxide, vaporized to dryness, and re-burnt at 450 °C for 12 h. The procedure was repeated four times. The resulting ash was dissolved in 10 ml of 1 M HNO_3. Nitric acid HNO_3 and hydrogen peroxide H_2O_2 were purchased from Poch S.A. (Poland). The content of Cd and Pb was determined by AAS in Varian SpectrAA 880 (Varian, Santa Clara, CA, USA) as described previously (Tomaszewska et al., 2015a,b). The quality of measurements was verified using blank determination and certified reference material CRM–185 R (bovine liver). The apparatus was calibrated according to the Merck standard (Merck, Darmstadt, Germany). The method described above is based on that reported previously (Winiarska-Mieczan, 2013).

2.5. Bone analysis

The bone length and weight were measured after removal of soft tissues from left femora. Each bone was wrapped in gauze soaked in isotonic saline and stored at −25 °C for further analysis.

2.5.1. Geometric parameters

Geometric properties such as the cross-section area (A), mean relative wall thickness (MRWT), and cortical index (CI) were estimated on the basis of horizontal and vertical diameter measurements of the mid-diaphyseal cross-section of bone using method described previously (Ferretti et al., 1993; Tomaszewska et al., 2015c).

2.5.2. Mechanical properties

The mechanical properties of the femur were determined for bones after 3-h thawing at room temperature using the three-point bending test. The mechanical properties were examined on a Zwick Z010 universal testing machine (Zwick GmbH & Company KG, Ulm, Germany) equipped with a measuring head (Zwick GmbH & Company KG, Ulm, Germany) with an operation range up to 10 kN, linked to a computer with TestXpert II 3.1 software (Zwick GmbH & Company KG, Ulm, Germany) registering the relationship between force perpendicular to the longitudinal axis of the bone and the resulting displacement. The distance between the supports was set at 40% of the total bone length. The measuring head loaded bone samples at a constant speed of 10 mm/min. The ultimate strength