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The lack of protective effects of tea supplementation on liver and jejunal epithelium in adult rats exposed to cadmium and lead

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ABSTRACT

Adult rats at the age of 12 weeks were divided into the control group and groups supplemented with green (GT), black (BT), red (RT), or white (WT) tea extracts. The diet (except that for the control) was mixed with 7 mg Cd/kg and 50 mg Pb/kg. The experiment lasted 12 weeks. Basal haematology and plasma biochemical parameters as well as the histomorphometrical parameters of jejunal epithelium and liver were determined. The lowest body mass was found in the RT and WT groups. Some functional (increased plasma ALT and AST, and the de Ritis coefficient) and structural changes in the liver (slight fatty degenerative changes, an increase in the intercellular space) were evident irrespective of the type of tea in the Cd and Pb poisoned rats. This toxic effect was visible especially in rats drinking black or red tea. However, the rats had no elevated LDH and ALT activities. The highest content of Cd and Pb in the liver and blood plasma was found in rats drinking red tea. Based on the results obtained, it is clear that long-term exposure of adult rats with a mature intestinal barrier to Cd and Pb contamination, under higher exposure conditions than the current estimates of weekly exposure of the general population to Cd and Pb through diet, causes a toxic effect, especially in the liver, and can change the structure of intestinal mucosa, irrespective of tea administration.

1. Introduction

Tea is the most commonly consumed beverage in the world. The economic and social interest in tea is obvious since approximately 18–20 billion cups of tea are consumed daily worldwide. Black and green teas are the most popular types. The four major types of tea, i.e. green, black, white, and red tea, differ in terms of production methods. Each type of tea has its own characteristics including a different taste and different health benefits. The chemical composition of tea is complex and includes alkaloids (caffeine, theophylline, and theobromine), amino acids, carbohydrates, proteins, chlorophyll, volatile compounds, minerals, and trace elements. Polyphenols are the main bioactive molecules in tea. The major polyphenolic compounds in tea include catechins, which are present in large amounts in green tea; therefore, the antioxidant capacity of green tea is greater than that of other types (Lee et al., 2002).

The tea beverage is considered a medicine because of its polyphenol content. Research on the effects of tea on human health is an effect of the growing need to provide naturally healthy diets that include plant-derived polyphenols (McKay and Blumberg, 2002). In some studies, tea is associated with antiallergic action and antimicrobial properties (Maeda-Yamamoto, 2013; Soichiro and Akikazu, 2002). Further studies have associated consumption of tea with a lower risk of several types of cancer including stomach, oral cavity, oesophagus, and lung malignancies (Chung et al., 2003). Therefore, tea appears to be an effective chemopreventive agent for toxic chemicals and carcinogens. Moreover, it has antioxidant properties that are even much stronger than vitamins E or C (Flora, 2002).

Free radicals are constantly generated due to radiation, chemicals, toxins, physical stress, and the oxidation process of drugs, food, and environmental pollutants. Cadmium (Cd) and lead (Pb) are non-functional heavy metals placed under the category of environmental pollutants due to their toxic effects on plants, animals, and

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; Cd, cadmium; DAB, 3′3′-diaminobenzidine tetrahydrochloride; LDH, lactate dehydrogenase; Hb, haemoglobin concentration; HT, haematocrit; Pb, lead; RBC, the number of red blood cells; TA, tannic acid; WBC, the number of white blood cells; WHO, World Health Organization.

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humans. A continuous intake of low amounts of heavy metals over a long period causes metal accumulation and damage to all organs such as kidney and liver. Both these heavy metals occur naturally in soils, especially in urban and industrial locations. For this reason, Cd and Pb are extensively studied and their effects on human health are regularly reviewed. Dietary Cd intakes vary widely in different countries. In European countries, the dietary Cd intake estimates are between 1.5 and 2.23 μg/kg b.w. per week (EFSA, 2012a), and human exposure to dietary Pb ranges from 2.8 to 4.13 μg/kg of body weight per week (EFSA, 2012b). However, this intake is growing in smokers or people living near heavy metal-polluted areas including high- or moderate-exposure groups (Satarug and Moore, 2004; Kaczmarek-Wdowiak et al., 2004). The doses used in our study are higher than the current estimates of weekly exposure of the general population to Cd and Pb through diet, but are still lower than in smokers or heavy metal-polluted areas.

This study examines the effect of different tea extracts on the jejunum and liver ultrastructure and haematological parameters in adult rats in the case of exposure to dietary Cd and Pb within the limits of human environmental exposure to these elements.

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 University of Life Sciences of Lublin, Poland. The experiment complied with the Guiding Principles for Research Involving Animals. All the efforts were made to minimize the number of animals used as well as their suffering.

2.2. Animals and basal diet

The experiment lasted 12 weeks. Sixty adult 12-week-old male Wistar rats weighing 425.1 ± 9.6 g were used. All the animals were weighed and observed in individual polypropylene cages (the dimensions of 380 mm × 200 mm × 590 mm) and acclimated in the laboratory for 7 days. The rats were kept in a room at a temperature of 21 ± 3 °C and 55 ± 5%, humidity. A 12:12 h day cycle was maintained. The rats had free access to water and were fed ad libitum with common laboratory animal feed mixed with 7 mg Cd/kg and 50 mg Pb/kg. The rats were weighed every 7 days. The weekly intake of feed provided the basis for the calculation of the average weekly supply of Cd and Pb. 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. The level of 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 including the high-exposure group (Tomaszewska et al., 2015; Winiarska-Mieczan, 2013).

The animals were randomly divided into the control group, used as a reference group (without Cd, Pb, and teas; $n = 12$), and four groups exposed to Cd and Pb and supplemented with different tea extracts – green tea (GT; $n = 12$), black tea (BT; $n = 12$), red tea (Puerh; RT; $n = 12$), and white tea (WT; $n = 12$).

Fresh drinking tea solutions were provided every two days. After 12 weeks, adult rats were starved for 24 h and weighed. Afterwards, the rats were euthanized by CO_2 inhalation. Immediately after euthanasia, blood was sampled directly from the heart into 6 mL Vacutest vacuum tubes with heparin-Li as a coagulant. The liver was isolated and weighed as well. Relative liver mass (%) was calculated as a ratio of liver weight and body weight.

2.3. Preparation of tea solutions

The teas used in the experiment were purchased from a commercial source tea and provided to the rates instead of drinking water, which the control group received. The brews of black tea (India, Lipton), green tea (China, Lipton), red tea (China, Lipton), and white tea (China, Lipton) were prepared in a manner described previously (Tomaszewska et al., 2015; Winiarska-Mieczan, 2013). The tea brew was divided into 30 mL samples, which were frozen at −20 °C. Each time the samples were used for making experimental solutions. After thawing, they were mixed with distilled water at room temperature in a proportion ensuring a 2% content of tannic acid (TA) (Winiarska-Mieczan, 2013; Tomaszewska et al., 2015).

2.4. Determination of the TA and total polyphenol content in tea

The content of TA in the tea was determined using the spectrocolorimetric method described in a previous article (Winiarska-Mieczan, 2013). The content of total polyphenols was determined as described previously (Tomaszewska et al., 2015). Total phenols were expressed as tannic acid equivalents.

2.5. Determination of the content of Cd and Pb in liver

Liver 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. The content of Cd and Pb was determined by AAS in a Varian SpectrAA 880 (Santa Clara, CA, USA). The quality of the measurements and method were described previously (Tomaszewska et al., 2015; Winiarska-Mieczan, 2013).

2.6. Haematological and plasma biochemical analyses

Blood samples were collected carefully for haematological and blood plasma biochemical analyses using standard venipuncture of the heart. The haematological analyses were performed with the use of an automatic haematological analyser MS9 (MELET SCHLOESING Laboratories, France). The numbers of white and red blood cells (WBC and RBC), haemoglobin concentration (Hb), and haematocrit (HT) were determined.

The plasma was immediately separated by centrifugation. Total protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) were determined by the colorimetric method using a Metrolab 2300GL random-access biochemical analyser (Metrolab SA, Buenos Aires, Argentina) and tests by BioMaxima (Lublin, Poland). Based on both AST and ALT activities, the relationship between AST and ALT was calculated as the de Ritis coefficient. Normally, this ratio is greater than one; when it falls below 0.9 or is markedly enhanced, it suggests liver injury (Śliwa et al., 2009; Cohen and Kaplan, 1975). Moreover, Fe, Pb, and Cd concentrations and total protein were determined. The concentration of Fe was determined by means of ready-made sets produced by BioMaxima (Lublin, Poland). The concentration of Cd and Pb were determined using the AAS method in a Varian SpectrAA 880 (Santa Clara, CA, USA) as described previously (Tomaszewska et al., 2015). For the analysis of Cd and Pb levels, the plasma was diluted with deionized water (Hydrolab, Gdansk, Poland) at a ratio of 1:10.