Abstract

The aim of this study was to investigate the accumulation of cadmium (Cd) in the kidneys and its excretion with urine, and possible effect of benzo(a)pyrene (B(a)P) and pyrene on these processes in rats. The studies were conducted on 60 Wistar male rats by short-term (28 d) and subchronic (90 d) experiments. Cadmium was given at doses 0.1 and 0.5 mg/kg. The samples of urine and kidneys were analysed by atomic absorption spectrophotometry. The obtained data showed that Cd in a dose of 0.5 mg/kg significantly increased its renal and urine content; however, the lower dose (0.1 mg/kg) administered during 28 d did not increase Cd urinary excretion levels. This indicates that Cd has a tendency to accumulate in the organism (in kidneys in particular), and when absorbed, is very slowly eliminated from the organism. In short term experiments both doses of Cd increased its accumulation in the kidneys. B(a)P (dose 0.00015 mg/kg) in 28 d and 90 d studies, and pyrene (dose 0.00075 mg/kg) in 90d studies had no effect on the accumulation of Cd in the kidneys and excretion with urine.

Key words: rats, cadmium, benzo(a)pyrene, pyrene, accumulation.

Cadmium (Cd) is an environmental pollutant, the presence of which significantly increased because of its widespread industrial use. It is known to damage a number of tissues (1–3, 10–13). Cd occurs in the air, water, plant, and animal tissues. The inhalation or absorption of Cd from various sources may lead to its accumulation in the human body (4, 14, 17). For the general population that is mainly exposed by oral and inhalation routes, the kidneys are critical organs, in which the first adverse effects of Cd occur. One of the earliest signs of Cd nephropathy is proteinuria, resulting from a decreased tubular reabsorption of low-molecular-weight proteins (15). The soluble salts of Cd accumulate in the organism and affect toxically not only the kidneys, but also the liver, brain, lungs, testicles and central nervous system (8, 15). Most of the absorbed Cd is accumulated in the liver, the rest of it being distributed in other tissues (20, 21). Cd is known to be a carcinogen; however, the possible mechanism of the carcinogenesis, with regards to the activation and inactivation of cancer related genes, has not yet been fully elucidated (16). Cd and polycyclic aromatic hydrocarbons (PAHs): benzo(a)pyrene (B(a)P) and pyrene are widely spread chemical environment contaminants (14, 17); therefore they were selected for the investigation. These three chemical agents meet the following criteria: Cd and PAHs (B(a)P and pyrene) are in different chemical classes, each of them has high environmental relevance and a potential effect on human health. They have the similar pollution sources and ways of entering into the human organism (5, 20).

In the present study the cadmium accumulation in the kidneys and its excretion with urine, and the possible influence of benzo(a)pyrene and pyrene on these processes, were investigated.
Material and Methods

The studies were conducted on 60 Wistar male rats, 6-7 weeks of age and 120 ±20 g of initial body weight. The animals were kept under standard laboratory conditions. They were allowed to acclimatize to the laboratory conditions for 4 d before the experiments began. The animals were treated in accordance with the law of the Lithuania Republic and the Guide for the Care and Use of Laboratory Animals.

The control groups (I, VII) were given drinking water *ad libitum*. The experimental groups (II, III, IV) were given cadmium chloride (CdCl₂, Fluka): 0.1 mg/kg for 28 d and 90 d, and 0.5 mg/kg for 28 d. The doses corresponded to 100 or 500 of the average daily intake per man (ADI). Groups V, VI, and VIII were fed: 0.1 mg/kg of Cd+0.00015 mg/kg of benzo(a) pyrene (B(a)P) for 28 d, 0.1 mg/kg of Cd+ 0.00015 mg/kg of B(a)P for 90 d, and 0.5 mg/kg of Cd+0.0015mg/kg of B(a)P for 28 d; respectively. B(a)P dose corresponded 100 or 1000 ADI per man. Group IX was feed 0.1 mg/kg of Cd+ 0.00075 mg/kg of pyrene for 90 d. Pyrene dose corresponded to 100 ADI. Group X was fed 0.1 mg/kg of Cd+0.00015 mg/kg of B(a)P+0.00075 mg/kg of pyrene.

The tested compounds were administered *per os* by gavages (5 d per week). The amount of cadmium in the kidney and urine samples was measured with an atomic absorption spectrophotometry SIMAA (Perkin Elmer, Germany). The urine was collected during the 24 h period according standard methods. No the presence of Cd was found in drinking water and the amount of Cd in feed was 0.025 mg/kg.

Results

The obtained data showed that with the increasing Cd dosage, its renal content considerably increased. When the dose was increased five times during 28 d (from 0.1 up to 0.5 mg/kg), Cd levels in the kidneys increased from 0.384 ±0.02 µg/g to 3.87 ±0.40 µg/g as compared to control (0.044 ±0.01 µg/g), (P<0.001) (Table 1).

In the short term experiment (0.5 mg/kg for 28 d), Cd levels in the renal cortex did not attain the critical level (200 µg/g) and could reach about 6 µg/g, indicating that the renal tubuli were not yet damaged and renal dysfunction had not manifested. When the rats were administered 0.1 mg/kg of Cd for 28 d or 90 d, Cd urinary excretion levels did not increase. When the animals were given a higher dose (0.5 mg/kg of Cd, 28 d), urinary excretion of Cd increased 3.6 times.

Histological investigation of the kidneys in the subchronic experiment revealed no significant impairment; the renal corpusculi and epithelium of cortical tubuli were thin, vacuolized, with disintegrated parts of the cytoplasma occurring in some parts of their cavity.

Discussion

With prolonging the exposure to Cd, its level in the kidneys exceeds that in the liver (6, 18). Cd is eliminated from the body very slowly and in almost equal parts with the faeces and urine. The most characteristic feature of Cd-induced renal tubular dysfunction is an increased urinary excretion of β 2–microglobulins, low molecular weight proteins. Cd is not harmful to the kidneys as long as its level in the renal cortex does not exceed the critical level of 200 ppm, i.e. 200 µg/g or 200 mg/kg (4). The presence of the critical level in the renal cortex in 10% of humans induces the renal dysfunction. Cd levels in the renal cortex are about 1.5 times higher than those in the kidneys.

Table 1

The effect of exposure to Cd on its accumulation in the kidneys and excretion with urine, and possible influence of benzo(a)pyrene (B(a)P) or pyrene (P) on these processes

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg)</th>
<th>Quantity of Cd in kidneys (µg/g)</th>
<th>Quantity of Cd in urine (µ/l)</th>
<th>Test duration (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Control (water)</td>
<td>-</td>
<td>0.044 ± 0.01</td>
<td>33.82 ± 8.49</td>
<td>28</td>
</tr>
<tr>
<td>II. Cd</td>
<td>0.1</td>
<td>0.384 ± 0.02***</td>
<td>49.81 ± 26.44</td>
<td>28</td>
</tr>
<tr>
<td>III. Cd</td>
<td>0.5</td>
<td>-</td>
<td>19.88 ± 3.86</td>
<td>90</td>
</tr>
<tr>
<td>IV. Cd</td>
<td>0.1 + 0.00015</td>
<td>3.870 ± 0.40***</td>
<td>118.20 ± 18.82**</td>
<td>28</td>
</tr>
<tr>
<td>V. Cd + B(a)P</td>
<td>0.311 ± 0.03***</td>
<td>30.15 ± 11.16</td>
<td></td>
<td>28</td>
</tr>
<tr>
<td>VI. Cd + B(a)P</td>
<td>0.5 + 0.0015</td>
<td>3.870 ± 0.35***</td>
<td>184.33 ± 54.40*</td>
<td>28</td>
</tr>
<tr>
<td>VII. Control (water)</td>
<td>-</td>
<td>23.01 ± 6.28</td>
<td>-</td>
<td>90</td>
</tr>
<tr>
<td>VIII. Cd + B(a)P</td>
<td>0.1 + 0.00015</td>
<td>-</td>
<td>30.63 ± 3.22</td>
<td>90</td>
</tr>
<tr>
<td>IX. Cd + P</td>
<td>0.1 + 0.00075</td>
<td>-</td>
<td>16.99 ± 4.26</td>
<td>90</td>
</tr>
<tr>
<td>X. Cd + B(a)P + P</td>
<td>0.1 + 0.00015</td>
<td>-</td>
<td>30.79 ± 3.54</td>
<td>90</td>
</tr>
</tbody>
</table>

Significant difference compared to the control: *P < 0.05; **P < 0.01; ***P < 0.001.
The critical level of Cd in the renal cortex (200 μg/g) is confirmed by the experimental data: Proteinuria accompanied by histopathological changes in the kidneys is evoked in rats at Cd doses of 1.2 mg/kg per os. For Cd detoxication, of particular significance is the low molecular weight metal-binding protein - metallothionein. Bound by the metallothionein synthesized in the liver, Cd is transported to the kidneys, filtered in the corpuscles and reabsorbed from the proximal tubular filtrate (when its quantity is not too large) or excreted with urine. Exogenous metallothionein is decomposed in the lysosomes. In the cases of degeneration and atrophy of the renal proximal tubuli, a relation between Cd levels in the blood, urine, and renal cortex has been found to change: Cd excretion with urine significantly increases, thus decreasing Cd and renal cortex has been found to change: Cd excretion in the kidneys is evoked in rats at Cd doses of 1.2 mg/kg accompanied by histopathological changes in the renal proximal tubuli (57x589). In the polluted areas the dose is ten times higher (150–200 μg/g) is confirmed by the experimental data: Proteinuria (150–200 µg) (2).

Benzo(a)pyrene, belonging to the group 2A carcinogenic factors (11), administered per os to rats is absorbed in their organism by 38-58%, pyrene absorption reaches 65-84% (19). B(a)P and pyrene are distributed in the hepatic, pulmonary, and renal protein fractions. B(a)P in the liver is decomposed into a number of metabolites; a part of them, with the help of glutathione-S-transferase, is excreted from the organism with the faeces and urine; a part of them is excreted into the bile and circulates in the enterohepatic circle.

The single studies show the ability of Cd and B(a)P to interact in all xenobiotic metabolism phases. In some cases, Cd acts as a genotoxicity bioactivator of this organic carcinogen (9), while in other cases, it acts as an inhibitor. Studies with Escherichia coli failed to show that PAHs cause derangements in the mechanism of action of metallothionein, which plays a basic role in Cd detoxication, whereas the studies with fish showed that B(a)P can suppress metallothionein synthesis. Due to this, Cd toxicity is increasing. There is no excretion from the organism; however, the single studies show that B(a)P can reduce Cd excretion with the bile: a relation has been found between an increased activities of glutathione-S-transferase in the rat liver under the effect B(a)P and a suppressed Cd excretion to the bile.

To highlight Cd accumulation in the kidneys and the excretion with urine, in our studies the animals were exposed to Cd alone and to its combination with B(a)P and/or pyrene. The results showed that B(a)P administered for 28 d did not change Cd accumulation in the kidneys (compared to Cd group). With increasing Cd doses, it increased from 0.311 ± 0.03 µg/g to 3.87 ± 0.35 µ/g vs control (0.044 ± 0.01 µg/g), (P < 0.001).

Cd levels in the rats’ urine did not increase when Cd + B(a)P, Cd + pyrene and Cd + B(a)P + pyrene combinations were given for 28 d and 90 d (100 ADI). Cd levels in the urine increased 5.6 times vs control, when the rats were administered Cd (0.5 mg/kg) + B(a)P (0.0015 mg/kg) for 28 d (500 and 1000 ADI). It is possible to conclude that the tested doses of B(a)P, pyrene, and their combinations given for 28 d and 90 d exerted no effect on Cd urinary excretion. B(a)P (0.00015 mg/kg) combined with respective Cd doses 0.1 mg/kg, increased Cd urinary excretion by 54.1%, Cd + B(a)P + pyrene to 55.9%, as compared with the respective Cd groups, although the increase was not statistically significant. No tendency of such increase was observed under the effect of Cd + pyrene complex. This tendency of an enhanced urinary excretion of a toxic metal can be partly explained by the data of the other authors, who showed the possibility B(a)P to suppress Cd excretion into the bile (7).

In a subchronic experiment, the histological examination of the kidneys revealed no renal damage by Cd + B(a)P and Cd + pyrene combinations; the structural components of the renal cortex and core showed no visible changes and did not differ from the control. Subchronic 100 ADI injured the proximal cortical tubuli which were partly decomposed, their cross-section was uneven, whereas the renal corpusculi and cortical tubuli showed no changes.

In the group of the animals, which was given Cd at the dose of 0.5 mg/kg, its excretion increased. The use of 0.1 and 0.5 mg/kg doses increased the accumulation of Cd in the kidneys. In conclusion, the obtained results showed that benzo(a)pyrene and pyrene, given in the combination with cadmium, had no influence on the accumulation of Cd in the kidneys and on its excretion with urine.

References


