SHORT COMMUNICATION

Acrylamide-Induced Changes in Femoral Bone Microstructure of Mice

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Summary

Acrylamide (AA) is one of the most common toxins in foods. Its effect on bone microstructure has not been investigated. The aim of our study was to analyze the impact of acute exposure to AA on femoral bone microstructure in mice. Adult animals were treated perorally with 2 doses of AA (E1 group, 1 mg/kg b.w.) in a 24-h period and with 3 doses of AA (E2 group, 1 mg/kg b.w.) in a 48-h period. Mice exposed to AA had smaller sizes of primary osteon’s vascular canals. Secondary osteons were significantly smaller in mice from E2 group; however their increased number (from 38 % to 77 %) was identified in both E1 and E2 groups. In these groups, a higher number of resorption lacunae (from 100 % to 122 %) was also found. The values for bone volume, trabecular number were increased and that for trabecular separation was decreased in mice administered AA. Significantly higher value of bone surface was observed in mice from E1 group whereas trabecular thickness was increased in E2 group. The effect of AA on microstructure of compact and trabecular bone tissues is different. In our study, one dose of AA was used and acute effects of AA were investigated. Therefore, further studies are needed to study mechanisms by which AA acts on bone.

Key words

Acrylamide • Bone • Mouse • Microstructure

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Acrylamide (AA, CAS Reg. No. 79-06-1), α,β-unsaturated reactive molecule, is an odorless crystalline solid (Erikson 2005, Parzefall 2008, Wei et al. 2014). Besides its utilization in industry, AA is a contaminant in foods prepared during high-temperature cooking (Pedreschi et al. 2014) like fried potatoes, bakery products, breakfast cereals and coffee. It can also be absorbed across the skin (Li et al. 2015).

There are many reports demonstrating various toxic effects of AA in experimental animals (Takami et al. 2012), including reproductive toxicity (Park et al. 2010), genotoxicity (Li et al. 2015) and neurotoxicity (Tyl et al. 2000). AA is a substance classified as a probable human carcinogen (Mucii and Wilson 2008).

Although AA belongs to the most commonly consumed toxins by humans, its effect on bone microstructure is still unknown. Therefore, our study was aimed to determine femoral bone microstructure of adult mice after an acute peroral exposure to AA.

In our experiment, twelve clinically healthy 12-week-old Swiss mice (males) were used. The animals were obtained from the accredited experimental laboratory of the Pedagogical University in Cracow. Adult mice were randomly divided into three experimental groups of 4 animals each. In E1 group
males were treated perorally with 2 doses of AA (1 mg/kg b.w.) in a 24-h period. In E2 group mice received 3 doses of AA (1 mg/kg b.w.) during a 48-h period. The dose of AA was chosen on the basis of the experiment conducted by Doerge et al. (2005) and our previous experiences (Kopatska et al. 2015). AA was dissolved in physiological saline. The third group without AA administration served as a control one (group C). All the applied procedures were approved by the First Local Ethic Committee on Experiments on Animals in Cracow (resolution number 175/2012).

Three hours after the last dose of AA, all mice were killed and their femora were used for microscopical analyses. Thin sections from femora (70-80 μm) were prepared according to the methodology of Martiniaková et al. (2008). The qualitative histological characteristics of the compact bone were determined according to the internationally accepted classification systems of Enlow and Brown (1956) and Ricqlès et al. (1991). The quantitative (morphometrical) parameters of the compact bone were assessed using the software Motic Images Plus 2.0 ML (Motic China Group Co., Ltd., Xiamen, China). We measured area, perimeter, maximum and minimum diameters of the vascular canals of primary osteons, Haversian canals and secondary osteons in all views (anterior, posterior, medi[al], lateralis) of thin sections.

Morphometrical characteristics of the trabecular bone and cortical bone thickness were determined using microCT system (μCT 50, Scanco Medical, Brüttisellen, Switzerland). μCT studies were performed on the distal femora to image trabecular bone morphometry. Trabecular bone was analyzed in a region of interest starting 1.2 mm from the end of the growth plate and extending 1.5 mm. Following parameters were measured: bone volume, trabecular number, trabecular thickness, trabecular separation and bone surface. Cortical bone thickness was analyzed in a region of interest starting 5.2 mm from the end of the growth plate and extending 1.5 mm at femoral midsh[ft].

Statistical analysis was performed using SPSS 8.0 software. All data were expressed as mean ± standard deviation. The unpaired Games-Howell's and/or Tukey's tests were used for establishing statistical significance (P<0.05) among all groups.

Endosteal surfaces of femora in mice from the C group consisted of non-vascular bone tissue (in anterior, medi[al] and lateralis views), which contained cellular lamellae and osteocytes. In pars posterior, primary vascular radial bone tissue was observed. This tissue included vascular canals (branching or non-branching) radiating from the marrow cavity. In the middle parts of the compact bone (mainly in anterior and lateralis views), a few primary osteons and several secondary osteons were identified. Non-vascular bone tissue was found only in pars medialis. Finally, the periosteal border was composed of non-vascular bone tissue (Table 1). These results are in accordance to those of other researchers (Treuting and Dintzis 2011, Enlow and Brown 1956, Reim et al. 2008).

In our study, differences in compact bone microstructure of mice from the E1 and E2 groups were identified. Mice from these groups had more intact secondary osteons (from 38 % to 77 %) in the middle part of the compact bone. In addition, more resorption lacunae (from 100 % to 122 %) were found in pars anterior of their periosteal border (Table 1).

The changes mentioned above can be explained as an adaptive response of the compact bone to AA toxicity. The results by Raju et al. (2015) showed that AA increases the oxidative stress and peroxidation of the lipids. Oxidative stress is characterized by an increased level of reactive oxygen species, which enhance bone resorption (Bai et al. 2005) and osteoclastogenesis (Garret et al. 1990). Also, a biochemical link between increased lipid peroxidation and reduced bone mineral density has been described (Basu et al. 2001).

In total, 353 vascular canals of primary osteons, 54 Haversian canals and 54 secondary osteons were measured. The results are summarized in Table 1. All measured variables (area, perimeter, maximal and minimal diameters) of the primary osteon's vascular canals were significantly decreased (P<0.05) in groups E1 and E2 when compared to the C group. Significant differences were also found between E1 and E2 groups. Haversian canals values did not differ significantly among all groups. On the contrary, the values of the secondary osteons were significantly smaller (P<0.05) in mice from the E2 group. Significant differences were also demonstrated between the E1 and E2 groups.

Vasoconstriction of the vascular canals of primary osteons in mice exposed to AA could be associated with deleterious effect of AA on blood vessels. According Raju et al. (2015) AA decreases the high density lipoprotein (HDL). Low HDL is associated with narrowing or blockage of the arteries and vessels (Miller et al. 1992).
Table 1. Results of compact bone microstructure in mice from the E1, E2 and C groups.

<table>
<thead>
<tr>
<th>Measured structures</th>
<th>Group</th>
<th>n</th>
<th>Area (μm²)</th>
<th>Perimeter (μm)</th>
<th>Max. diameter (μm)</th>
<th>Min. diameter (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vascular canals of primary osteons</td>
<td>C (1)</td>
<td>114</td>
<td>48.802±11.851</td>
<td>25.352±3.143</td>
<td>4.556±0.706</td>
<td>3.402±0.525</td>
</tr>
<tr>
<td></td>
<td>E1 (2)</td>
<td>115</td>
<td>39.135±10.124</td>
<td>22.336±2.994</td>
<td>3.889±0.624</td>
<td>3.176±0.469</td>
</tr>
<tr>
<td></td>
<td>E2 (3)</td>
<td>124</td>
<td>30.059±7.183</td>
<td>19.667±2.531</td>
<td>3.423±0.577</td>
<td>2.769±0.409</td>
</tr>
<tr>
<td>Games-Howell test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+ P&lt;0.05</td>
</tr>
<tr>
<td>Haversian canals</td>
<td>C (1)</td>
<td>13</td>
<td>32.477±6.594</td>
<td>20.385±2.008</td>
<td>3.500±0.424</td>
<td>2.946±0.456</td>
</tr>
<tr>
<td></td>
<td>E1 (2)</td>
<td>18</td>
<td>31.967±7.534</td>
<td>20.094±2.282</td>
<td>3.400±0.424</td>
<td>2.967±0.455</td>
</tr>
<tr>
<td></td>
<td>E2 (3)</td>
<td>23</td>
<td>27.839±4.357</td>
<td>18.869±1.472</td>
<td>3.265±0.299</td>
<td>2.691±0.292</td>
</tr>
<tr>
<td>Tukey test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>E1 (2)</td>
<td>18</td>
<td>370.783±115.228</td>
<td>68.511±10.456</td>
<td>11.961±1.992</td>
<td>9.706±1.567</td>
</tr>
<tr>
<td></td>
<td>E2 (3)</td>
<td>23</td>
<td>244.396±68.085</td>
<td>56.013±7.928</td>
<td>9.874±1.611</td>
<td>7.787±1.260</td>
</tr>
<tr>
<td>Games-Howell and/or Tukey test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1:3+; 2:3+</td>
</tr>
</tbody>
</table>

n, number of measurements; + P<0.05; NS, non-significant differences.

Significantly reduced sizes of the secondary osteons in mice from the E2 group could be related to decreased bone mineralization. The results by Wauquier et al. (2009) showed that AA increased the level of the hydrogen peroxide. The hydrogen peroxide significantly decreases the bone mineralization (Arai et al. 2007).

Our results also showed an insignificant effect of AA administration on cortical bone thickness (0.18±0.009 mm, 0.19±0.006 mm, 0.17±0.022 mm) in mice from the E1, E2 and C groups, respectively.

In the trabecular bone, the values for bone volume and trabecular number were significantly increased (P<0.05) in mice administered AA. On the contrary, the value for trabecular separation was significantly decreased in these mice. Significantly higher value (P<0.05) of bone surface was observed in mice from the E1 group whereas trabecular thickness was increased in mice from the E2 group. The results are summarized in Table 2. Representative reconstructed 3D images of the trabecular bone are also illustrated in Table 2.

According to Alturfan et al. (2011) AA significantly increases synthesis of tissue collagen in various parenchymatous organs (brain, lung, liver, kidney, testes). The collagen is a major organic component of mineralized bone matrix. Also, the results...
Table 2. Results of trabecular bone microstructure in mice from the E1, E2 and C groups.

<table>
<thead>
<tr>
<th></th>
<th>C group (1)</th>
<th>E1 group (2)</th>
<th>E2 group (3)</th>
<th>Tukey test</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BV/TV (%)</strong></td>
<td>0.08±0.02</td>
<td>0.13±0.01</td>
<td>0.18±0.04</td>
<td>1:2+; 1:3+</td>
</tr>
<tr>
<td><strong>Tb. N. (1/mm)</strong></td>
<td>3.97±0.45</td>
<td>4.76±0.09</td>
<td>4.89±0.48</td>
<td>1:2+; 1:3+</td>
</tr>
<tr>
<td><strong>Tb. Th. (mm)</strong></td>
<td>0.04±0.0014</td>
<td>0.04±0.0013</td>
<td>0.05±0.0019</td>
<td>1:3+; 2:3+</td>
</tr>
<tr>
<td><strong>Tb. Sp. (mm)</strong></td>
<td>0.25±0.03</td>
<td>0.20±0.01</td>
<td>0.19±0.02</td>
<td>1:2+; 1:3+</td>
</tr>
<tr>
<td><strong>Bs. (mm²)</strong></td>
<td>14.75±5.29</td>
<td>22.63±1.68</td>
<td>21.93±4.69</td>
<td>1:2+</td>
</tr>
</tbody>
</table>

n, number of measurements; * P<0.05; NS, non-significant differences; BV/TV, bone volume; Tb. N., trabecular number; Tb. Th., trabecular thickness; Tb. Sp., trabecular separation; Bs., bone surface.

by Raju et al. (2015) showed that AA increases a concentration of calcium, an important component of hydroxyapatite crystals (Koutsopoulos 2002), in the blood. Therefore, we suppose that AA could influence the trabecular bone microstructure through these mechanisms.

Our results suggest that acute peroral administration to AA significantly affects microstructure of compact and trabecular bone tissues. However, the impact of AA on microarchitecture of these tissues is different. The compact bone is more resorbed, trabecular bone is more robust. Therefore, it would be necessary to study mechanisms by which AA acts on bone. These mechanisms remain unclear. Anyway, the most evident changes were observed in mice treated with 3 doses of AA in a 48-h period. It can be concluded that the effect of AA on bone microstructure depends on the degradation rate. Our findings provide the first information related to AA impact on bone microarchitecture in experimental animals.

There are several limitations to our study. First, only one dose of AA was used. Second, we investigated only acute effect of AA on bone microstructure. Therefore, further studies are needed to find out the complex effect of AA in the bone.

**Conflict of Interest**

There is no conflict of interest.

**Acknowledgements**

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**References**


