Physiol. Res. 66: 1067-1071, 2017

SHORT COMMUNICATION

Acrylamide-Induced Changes in Femoral Bone Microstructure of Mice

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Received October 19, 2016
Accepted May 2, 2017
On-line September 22, 2017

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

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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 (Kopańska *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 Ricqlés *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*, *medialis*, *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 midshaft.

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, medialis* 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).

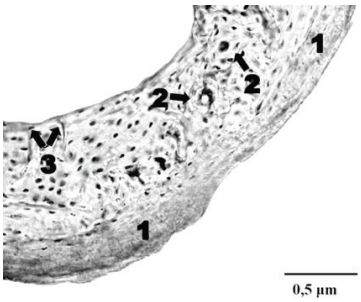
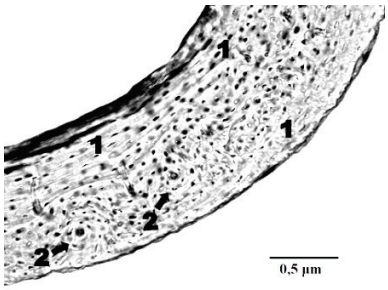
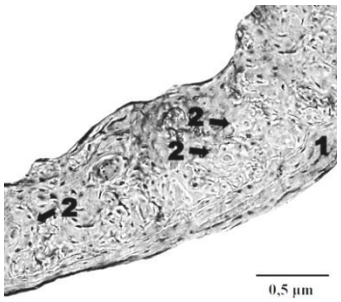
In our study, differences in compact bone microstructure of mice from the E1 and E2 groups were identified. Mice from these groups had more intact secondary osteons (from 38 % to 77 %) in the middle part of the compact bone. In addition, more resorption lacunae (from 100 % to 122 %) were found in *pars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

C group	E1 group	E2 group
		
1 – non-vascular bone tissue 2 – intact secondary osteons in the middle part of *substantia compacta* 3 – primary vascular radial bone tissue	1 – non-vascular bone tissue 2 – resorption lacunae near periosteal border	1 – non-vascular bone tissue 2 – intact secondary osteons in the middle part of *substantia compacta*

Measured structures	Group	n	Area (μm^2)	Perimeter (µm)	Max. diameter (µm)	Min. diameter (µm)
Vascular canals of primary osteons	C (1)	114	48.802±11.851	25.352±3.143	4.556±0.706	3.402±0.525
	E1 (2)	115	39.135±10.124	22.336±2.994	3.889±0.624	3.176±0.469
	E2 (3)	124	30.059±7.183	19.667±2.531	3.423±0.577	2.769±0.409
	Games-Howell test		1:2$^+$; 1:3$^+$; 2:3$^+$	1:2$^+$; 1:3$^+$; 2:3$^+$	1:2$^+$; 1:3$^+$; 2:3$^+$	1:2$^+$; 1:3$^+$; 2:3$^+$
Haversian canals	C (1)	13	32.477±6.594	20.385±2.008	3.500±0.424	2.946±0.456
	E1 (2)	18	31.967±7.534	20.094±2.282	3.400±0.424	2.967±0.455
	E2 (3)	23	27.839±4.357	18.869±1.472	3.265±0.299	2.691±0.292
	Tukey test		NS	NS	NS	NS
Secondary osteons	C (1)	13	439.153±189.217	73.323±16.554	12.723±2.750	10.515±2.611
	E1 (2)	18	370.783±115.228	68.511±10.456	11.961±1.992	9.706±1.567
	E2 (3)	23	244.396±68.085	56.013±7.928	9.874±1.611	7.787±1.260
	Games-Howell and/or Tukey test		1:3$^+$; 2:3$^+$	1:3$^+$; 2:3$^+$	1:3$^+$; 2:3$^+$	1:3$^+$; 2:3$^+$

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

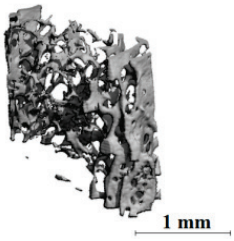
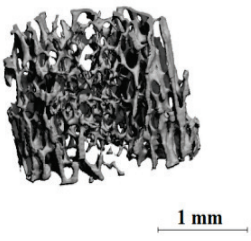
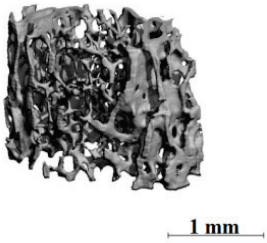
Significantly reduced sizes of the secondary osteons in mice from the E2 group could be related to decreased bone mineralization. The results by Wauquier *et al.* (2009) showed that AA increased the level of the hydrogen peroxide. The hydrogen peroxide significantly decreases the bone mineralization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

	C group (1)	E1 group (2)	E2 group (3)	Tukey test
				
BV/TV (%)	0.08±0.02	0.13±0.01	0.18±0.04	$1{:}2^{+}$; $1{:}3^{+}$
Tb. N. (1/mm)	3.97±0.45	4.76±0.09	4.89±0.48	$1{:}2^{+}$; $1{:}3^{+}$
Tb. Th. (mm)	0.04±0.0014	0.04±0.0013	0.05±0.0019	$1{:}3^{+}$; $2{:}3^{+}$
Tb. Sp. (mm)	0.25±0.03	0.20±0.01	0.19±0.02	$1{:}2^{+}$; $1{:}3^{+}$
Bs. (mm^2)	14.75±5.29	22.63±1.68	21.93±4.69	$1{:}2^{+}$

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

This work was supported by the Slovak Research and Development Agency under the contract No. SK-PL-2015-0032. The study was also supported by the projects VEGA 1/0653/16 and KEGA 031UKF-4/2016.

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