INFLUENCE OF ALPHA-KETOGLUTARATE ON CORTICAL BONE ATROPHY AFTER DENERVATION OF THE HUMERUS IN TURKEY

MARcin R. TATARA, PIOTR SILMANOWICZ1, PIOTR MAJCHER2, WITOLD KRUPSKI3 AND TADEusz STUDZIŃSKI

Department of Animal Physiology, 1Department of Animal Surgery, Faculty of Veterinary Medicine, Agricultural University of Lublin, 20 – 033 Lublin, Poland
2Department of Orthopaedy, Traumatology and Rehabilitation, Medical University of Lublin, 20 – 950 Lublin, Poland
32-nd Department of Radiology, Medical University of Lublin, 20 – 081 Lublin, Poland
e-mail: marcinta@o2.pl

Received for publication June 15, 2004.

Abstract

The purpose of this study was to determine the influence of alpha-ketoglutarate (AKG) daily administration via the digestive tract on cortical bone loss. Due to structural and physiological properties, the study was executed on humerus model in turkey. The neurectomy of the radial and mediano-ulnar nerves was applied to induce osteopenic effect in the right humerus. The experiment was conducted from the 22nd d after hatching, when the turkeys were subjected to neurectomy and AKG administration was started (0.4 g/kg b.w./d). After 14 weeks of the experiment, the animals were sacrificed. Right and left humeri were X-rayed, isolated and frozen at –25°C until further analyses. Using quantitative computed tomography (QCT) method, volumetric bone density of the cortical bone compartment was estimated. Moreover, geometrical and mechanical properties of the investigated bones were determined. The obtained results indicate that AKG daily administration inhibits cortical bone loss induced by denervation of the humerus in turkey.

Key words: turkey, humerus, denervation, cortical bone, alpha-ketoglutarate.

Osteoporosis, the most common metabolic bone disease of the skeletal system, is characterised by low bone mass and microarchitectural remodelling of bone tissue. Peak bone mass in human beings results from the amount of bone tissue acquired during growth and age-related bone loss which in woman rapidly accelerates after menopause (14). Numerous investigations showed that postmenopausal bone loss occurs both in the trabecular and the cortical bone compartments (8).

Due to different metabolic bone activity and structural properties of the compact and the trabecular bone compartments they fulfil different mechanical functions in organisms. The cortical bone has higher density than the trabecular bone and forms shafts of long bones. It is mainly responsible for mechanical endurance to bending forces, whereas the cancellous bone is present in epiphyse and metaphyses of long bones and vertebral bodies, providing resistance for traction and compressive stress (1). Considering presented physiological aspects and metabolic activity in bone tissue, it is necessary to investigate the trabecular bone and cortical bone compartments separately. Due to structural and physiological features, the humerus of turkey fulfils all the requirements indispensable for investigation on the cortical bone model. As opposite to the ulna, femur and tibia in turkey, pneumatic humerus consists of cortical bone only (15-17).

Investigations of alpha-ketoglutarate (AKG) showed great importance of this molecule in Krebs cycle. Moreover, it functions as an energy donor and ammonium ion scavenger. Besides, it is a source of glutamine that stimulates protein synthesis, inhibits protein degradation in muscles, and serves as an important metabolic fuel for cells of the gastrointestinal tract (9, 11, 12). Digestive processes in the intestine are influenced by AKG that serves as a substrate for synthesis of amino acids, especially proline (13). Due to increased hydroxylation of peptide-bound proline to hydroxyproline, the conversion of pro-collagen to collagen and bone matrix formation rise as well. Furthermore, the reductive amination processes of AKG in the liver provide glutamate (Glu) that is released from nerve fibers penetrating into bone tissue and regulates its metabolism through glutamate receptors (GluR) present on osteoblasts (3, 4, 19).

To investigate the effect of alpha-ketoglutarate administration on the cortical bone loss evoked by
denervation, volumetric bone density, morphological and mechanical properties of the humerus in turkey were evaluated.

Material and Methods

Experimental design and sampling procedure. The experiment was executed on growing HB-91 turkeys. The animals were kept under standard rearing conditions with free access to fresh water, appropriate feed supplied in accordance with the stage of production cycle, and air temperature set at the level of 28°C. The experimental birds were separated from the remaining farm stock with a special wire fencing construction, which assured identical feeding, visual and acoustic contact for all of the turkeys. The separated area was lined with straw, just like the rest of the building. The experiment was conducted from the beginning of the fourth week after hatching until the moment that the production cycle was completed (the 17th week of life). On the 22nd d of life, all the experimental turkeys were divided into two weight-matched groups and subjected to surgical procedure. The first group (group 1) consisted of 6 turkeys that were subjected to denervation of right humerus and received placebo (physiological saline – PhS). The second group (group 2 – had 6 turkeys with denervated right humerus) received orally alpha-ketoglutarate (AKG) in the dosage of 0.4 g/kg body weight per day. All left wings within both groups were surgically untouched. The denervation of the investigated bone was performed by neurectomy in the region of the proximal epiphysis of the humerus. To avoid nerve fibers regeneration, the neurectomy consisted of cutting out two sections of approximately 10 mm each of the radial and mediano-ulnar nerves. Both AKG and PhS were administered every day throughout 14 weeks at the same volumes, directly into the crop with the use of a tube, starting from the 22nd d of life. After 14 weeks of the experiment, the turkeys were slaughtered, humeri were X-rayed, isolated and frozen at −25°C for further analyses.

Volumetric bone density of cortical bone. Quantitative computed tomography (QCT) method and SOMATOM AR. T – SIEMENS apparatus supplied with VR 3 software were used for volumetric bone density determination of the cortical bone in the humerus. Bone density was measured for cortical bone using 2 mm thick, cross sectional, diaphysial QCT scans at 36% of humerus length along the longitudinal axis, measuring from the distal extremity of this bone.

Analysis of mechanical and geometrical properties. Mechanical properties of the investigated bones were estimated using three-point bending test, according to Ferretti’s et al. method in INSTRON 4302 apparatus linked with a computer, registering relationship between forces perpendicular to length of bone and resulting in displacement (6, 7). Results were presented graphically and maximum elastic strength (Wy) and ultimate strength (Wf) were estimated. On the basis of measurement of horizontal and vertical diameters of cross section of the humerus, both external and internal diameters, cross-sectional area (A), second moment of inertia (Ix), mean relative wall thickness (MRWT) and cortical index (CI) were estimated (2).

Statistical analysis. All data are presented as mean ± standard error (±S.E.). Statistical analyses were performed using Statistica software version 5.0. All investigated parameters in both groups of turkeys were found to be normally distributed in accordance with Kolomogorov-Smirnov test. The Student’s t-test was used to determine statistical significance level of differences in variables between investigated parameters. A significance level of P≤0.05 was used for all comparisons.

Results

At the age of 22 d the body weight of turkeys belonging to groups 1 and 2 reached 527.66 g (±16.39) and 531.33 g (±16.64), respectively. No significant difference was stated between control and AKG treated turkeys in the beginning of the experiment (P=0.89). The final mean body weight of turkeys at the age of 17 weeks reached 8698.33 g (±275.45) in group 1 and was not significantly different from 9108.33 g (±393.78) in group 2 that received alpha-ketoglutarate (P=0.50).

The mean weight of the left humeri in turkeys from group 1 reached 54.7 g (±2.88) and exhibited clear tendency to higher values when compared to 44.83 g (±3.18) found in denervated ones. Statistically significant difference in weight value was stated between denervated and physiologically innervated humeri in the AKG treated turkeys, in which these values reached 61.9 g (±1.16) and 52.26 g (±2.1), respectively (P<0.007). Moreover, the AKG-treated turkeys had significantly higher weight of denervated humerus, when compared to control birds (P=0.001). The length of the humeri showed no significant differences between left and right bones within both investigated groups and the differences of this parameter between AKG and control groups were not found as well (Table 1).

Volumetric cortical bone density (Cd) measurements (Table 1) showed strong tendency to lower Cd of denervated humerus in turkeys that received placebo, when compared to physiologically innervated left bone (P=0.07). Considering the AKG treated birds, insignificant difference was in this parameter between right and left humerus (P=0.34). Moreover, a significant increase of Cd value of denervated humerus in AKG group was noted when compared to this bone in the control group (P=0.001). Considering cross-sectional area (A), mean relative wall thickness (MRWT), and cortical index (CI) of the investigated bones within group 1, significantly decreased values in all of these geometrical parameters were observed in right denervated humerus (Table 1). Similar differences were obtained analysing mechanical properties like maximum elastic strength (Wy) and ultimate strength (Wf) in this group of turkeys (both P<0.05) (Table 1). Comparing
right and left humeri in birds from group 2, no significant differences were noted both in geometrical and mechanical properties (Table 1). Moreover, right humeri reached significantly higher values of geometrical and mechanical parameters in AKG treated birds, when compared to right humeri of control animals from group 1 (P≤0.005) (Table 1).

Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1 (control group)</th>
<th>Group 2 (AKG treated group)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left humerus (innervated)</td>
<td>Right humerus (denervated)</td>
</tr>
<tr>
<td>Number of investigated bones</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Length (mm)</td>
<td>136.33±2.4</td>
<td>134±1.94</td>
</tr>
<tr>
<td>Cortical bone density (g/cm³)</td>
<td>1.835±0.087</td>
<td>1.600±0.044</td>
</tr>
<tr>
<td>Cross-sectional area (mm²)</td>
<td>67.24±5.46</td>
<td>47.01±4.14</td>
</tr>
<tr>
<td>Second moment of inertia (mm⁴)</td>
<td>1093±136.5</td>
<td>742.1±88.9</td>
</tr>
<tr>
<td>Mean relative wall thickness</td>
<td>0.346±0.027</td>
<td>0.231±0.020</td>
</tr>
<tr>
<td>Cortical index</td>
<td>25.29±1.42</td>
<td>18.43±1.39</td>
</tr>
<tr>
<td>Maximum elastic strength (N)</td>
<td>854.1±89.2</td>
<td>549.1±72.9</td>
</tr>
<tr>
<td>Ultimate strength (N)</td>
<td>1347.5±133.4</td>
<td>913.3±103.1</td>
</tr>
</tbody>
</table>

* Differences between mean values are significant when means are not marked with the same letter. The significance level of P≤0.05 was used for all comparisons.

Discussion

Due to signalling molecules that may act as efferent agents on bone cells, great importance of the nervous system in bone metabolism and anabolism regulation was reported in numerous investigations in recent years (4, 5, 10). The identification of the nerve-derived signalling molecules (neuropeptides) capable of modulating cellular activities of bone cells, facilitates a novel approach to study the biology of skeletal innervation (10). Among many neuropeptides that regulate bone tissue metabolic processes, the best known are glutamate (Glu), calcitonin gene-related peptide (CGRP), substance P (SP), and vasoactive intestinal peptide (VIP) (3-5, 10). In vitro and in vivo studies provided evidence that glutamatergic signalling is a necessity for normal osteoblast function. Recent studies have shown that osteoblasts not only express a wide number of glutamatergic proteins but also possess the ability to both regulate glutamate release and actively recycle extracellular glutamate (19). Other investigations proved that bone loss induced in a model of sciatic neurectomy in growing rats is associated with the decrease in glutamatergic innervation. The manipulation of Glu action in bone may, therefore, serve as a new therapeutic target for the pathology associated with modifications of bone remodeling (3).

The performed investigation showed that partial elimination of innervation of the humerus evokes decreased bone weight and cortical bone density. Moreover, significantly lowered geometrical and mechanical properties of denervated humerus indicate osteopenic changes in the cortical bone compartment. These results confirm that proper function of the nervous system is responsible for bone normal growth, development, and mineralisation processes. Considering the effect on bone tissue of alpha-ketoglutarate administration via the digestive tract in turkeys, the protective influence of this molecule on cortical bone loss must be concluded. It was found that due to AKG administration, cortical bone density, geometrical parameters, and mechanical endurance obtained similar values both in left innervated and right denervated humeri of turkeys. Anti-osteopenic effect of AKG seems to be more highlighted when compared with analysed parameters of denervated humeri between AKG treated and control birds. It is worth to underline that the obtained results were not influenced by differences in body weight values between the investigated groups of turkeys. The obtained results are in accordance with other investigations showing the beneficial effect of AKG on bone tissue, not only in cortical bone compartment but in trabecular bone as well. (15-18).
Considering current knowledge of AKG, its metabolites and peripheral nervous system functions, it can be concluded that increased glutamate synthesis and its utilisation as neuro-signalling molecule in bone tissue may protect bone loss in the cortical bone compartment. The other mechanism that may be involved in the positive influence of AKG on bone tissue homeostasis maintenance is the increased collagen formation as the result of higher proline synthesis and its following conversion to hydroxyproline, which was previously reported (13).

In conclusion, the dietary administration of AKG in turkeys improves skeletal system quality under condition of bone’s denervation and guarantees bone tissue homeostasis maintenance. Furthermore, better mechanical endurance of skeletal system provided by dietary AKG may be considered as an effective way in the prevention of bone pathological processes.

References