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The effect of levetiracetam on rat bone mineral density, bone structure and biochemical markers of bone metabolism

Iva Karesova[a,*], Julius Simko[b], Sona Fekete[a], Eva Zimcikova[c], Jana Malakova[a], Helena Zivna[d], Ladislava Pavlikova[a], Vladimir Palicka[a]

[a] Institute of Clinical Biochemistry and Diagnostics, Charles University in Prague, Faculty of Medicine and University Hospital, Hradec Kralove, Czech Republic
[b] Department of Neurology, Charles University in Prague, Faculty of Medicine and University Hospital, Hradec Kralove, Czech Republic
[c] Department of Social and Clinical Pharmacy, Faculty of Pharmacy in Hradec Kralove, Charles University in Prague, Czech Republic
[d] Radioisotope Laboratories and Vivarium, Faculty of Medicine, Hradec Kralove, Czech Republic

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ABSTRACT

Some data suggest that exposure to levetiracetam (LEV) might be associated with a risk for bone health in the model of orchidectomized rats. The aim of this study was to investigate if there is any significant risk of LEV for bone health in the model of gonadally intact animals.

Wistar rats were divided into a control group and a test group, 8 rats in each group. The control rats received standard laboratory diet (SLD) while rats in the test group were fed SLD enriched with LEV for 12 weeks. Dual energy X-ray absorptiometry was used to measure BMD of the whole body, femur and lumbar vertebrae. The concentrations of bone markers were examined in bone homogenate. Both femurs and tibiae were used for biomechanical testing.

We found in the LEV group significantly decreased absolute and relative values of adipose tissue, higher whole-body BMD, higher right tibia cortical thickness, and a significantly increased concentration of Bone Alkaline Phosphatase (BALP) and cross-linked C-telopeptide of type I collagen (CTX-I) compared with the control group.

The results suggest that the long-term administration of LEV in the model of gonadally intact rats does not have a negative effect on bone. Significant increase in BMD and cortical thickness of the right tibia may indicate even a positive influence on the properties of bone. Further studies will be necessary in animals and humans to confirm these findings.

1. Introduction

Epilepsy is one of the most common chronic neurological disorders, characterized by recurrent seizures of cerebral origin (Hamed et al., 2014). The disease affects more than 50 million people worldwide (Shen et al., 2014). Anti-epileptic drugs (AEDs) are the main form of treatment for people with epilepsy and the treatment with AEDs is frequently lifelong (Shen et al., 2014; Fitzpatrick, 2004). However AEDs are prescribed as first-line treatment also for a variety of non-epileptic conditions as well, mainly bipolar spectrum disorders and chronic pain states (Reimers, 2014). Only one out of three AED users takes these drugs for epilepsy (Reimers, 2014). AEDs are associated with adverse effects on bone health and with increased risk of fracture (Koo et al., 2013). It is estimated that patients with epilepsy have a 2–6 times greater risk of bone fractures compared with the general population (Svalheim et al., 2011). Aetiology of osteopathy in epileptic patients is multifactorial with an important role of a usually lower level of physical activity and probably also a shorter period of the exposure to the sun. The probability of a decrease in BMD increases with the length of AEDs medication, combination of AEDs, institutionalization and the presence of other risks for bone tissue in the case history (Desai et al., 1996). Enzyme-inducing AEDs are particularly implicated in bone loss, but some studies suggest that also valproate, an enzyme inhibitor, is associated with reduced bone mineral density (BMD) and bone loss (Anwar et al., 2014). Evidence for the effect of newer AEDs on bone metabolism and BMD is limited and further investigation is required (Anwar et al., 2014).

Levetiracetam (LEV) is a relatively new and one of the most widely-prescribed non enzyme-inducing AEDs, effective in the treatment of partial and generalized seizures (Artemiadis et al., 2016; Anwar et al., 2014). LEV is an analogue of the nootropic agent piracetam and has anti-seizure properties (Shetty, 2013; Erbaş et al., 2016). It has also

* Corresponding author.
E-mail address: karesova.iva@gmail.com (I. Karesova).

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been proposed that the attractiveness of LEV as an AED is boosted by its beneficial pharmacokinetic attributes, including excellent oral bioavailability not dependent on the P450 cytochrome system, linear kinetics, minimal plasma protein binding, and rapid achievement of a steady state concentration (Shetty, 2013; Peyrl et al., 2015). The drug is safe and generally well tolerated (Koo et al., 2013). Literature data suggest that LEV may have minimal (if any) adverse effects on bone (Artemiadis et al., 2016; Koo et al., 2013; Erbaş et al., 2016; Nissen-Meyer et al., 2008), and the effect on bone may even be positive, as demonstrated by a recent retrospective cohort study (Phabphal et al., 2013). However, in orchidectomised rats we have demonstrated that LEV treatment may significantly reduce BMD and bone mineral content (BMC), and have an adverse effect on bone metabolism (Fekete et al., 2013). In this study we have exposed gonadally intact animals to LEV to investigate whether the effect applies also in the model of gonadally intact animals.

2. Materials and methods

2.1. Animals

The experiment used eight-week-old male albino Wistar rats (Biotest s.r.o., Konarovice, Czech Republic). The animals were hosted in groups of 4 in plastic cages. During the experimental period the animals were maintained in controlled conventional conditions (12 h light and 12 h dark, temperature 22 ± 2 °C, air humidity 30–70%). Tap water and standard laboratory diet (SLD, VELAS, a.s., Lysa nad Labem, Czech Republic) or SLD enriched with LEV were given ad libitum. Drinking water was available ad libitum. The weights of the rats were monitored once a week. All animals received humane care in accordance with the guidelines set by the Institutional Animal Use and Care Committee of Charles University, Prague, Faculty of Medicine in Hradec Kralove, Czech Republic. The protocol of the experiment was approved by the same committee.

2.2. Experiment design

The rats were divided into two groups of 8 animals: 1st group (Control group): rats fed with SLD; and 2nd group (LEV group): rats fed with SLD enriched with LEV (101 mg/25 g of the diet; Levetiracetam, UCB Pharma). On the second day the LEV group began to receive SLD enriched with LEV and the Control group only SLD, both diets ad libitum. After 12 weeks, the animals were killed by blood withdrawal from the abdominal aorta under ether anesthesia, and the obtained serum was aliquoted and stored at − 80 °C for ensuing biochemical analyses. After kill of the rats, both tibiae and femurs were dissected free of soft tissue, wrapped in gauze moistened with saline and frozen to −80 °C till required for analysis.

2.3. Analysis of serum and bone homogenates

Blood serum levels also of levetiracetam were determined at the end of the experiment. Concentrations of levetiracetam in the samples were determined by the modified high-performance liquid chromatography method with UV photodiode-array detection (Lancelin et al., 2007). Levetiracetam and internal standard UCB 17025 were extracted after alkalization of the sample (0.05 ml) into dichloromethane. Organic solvent was evaporated and the residue was dissolved and injected for HPLC analysis. Compounds were separated on a Zorbax SB-C8 column (Agilent Technologies, USA) at flow rate 1.1 ml/min. The mobile phase was composed of 10% acetonitrile, 7% methanol and 83% of a 20 mM phosphate buffer pH 6.7 with 0.1% triethylamine. UV detection was performed at a wavelength of 200 nm.

Bone homogenate was prepared from the femur. After animal kill, both femurs were carefully excised; after removal of all the surrounding skin, muscle and other soft tissue, they were stored at −80 °C until required. The diaphysis part of the femur (0.1 g) was disrupted and homogenized by TissueLyser II (Qiagen, Netherlands). The homogenization consisted of the following steps. Liquid nitrogen was poured into the grinding jar over the ball and femur sample and 0,5 ml of phosphate buffer was added (PBS, PENTA Prague, Czech Republic). The femur tissue was ground at 30 Hz for 1 min. After this procedure a further 1,5 ml of phosphate buffer was added and ground at 10 Hz for 15 s. The raw tissue homogenate was centrifuged at 10,000 g at 4 °C for 10 min, and the resulting supernatant was collected and stored at − 80 °C.

Levels of the markers of cross-linked C-telopeptide of type I collagen (CTX-I), amino-terminal propeptide of procollagen type I (PINP), sclerostin (SOST), Bone Alkaline Phosphatase (BALP) and Receptor Activator of Nuclear Factor Kappa B Ligand (RANKL) were analyzed in this bone homogenate using the ELISA method. Bone-marker levels were determined using kits from the firm Uscn Life Science Inc., Wuhan, China (PINP, ng/ml; CTX-I, pg/ml; SOST, pg/ml; RANKL, pg/ml), except for levels of BALP which were determined using a kit from the firm BlueGene Biotech, Shanghai, China (BALP, ng/ml).

2.4. Dual energy X-ray absorptiometry analysis

The rat bone mineral density (BMD, g/cm^2) was measured by means of dual energy X-ray absorptiometry (DEXA) on a Hologic Delphi A device (Hologic, MA, USA) at the Osteocentre of the Faculty Hospital Hradec Kralove, Czech Republic. Before measurements, a tissue calibration scan was performed with the Hologic phantom for the small animal. BMD of the whole body, in the lumbar vertebrae and in both femurs (Fig. 1), and the total lean and fat masses were evaluated by computer using the appropriate software program for small animals (DEXA; QDR-4500A Elite; Hologic, Waltham, MA, USA). All animals were scanned by the same operator.

2.5. Biomechanical testing procedure

Mechanical testing of the rat femoral shaft and femoral neck was done with a special electromechanical custom-made testing machine

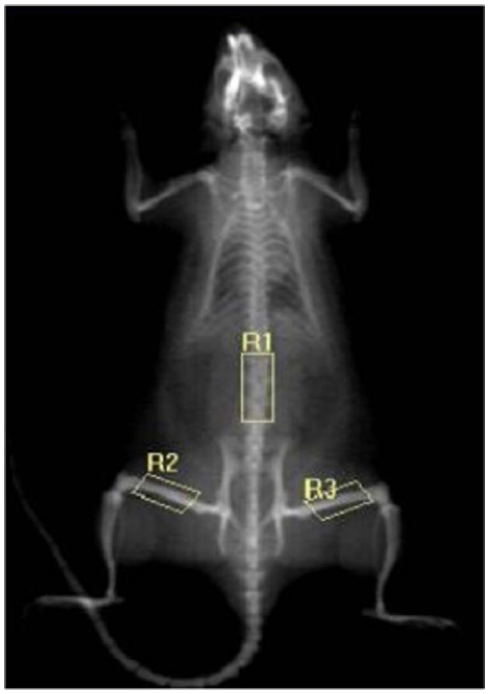

Fig. 1. Evaluation of BMD in three areas of the rat skeleton R1 – lumbar columna (L3-L5); R2 – left femur; R3 – right femur.