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The effect of lamotrigine and phenytoin on bone turnover and bone strength: A prospective study in Wistar rats

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ABSTRACT

Objective: Some data suggest that exposure to lamotrigine (LTG) might be associated with impaired bone health in an orchidectomized rat model. The aim of this study was to determine if LTG poses any significant risk for bone in a gonadally intact animals and to compare the effect of LTG with that of phenytoin (PHT).
Method: Twenty-four rats were divided into control and test groups, (n = 8 per group). Control rats received a standard laboratory diet (SDL), while rats in the test groups were fed a SLD enriched with LTG or PHT for 12 weeks. Dual energy X-ray absorptiometry was used to measure bone mineral density (BMD). The concentrations of bone turnover markers (BTM) were assayed in bone homogenates. The femurs were measured and biomechanically tested.
Results: Treatment with either LTG or PHT had no significant effect on BMD or on the biomechanical strength of the bones. In contrast to the effect of LTG, we did find significant changes in BTM in the PHT group: a highly significant decrease in the osteoprotegerin/receptor activator of nuclear factor kappa B ratio ($p < 0.01$) and highly significant increases in bone alkaline phosphatase and amino-terminal propeptide of procollagen type I ($p < 0.001$, $p < 0.01$, respectively). In the LTG group, the only significant change was a decrease in sclerostin ($p < 0.05$). The PHT level was 19.0 (15.6–19.5) μmol/l, which represents the lower end of the therapeutic range used in humans. The level of LTG was 60.7 (58.5–61.8) μmol/l.
Conclusions: LTG has no effect on the BMD, BTM or mechanical strength in gonadally intact animals. Although a low dose of PHT was associated with enhanced BTM, it did not affect BMD or the biomechanical properties of the bones, similar to the results observed for LTG.

1. Introduction

Many patients with epilepsy are required to chronically take antiepileptic drugs (AEDs). For this reason, understanding the undesirable effects of long-term medication is important. Osteopathies associated with long-term antiepileptic treatment were first noted in the late sixties (Schmid, 1967; Kruse, 1968). Since that time, a number of theories have been proposed to explain why AEDs affect bones, but none have explained all the reported effects (Pack, 2008). Most of the studies on the effects of AEDs on bone tissue are cross-sectional, and there are only a few longitudinal studies on this topic. There is also limited evidence that the use of newer AEDs, namely, topiramate, zonisamide, oxcarbazepine and gabapentin, may have adverse effects on bone mineral density (BMD) and bone metabolism, increasing the risk of fractures (Takahashi et al., 2003; Mintzer et al., 2006; Ensrud et al., 2008; Coppola et al., 2009; Heo et al., 2011; Babacan et al., 2012). Current studies suggest that lamotrigine (LTG) could have limited (if any) negative impacts on bone health (Kim et al., 2007; Sheth and Hermann, 2007; Pack et al., 2008), except in combination with valproate (Guo et al., 2001). However, in an orchidectomized rat model, we have shown that LTG may significantly reduce BMD and impair the mechanical strength of bones (Simko et al., 2014).

Therefore, in the current study we exposed gonadally intact animals to LTG to determine whether LTG has clinically

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significant effects on bone that are exclusive to gonadectomized rats or whether the effects are also present in a gonadally intact animal model.

The AEDs most commonly reported to cause decreased BMD and disorders of bone metabolism are those that induce cytochrome P450. Thus, another aim of this study was to determine whether there are significant differences between the effects of LTG and phenytoin (PHT), which is known to induce CYP450, on bone health.

2. Material and methods

2.1. Animals

This experiment used 24 eight-week-old male albino Wistar rats (Biotest s.r.o., Konarovice, Czech Republic). The animals were housed in groups of 4 in plastic cages. During the experimental period, the animals were maintained under controlled, standard conditions (12 h light and 12 h dark, temperature: 22 ± 2 °C, air humidity: 30–70%). Tap water and a standard laboratory diet (SLD, VELAS, a.s., Lysa nad Labem, Czech Republic) or a SLD enriched with LTG or PHT were available ad libitum. Rats were weighed 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 experimental protocol was approved by this same committee.

2.2. Experiment design

Rats were divided into three groups: 1) CONTROL, fed a SLD); 2) LTG, fed a SLD enriched with LTG (35 mg/25 g of food; Plexxo, Desitin); and 3) PHT, fed a SLD enriched with PHT (85 mg/25 g of food; Epilan D, Gerot). After 12 weeks, the animals were sacrificed via blood withdrawal from the abdominal aorta under ether anaesthesia, and the serum obtained from the samples was aliquoted and stored at −80 °C for subsequent biochemical analyses. Then, both tibias and femurs were dissected free of soft tissue, wrapped in gauze moistened with saline and frozen at −80 °C until required for analysis. The three-month follow-up period, should be sufficient for accurate assessing of changes in the BMD and likely also for the biomechanical properties of bone tissue (Sengupta, 2013).

2.3. Open field test (OFT)

An open field apparatus made of a wooden box (60 × 60 × 60 cm) was used to test the open-field exploratory behaviour of rats. The floor of the apparatus was white and was divided by lines into 16 evenly spaced squares, and the surrounding walls were painted black. In the novel test situation, each animal was placed in the lower right corner of the test apparatus for a maximum of 5 min to observe the following behaviours:

Ambulation: the number of squares crossed by the rat.

Rearing: the number of times the rat stood on its hind limbs.

Self-grooming: the number of grooming, scratching, licking and washing behaviours made by each individual rat.

Excretion: the number of faecal pellets excreted by each individual rat.

Central latency: the time to enter the central part of the apparatus (i.e., the four squares in the centre of the apparatus).

Before each trial, the floor and the walls were cleaned with cotton soaked in 70% alcohol.

2.4. Analysis of serum and bone homogenates

Blood serum levels of osteoprotegerin (OPG) and receptor activator of nuclear factor kappa B ligand (RANKL) were determined via ELISA (enzyme-linked immunosorbent assay). Blood serum levels of LTG were also determined in the middle and at the end of the experiment. LTG concentrations in the samples were determined using a modified high-performance liquid chromatography method with UV photodiode-array detection (Malakova et al., 2007) Liquid-liquid extraction of a 0.05 ml alkalinized sample was carried out into ethyl acetate. After the evaporation of the organic phase, the residue was dissolved in methanol. LTG and the internal standard BW 725C 78 were separated on a Symmetry C18 column (Waters, USA, 150 × 4.6 mm I.D., 5 μm particle size) with a Symmetry C18 guard column (20 × 3.9 mm I.D.). The mobile phase, at an isocratic flow rate of 1 ml/min, contained acetonitrile (28%) and a 6 mM phosphate buffer at pH 6.8 (72%). The eluate was monitored at a wavelength of 306 nm. Bone homogenates were prepared from the femurs. The proximal part of each femur (0.1 g) was disrupted and homogenized using a TissueLyser II (Qiagen, Netherlands). To homogenize the femur samples, liquid nitrogen was poured into the grinding jar over the ball and the femur sample and then 0.5 ml of phosphate-buffered saline (PBS, PENTA Prague, Czech Republic) was added. The femur samples were ground at 30 Hz for 1 min. After this, another 1.5 ml of PBS was added and the samples were 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. The levels of the bone-metabolism markers cross-linked C-telopeptide of type I collagen (CTX-I), amino-terminal propeptide of procollagen type I (PINP), sclerostin (SCL), and bone alkaline phosphatase (BALP) in the bone homogenate were analysed via ELISA. Bone-metabolism marker levels were determined using kits from Uscn Life Science Inc., Wuhan, China (PINP, ng/mL; CTX-I, pg/mL; SCL, pg/mL; RANKL, pg/mL) and from BlueGene Biotech, Shanghai, China (BALP, ng/mL).

2.5. Dual energy X-ray absorptiometry analysis

Rat BMD (g/cm2) was measured via 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 the measurements, a tissue calibration scan was performed using a Hologic small-animal phantom. The BMD of the whole body, the lumbar vertebrae and both femurs (Fig. 1), and the total lean and fat mass of the rats were evaluated using software appropriate for analysing small animals (DEXA; QDR-4500A Elite; Hologic, Waltham, MA, USA). All animals were scanned by the same operator.

2.6. Biomechanical testing procedure

Mechanical testing of the rat femoral shaft and femoral neck was performed with a special electromechanical, custom-made testing machine (Martin Kosek & Pavel Trnecka, Hradec Kralove, Czech Republic) according to our previously described methods (Gradosova et al., 2011). For the three-point bending test, the femur was placed on a holding device with the two support points 18 mm apart. A small stabilizing preload of up to 10 N was used to fix the bone between the contacts. A constant deformation rate of 6 mm/min was generated until maximal load failure, and the breaking strength (maximum load, N) was recorded. When the bone was broken, the thickness of the cortical section of the bone was measured using a sliding micrometre (OXFORD 0–25 mm 30DEG POINTED MICROMETER, Victoria Works, Leicester, Great Britain). The proximal section of the femur was used for a compression test of the femoral neck. The diaphysis of the bone was embedded in a container using a methacrylate resin, and a vertical load was applied to the top of the femoral head. A small stabilizing preload of up to 10 N was applied and increased at a constant speed of 6 mm/min until failure of the femoral neck. The breaking strength