Journal of the Neurological Sciences 340 (2014) 80–85

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

journal homepage: www.elsevier.com/locate/jns

The effect of topiramate and lamotrigine on rat bone mass, structure and metabolism

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ARTICLE INFO

Article history:
Received 2 October 2013
Received in revised form 6 February 2014
Accepted 25 February 2014
Available online 3 March 2014

Keywords:
Antiepileptic drugs
Lamotrigine
Topiramate
Bone mineral density
Bone markers

ABSTRACT

There is only limited data concerning the effect of the newer antiepileptic drugs on bone. The objective of this study was to determine the effect of topiramate (TPM) and lamotrigine (LTG) monotherapy on bone mineral density (BMD), mineral content (BMC), bone markers, body composition and bone mechanical strength in the orchidectomized (ORX) rat model.

24 orchidectomized Wistar rats were divided into control and test groups, 8 rats in each group. The control rats received standard laboratory diet (SLD) while rats in the test group were fed with SLD enriched with LTG or TPM for 12 weeks. Dual energy X-ray absorptiometry was used to measure bone mineral density. The concentrations of bone metabolism markers were assayed in bone homogenate. In addition, both femurs were measured and used for biomechanical testing.

Compared to the control group, both test groups had significantly lower weight, fat mass, whole body and femur BMD, BMC and reduced mechanical strength of bone. All of these changes were more pronounced in rats exposed to LTG.

In conclusion, both LTG and TPM significantly reduce BMD and body weight and impair mechanical strength of bone. A question arises as to the degree of dependence of the effect on the dose. Further studies are warranted to establish whether LTG and TPM may have a clinically significant effect on BMD exclusively in the model of gonadectomized rats, or whether the effect applies also in the model of gonadally intact animals, and in the respective human models.

1. Introduction

Many patients with epilepsy are required to take chronically antiepileptic drugs (AEDs). For this reason the issue of undesirable effects due to long-term medication is important. Osteopathies occurring with long-term chronic antiepileptic treatment were first noted in the late sixties [1,2]. Since that time a number of theories have been proposed to explain why AEDs affect the bone, but none explains all the reported effects [3]. Most studies of the effects of AEDs on bone tissue are cross-sectional; there are only a few longitudinal studies. There is limited evidence also that use of the newer AEDs, namely topiramate (TPM), zonisamide, oxcarbazepine and gabapentin, may have adverse effects on bone mineral density (BMD) and bone metabolism [4–9]. Current studies suggest that lamotrigine (LTG) could have limited (if any) negative impact on bone health (10–12), except in combination with valproate [13].

Decline of the sex steroids is an important risk factor of osteoporosis in the elderly. In older women as well as in older man the use of AEDs was independently associated with increased rates of bone loss [9,14]. In gonadally intact subject, no negative impact of LTG on the bone has been reported so far. We have thus selected the orchidectomized rat model (the model for androgen-deficient osteoporosis), in which higher sensitivity can be expected for detection of a possible risk of xenobiotics for bone health.

We report here our findings in orchidectomized (ORX) rats fed with TPM or LTG-enriched diet for 12 weeks, on the impact of TPM or LTG on BMD, bone mineral content (BMC), bone metabolism markers, and biomechanical properties of bone.

In the same study design we have previously reported that administration of levetiracetam can have a negative effect on bone as judged by

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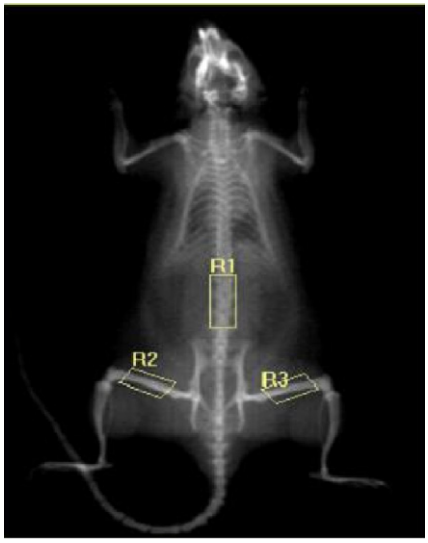
http://dx.doi.org/10.1016/j.jns.2014.02.032
0022-510X/



R1 – lumbar columna (L3-L5);

R2 – left femur

R3 – right femur

Fig. 1. Evaluation of BMD in three areas of the rat skeleton.

reduced femoral BMD, decreased serum levels of OPG (marker of bone formation) and increased levels of CTX-I (marker of bone resorption), but this study failed to show any change in biomechanical bone strength [15] (in the same study design, we have also observed a significant loss of BMD at the left and right femur areas in the lacosamide group, again without any change in biomechanical bone strength — publication in the peer review process).

2. Methods

2.1. Animals

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. The experiments 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 under controlled conventional conditions (12 h of light and 12 h of dark, temperature 22 ± 2 °C, air humidity 30–70%). Tap water, standard laboratory diet (SLD, VELAS, a.s., Lysa nad Labem, Czech Republic), SLD enriched with LTG and SLD enriched with TPM were given *ad libitum*. The weights of the rats were monitored once a week.

2.2. Experiment

The rats, weighing 270 ± 7 g at the beginning of the experiment, were divided into three groups of 8 animals:

1. CON-R: orchidectomized rats control fed with SLD;
2. LTG-R: orchidectomized rats fed with SLD enriched with LTG (39 mg/25 g of the diet; Lamotrigine, Glenmark); and
3. TPM-R: orchidectomized rats fed with SLD enriched with TPM (23 mg/25 g of the diet; Topiramat, Glenmark).

At the beginning of the experiment, the rats (CON-R, LTG-R and TPM-R) underwent bilateral orchidectomy under ether anesthesia. On the second day after operation, the LTG-R began to receive SLD enriched with LTG; the TPM-R began to receive SLD enriched with TPM; and the CON-R only SLD, both diets *ad libitum*. After 12 weeks, the animals were sacrificed 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 sacrificing the rats, both tibiae and femurs were dissected free of soft tissue, wrapped in gauze moistened with saline and frozen to −80 °C till the time of analysis.

2.3. Analysis of serum and bone homogenates

In the blood serum, the levels of osteoprotegerin (OPG) and insulin-like growth factor 1 (IGF-1) were determined by the ELISA (enzyme-linked immunosorbent assay) method. Concentrations of lamotrigine were measured using a modified method of high-performance liquid chromatography with UV photodiode-array detection [16]. Liquid–liquid extraction of a 0.05 ml alkalinized sample was carried out into ethyl acetate. After evaporation of the organic phase, the residue was dissolved in methanol. Lamotrigine 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 and Symmetry C18 guard column (20 × 3.9 mm I.D.). The mobile phase at isocratic flow rate of 1 ml/min contained acetonitrile (28%) and 6 mM phosphate buffer pH 6.8 (72%). The eluate was monitored at a wavelength of 306 nm. Determination of topiramate in the samples was performed using the gas chromatography-mass spectrometry method. This method was a modification of a bioanalytical method published previously [17]. The procedure included liquid–liquid extraction of 0.05 ml of the alkalinized sample with ethyl acetate. Trimethylanilinium hydroxide was used for flash methylation of topiramate and internal standard 5-(*p*-methylphenyl)-5-phenylhydantoin. Ions of m/z 352 (for the topiramate derivative) and m/z 296 (for the internal standard derivative) were recorded for data evaluation.

In the bone homogenate, the levels of the markers carboxy-terminal cross-linking telopeptide of type I collagen (CTX-I), aminoterminal propeptide of procollagen type I (PINP), bone alkaline phosphatase (BALP) and bone morphogenetic protein 2 (BMP-2) were determined, also using the ELISA method. The homogenate was prepared from the tibia. After animal sacrifice, both tibias were carefully excised; after removal of all the surrounding skin, muscle and other soft tissue, they were stored at −80 °C until required. The proximal part of the bone (0.1 g) was disrupted and homogenized in 1.5 ml of phosphate buffer (PBS, PAA Laboratories GmbH, Pasching, Austria) with a MagNA Lyser instrument (Roche Applied Science, Germany) at 6500 rpm for 20 s and cooled on a MagNA Lyser cooling block. This procedure was repeated three times.

The tissue homogenate was centrifuged at 10,000 *g* at 4 °C for 10 min. The supernatant was collected and stored at −80 °C. Kits from the firm Uscn Life Science Inc., Wuhan, China were used for