REVIEW

How can antiepileptic drugs affect bone mass, structure and metabolism? Lessons from animal studies

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Summary   Patients with epilepsy, treated with antiepileptic drugs (AEDs) are at increased risk of fractures. Although several commonly used AEDs reduce bone mass in patients, the mechanisms are only scarcely known. In this review, we focus on the usefulness of animal models to explore the skeletal effects of AEDs. Moreover, we report our findings from a recent study comparing the effect of levetiracetam (LEV), phenytoin (PHT) and valproate (VPA) on various aspects of bone health in actively growing female rats. Our data indicate that these AEDs act differently on bone mass, structure and metabolism. A novel finding is that LEV reduces bone strength and bone formation without altering bone mass. Based on these results we propose that epidemiological fracture studies of patients treated with LEV are needed, and that these patients should be evaluated regularly to identify possible bone-related side effects.
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Introduction

An association between antiepileptic drug (AED) therapy, lowered bone mineral density (BMD) and fracture risk has been repeatedly reported over the last 40 years (reviewed in[1]). The most common fracture sites are spine and hip but the relative risk of any fracture is increased.[1,2] AED-related bone disease affects both genders, although female epilepsy patients have the highest risk of fracture because of the added burden of postmenopausal osteoporosis.

Animal models

The mechanisms responsible for AED-related bone fragility are multiple and still inadequately understood. Animal studies are helpful in this respect, because they allow us to eliminate all the possible variation caused by life style, genetics, individual disease characteristics and compliance. However, extrapolation of results from animal model studies to the human clinical situation require careful consideration of the relevance of the animal model, including the age of the animals, duration of treatment and the concentrations of drugs used in the experiments.

The laboratory rat has been widely used to study the effects of drugs on bone health,[3] and Refs. in.[4,5] However, relatively few animal studies have investigated the effects of common AEDs like valproate (VPA) and phenytoin (PHT), representing enzyme-inducing and non-inducing drugs, respectively. Moreover, most previous studies were conducted in male animals,[6–8] and whether AEDs cause gender-specific effects on bones is unknown.

PHT causes bone loss due to elevated bone resorption in intact animals,[7,8] but has also been shown to have direct effects on osteoblast proliferation and differentiation in cell culture experiments.[9–12] The reported effects of VPA on human bone turnover differ widely (see [2] for review), and also effects of VPA on cultured bone cells vary from inhibition[13] to enhancement.[14] The newer AEDs have been little studied.[15] With respect to levetiracetam (LEV), no adverse skeletal effects were reported by the manufacturer before registration of Keppra® (UCB Pharma).

Evaluation of bone quality

Although the diagnosis of osteoporosis or AED-induced osteopenia currently is based on measurement of bone mineral density (BMD), additional factors are known to increase fracture risk, independent of BMD.[16,17] Among these, bone turnover reflects changes in the rate of bone remodelling, and is assessed by measurements of alterations in biochemical markers of bone formation and resorption. Bone strength also depends upon structural and material properties (e.g. micro-architecture, geometry and matrix composition),[18] which are assessed by histomorphological methods. Whereas in human studies fracture risk is the primary clinical endpoint, methods for *in vivo* bone quality evaluation are not available in a clinical setting. Instead, surrogate measures like biomechanical strength (three-point bending) can be applied in animal models.

Adolescent female rats treated with AEDs

In a recent study we investigated various aspects of bone health in growing female rats treated with clinically relevant doses of LEV, PHT and VPA.[19] We used female Wistar rats at 80 days of age, comparable to human adolescents.[4,5] To ascertain that the dose of the respective AEDs reproduced serum concentrations corresponding to the therapeutic ranges for humans, serum concentrations were measured and the doses adjusted accordingly (Table 1).[19] The chosen drug dose duration (90 days, representing about 20 oestrous cycles in rats, corresponding to ca. 2 years of menstrual cycles in women) was considered sufficient to mimic long-term AED treatment in humans, well above the minimum duration for women taking part in studies of AEDs and endocrine effects.[20] As shown in Table 1, weight gain was normal with no significant differences between the treatment groups.

Table 1 Weight and AED serum concentrations after 90 days of drug administration

Groups	(1) Low-dose LEV ($n = 14$)	(2) High-dose LEV ($n = 19$)	(3) PHT ($n = 15$)	(4) VPA ($n = 17$)	(5) Control ($n = 23$)
Administered dose (mg/kg)	50	150	50	300	—
Weight (g; mean ± S.D.)	249 ± 10	253 ± 26	242 ± 8	252 ± 17	252 ± 15
Serum drug concentration (μmol/l; mean ± S.D.)	122 ± 41	277 ± 65	37 ± 14	431 ± 248	—

The dose (administered twice daily) for each drug is shown, together with weight at the end of the study and AED serum concentrations (measured 4 h after last dose administration). Drug doses fed to rats were higher than those commonly used in humans, whereas the actual serum drug concentrations were not. Although serum levels of LEV in the high-dose group exceeded the commonly used therapeutic range, such levels can be observed in patients without side-effects. Reproduced with permission from.[19.]

Measurements of bone mass by DXA (Dual energy X-ray Absorptiometry) demonstrated that BMC/BMD tended to be reduced by VPA and PHT, whereas LEV did not induce changes in these parameters (Figure 1A). Biomechanical strength (assessed by three-point cantilever bending) of the femoral neck was significantly reduced by low-dose LEV only (Figure 1B). By comparison, no significant effects were observed in the strength of the femoral shaft (diaphysis) for any of the AEDs, although PHT, VPA and high-dose LEV tended to reduce strength (Figure 1C). Bone turnover was assessed by monitoring changes in serum levels of osteocalcin (bone formation marker) and RatLaps (bone resorption marker; collagen degradation products). The results showed that VPA increased bone turnover, whereas low-dose LEV reduced bone formation (Figure 1D). Histomorphometric analysis of the femoral neck indicated increased content of cartilage remnants in the metaphysis from LEV-treated versus control rats, whereas the relative trabecular bone volume and bone marrow space tended to be decreased in
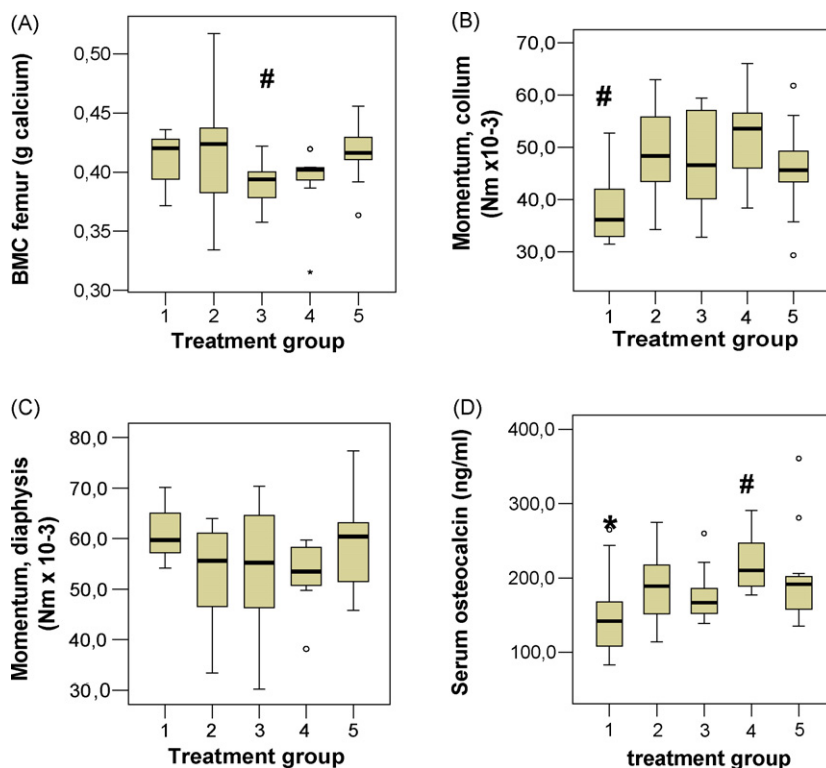

Figure 1 Bone quality parameters evaluated in AED-treated rats. (A) Bone mineral content (BMC) of total femur. $^{\#}p < 0.05$ when all groups were compared using Kruskal—Willis test. Post hoc tests for comparing single groups were not significant. (B) Biomechanical strength (ultimate momentum) of femoral collum. $^{\#}p < 0.05$ compared to groups 2 and 4 (MANOVA/Bonferroni post hoc test). (C) Ultimate momentum of the femoral diaphysis. (D) Serum concentration of the bone formation marker osteocalcin. Comparison of all groups with Kruskal—Wallis test: $p = 0.007$. $^{*}p < 0.05$ compared to group 5 (Dunnett t-test), $^{\#}p < 0.05$ compared to group 1 (Bonferroni). Treatment groups: (1) low-dose LEV; (2) high-dose LEV; (3) PHT; (4) VPA; (5) Control. The figure is modified from.[19]
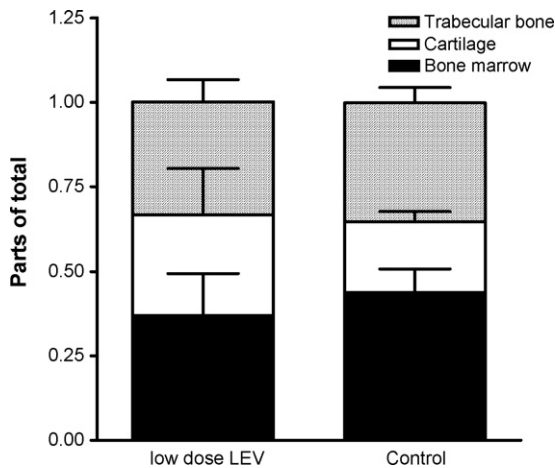
Figure 2 Morphological parameters of femurs from rats treated with low-dose LEV compared to vehicle-treated controls. Results represent the mean ± S.D. of 6 animals in each group. Reproduced with permission from.[19]

the LEV-treated bones (Figure 2). These structural differences in the properties of bone and cartilage are likely of importance for the observed change in biomechanical strength induced by low-dose LEV (Figure 1B).

Effect of VPA and PHT on adolescent female rats

Our findings that VPA and PHT treatment resulted in reduced bone mass at various skeletal sites, paralleled by increased bone turnover (for VPA), and a slightly reduced mechanical strength in the femoral shaft of these bones,[19] are consistent with the increased fracture risk associated with these drugs in humans.[1,2,16] Furthermore, they are in concordance with experimental data from male rats treated with AEDs,[6,8] and indicate different mechanisms of action for VPA and PHT in bone.

LEV has a biphasic effect on bone

In this first report on effects of clinically relevant concentrations of LEV on bone,[19] our data demonstrate that low-dose LEV causes selective reduction of biomechanical strength of the femoral neck without affecting bone mass. Moreover, bone formation is significantly reduced in LEV-treated rats as assessed by decreased serum osteocalcin levels relative to controls. Although previous studies did not report any effect of LEV on bone mass,[15,21] these results suggest that skeletal health may still be compromised by this drug. Since high-dose LEV does not impact these bone parameters, our data sug-

gests a dose-dependent, biphasic effect of LEV in bone. Similarly, the more profound effect of low-dose than high-dose LEV on serum estradiol levels in the same rats [22] support this notion.

The femoral neck represents metabolically active trabecular bone, and is also the site where epilepsy patients are at increased risk for fractures.[1,2] One possible mechanism by which LEV might cause impaired strength of the femoral neck is by alteration of bone microstructure/architecture. Consistent with this, histomorphometric data indicated increased content of cartilage remnants in the metaphysis of LEV-treated animals versus controls.[19] Bone trabeculae containing relatively more cartilage and therefore relatively less mineralized bone, would likely show impaired bone strength of the femoral neck. Prepubertal rather than fertile animals are susceptible to the effects of LEV on endocrine function.[22,23] Taken together, these animal studies suggest that young individuals, in particular, should be carefully monitored in future clinical studies for potential impairment of bone growth due to disturbances in the microstructure of the epiphyseal growth plate.

Thus, the effect of low-dose LEV on bone strength in skeletally immature rats may have consequences for longitudinal skeletal growth and fracture risk. Because of the differences in bone growth and metabolism between rats and humans, human observations in epidemiologic studies including fracture data are needed to clarify this issue. LEV prescription for children and adolescents with epilepsy is growing, therefore these patients should be monitored for signs of AED-related skeletal disturbances. Recommendations for monitoring of bone health in AED-treated patients focus on DXA measurements to detect reduced bone mass,[16,24] but official guidelines for prevention of bone loss in epilepsy patients do not exist. The knowledge that LEV-treated rats display compromised bone strength, reflecting alterations in bone structure/material properties and bone turnover rate, despite normal BMD, suggests increased fracture risk for long-term users of this drug.[2,25] Thus, controlled clinical studies are necessary to assess the effect of LEV on bone development and growth in children and adolescents, eventually resulting in new therapeutic guidelines.

Conclusion

Accumulating evidence that AED treatment in epilepsy patients not only reduces bone mass, but also affects bone quality and strength,[2] emphasize the need for experimental animal models to assess the

safety and mechanisms of action for new AEDs in the skeleton. Monitoring of bone quality is problematic in humans; animal models provide an additional system in which the properties of bone and the influence of drugs can be explored, isolated from disease, lifestyle and other confounding factors. Although side effects of the newer AEDs may seem subtle, they might be important in an epidemiologic perspective. LEV, PHT and VPA have differential effects on bone mass, structure and metabolism.[19] Notably, our data indicate that LEV does not affect bone mass, but has a biphasic effect on bone formation indices and biomechanical bone strength of the femoral neck (mainly trabecular bone). Finally, the lack of treatment guidelines for patients on AED therapy in order to reduce fracture risk is discussed.

Conflict of interest statement

None.

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