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# EFFECTS OF VIGABATRIN ON THE SKELETAL SYSTEM OF YOUNG RATS

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**Abstract:** Long-term administration of antiepileptic drugs may be connected with the risk of impairment of bone remodeling. Contrary to the reported unfavorable effect of classic antiepileptic drugs on bone metabolism, little is known about the effect of the next generation antiepileptics on bone remodeling. The aim of the present study was to investigate the effect of vigabatrin, as a representative of new antiepileptics, on the skeletal system of young rats, in comparison with conventional drugs – phenytoin and valproic acid. The experiments were carried out on 4-week-old male Wistar rats, divided into the control rats, and rats receiving vigabatrin (250 mg/kg *p.o.* daily), phenytoin (20 mg/kg *p.o.* daily) or valproic acid (250 mg/kg *p.o.* daily). The drugs were administered for 28 days. Histomorphometric parameters of the tibia and femur, mechanical properties of the femur, and bone length, diameter, mass, content of mineral substances and calcium were examined. After administration of phenytoin or valproic acid, the investigated bone parameters did not significantly differ from those observed in the control rats. Administration of vigabatrin caused profound impairment of bone accrual with impairment of bone histomorphometric parameters, along with the significant decrease in the body mass gain.

**Keywords:** vigabatrin, phenytoin, valproic acid, skeletal system, rats

Antiepileptics belong to the drugs which have traditionally been considered as bone damaging (1, 2). The connection between the use of antiepileptic drugs and bone disease has been recognized for almost 40 years (3). Administration of antiepileptic drugs may lead to the development of osteomalacia (rickets in children) or osteoporosis (4). However, little is known about the mechanisms leading to the unfavorable bone changes. The osteopathia associated with antiepileptic drug administration affects patients of both sexes and at all ages (3). Children are especially susceptible to negative effects on the skeleton because they are in a growth phase (4).

In the recent years, numerous drugs have been introduced to the treatment of epilepsis. Antiepileptic drugs generally may be divided to the older, classic antiepileptic drugs (such as benzodiazepines, carbamazepine, phenytoin, phenobarbital, primidone, valproic acid) and the newer, next generation, antiepileptic drugs (gabapentin, lacosamide, lamotrigine, levetiracetam, oxcarbazepine, rufinamide, topiramate, vigabatrin, zonisamide). The skeletal effects of the newer drugs are much less recognized [5–7]. The aim of the present study was to investigate the effect of a new generation drug, vigabatrin, on the skeletal system of young, rapidly growing rats. Effects of vigabatrin on the skeletal system have not been reported so far. For comparison, we also studied the effects of classic antiepileptic drugs, phenytoin and valproic acid.

## METHODS

The experiments were carried out on 4-week-old male Wistar rats (body mass at the beginning of the experiment: 75–95 g), fed a standard diet (Labofeed B) *ad libitum*. The diet contained: calcium 0.87%, phosphorus 0.74% and vitamin $D_3$ 1600 IU/kg. The rats were obtained from the Center of Experimental Medicine, Medical University of Silesia. The procedure of the experiments on animals was approved by the Local Ethics Commission, Katowice, Poland.

The animals were divided into four groups (n = 10): I – control rats; II – rats treated with vigabatrin (Sabril, Marion Merrell Ltd., 250 mg/kg *p.o.* daily); III – rats treated with phenytoin (Phenytoinum, WZF Polfa, 20 mg/kg *p.o.* daily); IV – rats treated with valproic acid (Convulex 300, Gerot Pharmazeutica GmbH, 250 mg/kg *p.o.* daily).

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The drugs were dissolved or suspended in distilled water with addition of Tween 20. The drugs were administered by a stomach gavage once daily for 4 weeks. The control rats received the vehicle in the same volume of 2 mL/kg *p.o.* daily. The animals were weighed every day.

In order to mark the calcification front, one day before the start and on the last day of administration of the drugs or the vehicle, the animals were given tetracycline hydrochloride (Sigma-Aldrich, 20 mg/kg *i.p.*). After 4 weeks of administration of the antiepileptics or vehicle, the animals were killed and

Table 1. Effects vigabatrin, phenytoin and valproic acid, administered for 28 days, on the body mass, and bone mass, length and diameter in young rats.

| | Control | Vigabatrin 250 mg/kg *p.o.* daily | Phenytoin 20 mg/kg *p.o.* daily | Valproic acid 250 mg/kg *p.o.* daily |
|---|---|---|---|---|
| Body mass [g] | | | | |
| – initial | 87.0 ± 1.8 | 84.1 ± 1.6 | 84.4 ± 1.2 | 83.5 ± 1.0 |
| – after 4 weeks | 212.0 ± 2.1 | 174.0 ± 7.1*** | 217.5 ± 3.7 | 214.3 ± 5.2 |
| Bone mass [mg] | | | | |
| – femur | 623.9 ± 9.4 | 528.9 ± 17.5*** | 618.2 ± 8.5 | 630.6 ± 9.5 |
| – tibia | 438.9 ± 8.1 | 371.0 ± 10.3*** | 427.6 ± 10.0 | 441.5 ± 8.1 |
| – L-4 vertebra | 171.0 ± 5.9 | 141.3 ± 7.2** | 160.5 ± 6.2 | 157.6 ± 6.7 |
| Bone mass/body mass [mg/100 g body mass] | | | | |
| – femur | 294.5 ± 4.7 | 305.6 ± 7.6 | 284.7 ± 4.6 | 295.2 ± 6.7 |
| – tibia | 207.0 ± 2.6 | 214.8 ± 5.6 | 197.0 ± 5.4 | 206.9 ± 6.1 |
| – L-4 vertebra | 80.8 ± 3.0 | 81.2 ± 2.1 | 73.9 ± 2.9 | 73.8 ± 3.4 |
| Bone length [mm] | | | | |
| – femur | 32.6 ± 0.1 | 3`0.4 ± 0.4*** | 32.5 ± 0.2 | 32.2 ± 0.2 |
| – tibia | 35.1 ± 0.3 | 33.1 ± 0.4** | 35.4 ± 0.4 | 35.0 ± 0.2 |
| Bone diameter [mm] | | | | |
| – femur | 3.21 ± 0.03 | 3.11 ± 0.04* | 3.24 ± 0.03 | 3.26 ± 0.02 |
| – tibia | 2.43 ±.0.02 | 2.31 ± 0.03** | 2.42 ± 0.02 | 2.39 ± 0.02 |

Results are the means ± SEM (n = 10). One way ANOVA followed by Duncan's test or, when appropriate, Kruskal-Wallis ANOVA followed by Mann-Whitney U test were performed to evaluate the significance of the results. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ – significantly different from the control rats.

Table 2. Effects vigabatrin, phenytoin and valproic acid, administered for 28 days, on mass of mineral substances and calcium content in bones of young rats.

| | Control | Vigabatrin 250 mg/kg *p.o.* daily | Phenytoin 20 mg/kg *p.o.* daily | Valproic acid 250 mg/kg *p.o.* daily |
|---|---|---|---|---|
| Mass of bone mineral [mg] | | | | |
| – femur | 200.6 ± 3.8 | 172.5 ± 5.6** | 192.7 ± 2.8 | 201.1 ± 2.7 |
| – tibia | 141.7 ± 2.6 | 123.8 ± 3.6*** | 138.0 ± 3.7 | 144.7 ± 2.5 |
| – L-4 vertebra | 52.2 ± 0.7 | 42.6 ± 1.9*** | 48.6 ± 0.9 | 49.6 ± 1.4 |
| Mass of bone mineral/bone mass [mg/100 mg bone mass] | | | | |
| – femur | 32.2 ± 0.5 | 32.6 ± 0.4 | 31.2 ± 0.4 | 31.9 ± 0.3 |
| – tibia | 32.3 ± 0.4 | 33.4 ± 0.3 | 32.3 ± 0.4 | 32.8 ± 0.2 |
| – L-4 vertebra | 30.8 ± 0.8 | 30.3 ± 0.6 | 30.5 ± 0.6 | 31.6 ± 0.6 |
| Calcium content [mg/g bone mineral] | | | | |
| – femur | 364.8 ± 11.7 | 351.0 ± 8.0 | 366.6 ± 10.9 | 361.2 ± 11.4 |
| – tibia | 319.4 ± 8.0 | 325.4 ± 8.8 | 318.9 ± 12.5 | 308.9 ± 5.4 |
| – L-4 vertebra | 322.0 ± 6.4 | 296.3 ± 14.9 | 309.1 ± 12.4 | 286.1 ± 6.6 |

Results are the means ± SEM (n = 10). One way ANOVA followed by Duncan's test or, when appropriate, Kruskal-Wallis ANOVA followed by Mann-Whitney U test were performed to evaluate the significance of the results. ** $p < 0.01$, *** $p < 0.001$ significantly different from the control rats.

the right and left tibial and femoral bones and L-4 vertebra were isolated. In the isolated left bones, mass and macrometric parameters were determined (length, diameter of the diaphysis in the mid-length). Histological specimens were prepared from the right femoral and tibial bones, as previously described [8–10]. The histomorphometric measurements were made using an Optiphot-2 microscope (Nikon), connected through an RGB camera (Cohu) to a personal computer (program Lucia G 4.51, Laboratory Imaging), with final magnifications of 200 and 500 times, and a lanameter (magnification 50 times). Transverse cross-sections made from the tibial diaphysis served for the measurements of the periosteal and endosteal transverse growth, the width of periosteal and endosteal osteoid, the area of the transverse cross-section of the diaphysis and the area of the transverse cross-section of the marrow cavity. In the longitudinal preparations from the distal femoral epiphysis, the width of epiphyseal cartilage and the width of trabeculae in the epiphysis and metaphysis were determined.

Bone mechanical properties were assessed using the set constructed at the Department of Pharmacology, Medical University of Silesia, in cooperation with Hottinger Baldwin Messtechnik GmbH. Mechanical properties of the whole femur and the femoral neck were examined, as previously described (10–12).

Mechanical properties of whole left femurs were studied using a bending test with three-point loading. The load was applied perpendicularly to the long axis of the femur in the mid-length of the bone supported on its epiphysis. The load increased at a rate of 100 N/min. The load-deformation curves, obtained for each bone, representing the relationships between load applied to the bone and deformation in response to the load, were analyzed. The extrinsic stiffness of bone (the slope of the load-deformation curve), the ultimate load (the maximum load sustained by the bone) and the deformation caused by the ultimate load were determined.

Mechanical properties of the right femoral neck were studied using a compression test. The

Table 3. Effects of vigabatrin, phenytoin and valproic acid, administered for 28 days, on bone histomorphometric parameters in young rats.

| | Control | Vigabatrin 250 mg/kg *p.o.* daily | Phenytoin 20 mg/kg *p.o.* daily | Valproic acid 250 mg/kg *p.o.* daily |
|---|---|---|---|---|
| Width of trabeculae in the femur [μm]: | | | | |
| – epiphysis | 58.3 ± 1.1 | 54.6 ± 0.7* | 55.9 ± 0.9 | 55.9 ± 0.7 |
| – metaphysis | 35.9 ± 0.6 | 32.5 ± 0.4*** | 33.6 ± 0.3* | 36.5 ± 0.9 |
| Width of epiphyseal cartilage in the femur [μm]: | 150.0 ± 9.5 | 151.6 ± 7.2 | 144.0 ± 8.0 | 145.5 ± 9.0 |
| Width of osteoid in the tibia [μm] | | | | |
| – periosteal | 13.6 ± 0.9 | 12.5 ± 0.6 | 14.1 ± 0.7 | 13.1 ± 0.9 |
| – endosteal | 11.1 ± 0.5 | 9.8 ± 0.3 | 12.3 ± 0.7 | 10.8 ± 0.4 |
| Transverse growth in the tibia [μm] | | | | |
| – periosteal | 176.0 ± 8.4 | 147.1 ± 6.1* | 162.0 ± 7.5 | 177.3 ± 9.3 |
| – endosteal | 72.5 ± 3.6 | 58.3 ± 2.1** | 71.4 ± 4.7 | 75.9 ± 4.9 |
| Transverse cross-section area of the tibial diaphysis [$mm^2$] | 3.5 ± 0.1 | 3.2 ± 0.1** | 3.6 ± 0.1 | 3.6 ± 0.0 |
| Transverse cross-section area of the tibial marrow cavity [$mm^2$] | 1.1 ± 0.1 | 0.9 ± 0.0* | 1.1 ± 0.1 | 1.0 ± 0.0 |
| Transverse cross-section area of the marrow cavity/transverse cross-section area of the diaphysis ratio in the tibia | 0.316 ± 0.014 | 0.299 ± 0.014 | 0.315 ± 0.011 | 0.287 ± 0.011 |

Results are the means ± SEM (n = 10). One way ANOVA followed by Duncan's test were performed to evaluate the significance of the results. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ significantly different from the control rats.

Table 4. Effects of vigabatrin, phenytoin and valproic acid, administered for 28 days, on the mechanical properties of the femur in young rats.

| | Control | Vigabatrin 250 mg/kg *p.o.* daily | Phenytoin 20 mg/kg *p.o.* daily | Valproic acid 250 mg/kg *p.o.* daily |
|---|---|---|---|---|
| Extrinsic stiffness of the femur [N/mm] | 169.8 ± 7.0 | 148.5 ± 9.7 | 156.0 ± 6.1 | 185.5 ± 13.5 |
| Ultimate load [N] | 77.7 ± 3.2 | 70.9 ± 3.2 | 75.7 ± 2.7 | 82.4 ± 3.7 |
| Deformation at ultimate load [mm] | 0.53 ± 0.04 | 0.56 ± 0.04 | 0.56 ± 0.03 | 0.50 ± 0.05 |
| Load at fracture of the femoral neck [N] | 77.7 ± 5.2 | 70.1 ± 6.4 | 74.1 ± 3.2 | 72.2 ± 4.1 |

Results are the means ± SEM (n = 10). One way ANOVA followed by Duncan's test were performed to evaluate the significance of the results.

load was applied to the head of the femur along the long axis of the femur. The load causing the fracture of the femoral neck was determined.

To determine the content of mineral substances in bones, the L-4 vertebra, left tibia and femur were mineralized at the temperature of 640°C for 48 h and weighed. Calcium content in the mineralized bones dissolved in 6 M HCl was then determined colorimetrically, using a kit produced by Pointe Scientific Inc.

Results are presented as the mean ± SEM. Statistical estimation was performed using ANOVA followed by *post-hoc* Duncan's test. When appriopriate (lack of homogeneity of variance), Kruskal-Wallis ANOVA, followed by Mann-Whitney U test, was used to determine specific differences.

## RESULTS

### Effects on the body mass gain, bone mass and macrometric parameters

Vigabatrin (250 mg/kg *p.o.* daily for 4 weeks) decreased body mass gain (Table 1). The mass, length and diameter of the isolated bones were also decreased in comparison with the control rats. However, the ratio of the bone mass to body mass remained unchanged.

Phenytoin (20 mg/kg *p.o.* daily for 4 weeks) and valproic acid (250 mg/kg *p.o.* daily for 4 weeks) did not affect body mass gain, bone mass and macrometric parameters.

### Effects on the mass of mineral substances and calcium content in bones

Only after administration of vigabatrin, the mass of bone mineral was decreased in comparison with the control rats (Table 2). However, vigabatrin did not affect the ratio of the mass of bone mineral to bone mass, and the calcium content in the bone mineral. Phenytoin and valproic acid did not affect bone mineral mass, mineralization and calcium content.

### Effects on bone histomorphometric parameters

Administration of vigabatrin significantly affected both the cancellous and compact bone (Table 3). It caused decreases in the width of trabeculae in the femoral epiphysis and metaphysis, and decreases in the periosteal and endosteal transverse growth, transverse cross-section area of the marrow cavity and the diaphysis in the tibia in comparison with the control rats. However, the ratio of the transverse cross-section area of the marrow cavity to the transverse cross-section area of the diaphysis remained unaffected.

Phenytoin caused only a significant decrease in the width of metaphyseal trabeculae in the femur. Valproic acid did not affect the bone histomorphometric parameters in rats.

### Effects on bone mechanical properties

None of the investigated antiepileptics significantly affected the mechanical properties of the femur (Table 4). Only vigabatrin tended to worsen the strength of the whole femur and the femoral neck.

## DISCUSSION

Vigabatrin is an analogue of γ-aminobutyric acid (GABA), which irreversibly inhibits GABA transaminase, increasing GABA levels. Vigabatrin

may be used in infantile spasms and as adjunctive therapy for adult patients with refractory complex partial seizures who have inadequately responded to several alternative treatments (6). However, it may cause severe adverse effects; a high proportion of patients treated with vigabatrin have developed irreversible visual field defects. Vigabatrin should be used when the potential benefits outweigh the risk of vision loss (6, 13). The effects of vigabatrin on the skeletal system have not been studied so far.

In the present study, the experiments were performed on young rats (4-week-old in the beginning of the study), because epilepsy frequently occurs in children. Childhood is a critical period for the accrual of bone mineral density. Its deficits acquired during childhood may potentially increase the risk of developing osteoporotic fractures later in life (14). Bone mineral content or density may be decreased in children with epilepsy either as a consequence of the disease, the condition that caused it, or the treatment of the disease (4).

Vigabatrin and valproic acid were used at the dose 250 mg/kg *p.o.* daily, which was similar to those used in previous experimental studies on rats to exert significant central effects (15–19). Phenytoin (20 mg/kg *p.o.* daily) was used at the dose reported to exert central effects [20] and to affect the skeletal system after *s.c.* administration (21).

Numerous studies suggest that patients with epilepsy treated with antiepileptic drugs may be at

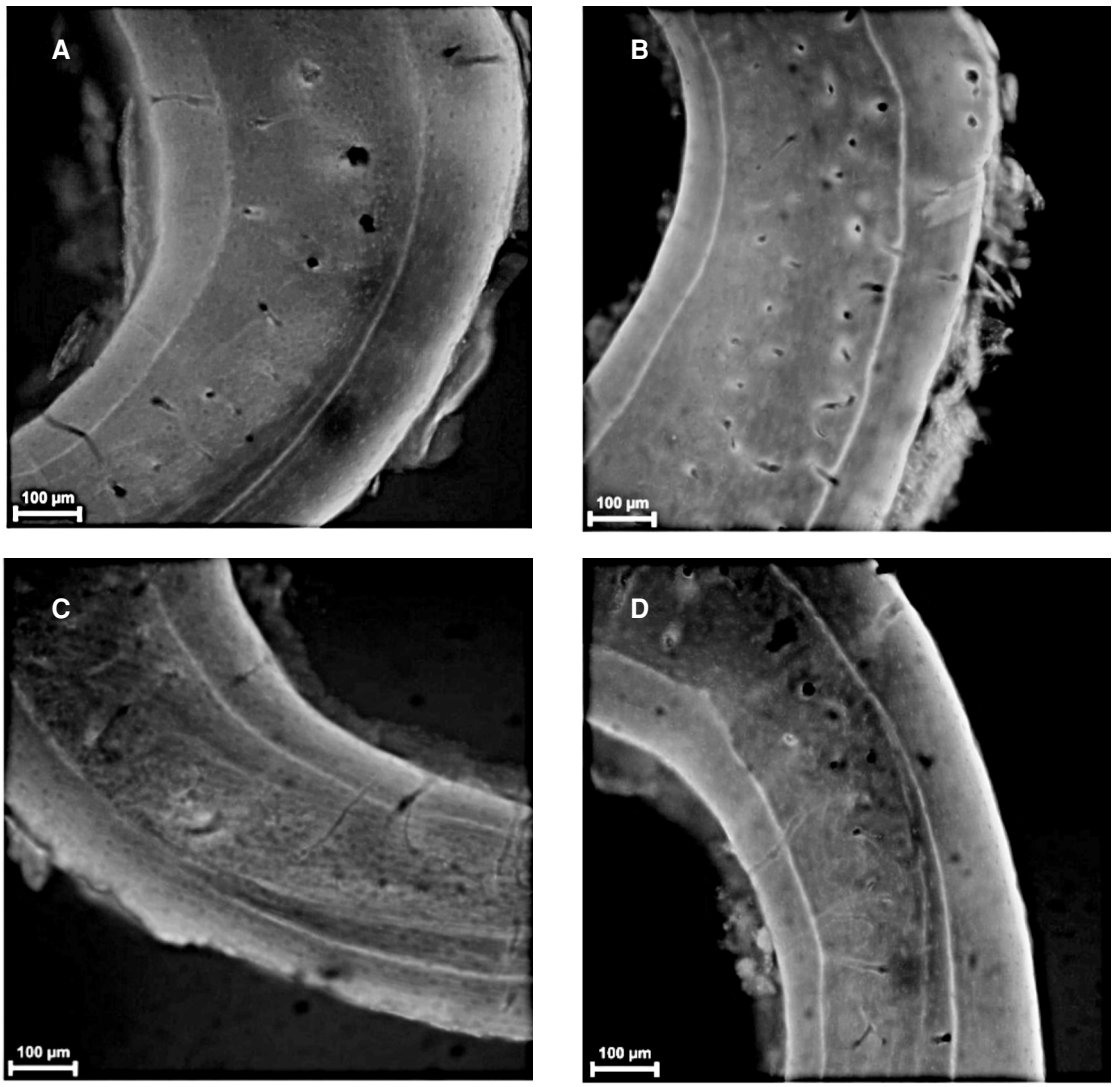


Figure 1. Transverse growth in the tibial diaphysis of young rats treated with antiepileptic drugs for 4 weeks. Tetracycline hydrochloride was administered on days 0 and 28. The distance between tetracycline stripes was measured on the periosteal and endosteal side, in UV light. From the left, preparations from: a control rat (A), rat treated with vigabatrin (250 mg/kg daily) (B), rat treated with phenytoin (20 mg/kg daily) (C), rat treated with valproic acid (250 mg/kg daily) (D)

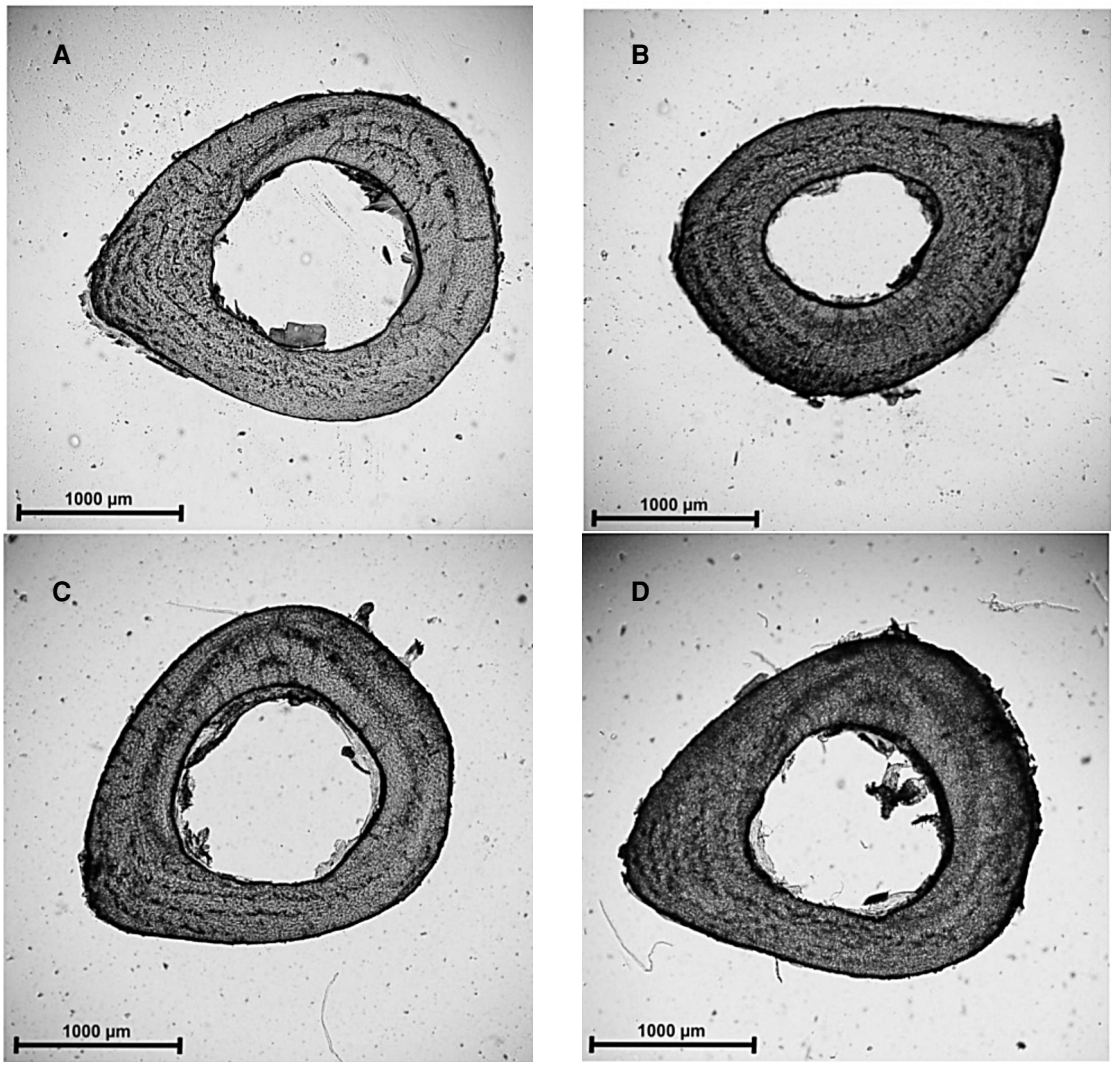


Figure 2. Transverse cross-sections of the tibial diaphysis of young rats treated with antiepileptic drugs for 4 weeks. From the left, preparations from: a control rat (A), rat treated with vigabatrin (250 mg/kg daily) (B), rat treated with phenytoin (20 mg/kg daily) (C), rat treated with valproic acid (250 mg/kg daily) (D)

an increased risk for bone disease including changes in bone turnover, osteoporosis, alterations in bone quality, and fracture (22–29). However, there is still a very limited understanding of the mechanisms of the effect of antiepileptic drugs on bone health, as well as on the nature of the bone disease (osteoporosis or osteomalacia) (28). Moreover, patients with epilepsy have an increased risk of fractures; this increase is mainly linked to fractures sustained during seizures. Recent studies have only demonstrated a very limited increase in the risk of fractures with the use of some but not all antiepileptic drugs (30). Contrary to other drugs unfavorably affecting the skeletal system (for example glucocorticosteroids or immunosuppressants), there are only a few experimental studies concerning the skeletal effects of antiepileptic drugs (21, 31–40)

Several mechanisms responsible for the effects of conventional antiepileptic drugs on the skeletal system have been proposed (41). The most often proposed mechanism is causing vitamin D deficiency, attributed to acceleration of vitamin D catabolism. Phenytoin and phenobarbital and some other antiepileptic drugs, like carbamazepine, oxcarbazepine and primidone are inducers of hepatic enzymes, however, clonazepam, ethosuximide, lamotrigine, tiagabine, topiramate, valproate and vigabatrin are not (4). Other possible mechanisms are: secondary hyperparathyroidism resulting from decreased calcium owing to hypovitaminosis D, cal-

citonin deficiency, vitamin K deficiency, deprived estrogen levels (41). Phenytoin was reported to directly affect bone cell activity 42–44).

In the present study, the investigated bone parameters after administration of phenytoin (20 mg/kg *p.o.* daily) or valproic acid (250 mg/kg *p.o.* daily) for 4 weeks did not significantly differ from those observed in the control rats (with the exception of a slight unfavorable effect of phenytoin on the width of femoral metaphyseal trabeculae). Both drugs are among the most frequently reported to worsen bone properties in epileptic patients. However, there is a problem with interpretation of adverse bone effects in patients treated with antiepileptics, since the disease itself may lead to the symptoms. According to Vestergaard (30), much of the increase in fracture risk may be due to the underlying disorder and the severity of seizures rather than to the drugs used to treat epilepsy and, from a fracture point of view, most antiepileptic drugs seem to be relatively safe.

The duration of the drug treatment in the present study (4 weeks) was sufficient to develop significant skeletal changes due to estrogen deficiency, glucocorticoid excess, and drug treatment in our previous studies (8–12). However, it must be stated that phenytoin and valproic acid were reported to exert unfavorable effects on the skeletal system of adult rats after longer treatment periods (32, 33). Decreases of bone mineral density were also observed in young rats after administration of phenytoin (20 mg/kg *s.c.*) for 5 weeks (21).

Although the conventional drugs did not affect the skeletal system of the growing rats in the present study, vigabatrin, a next generation antiepileptic drug, caused significant changes.

Administration of vigabatrin (250 mg/kg *p.o.* daily) in immature rats caused decreased body mass gain and inhibition of compact bone growth with concomitant worsening of histomorphometric parameters of the cancellous bone. Bone macrometric parameters and mass were significantly decreased, however, the ratio of bone mass to the body mass remained unaffected. The decrease in the width of trabeculae in the femoral epiphysis and metaphysis might have been the effect of the decreased bone formation and/or increased bone resorption. In the compact bone of the tibia, the periosteal and endosteal transverse growth as well as the transverse cross-section area of the tibial diaphysis were decreased (decreased bone formation), and the transverse cross-section of the tibial marrow cavity was decreased. Since the ratio of the transverse cross-section area of the marrow cavity to the diaphysis area in the tibia remained unaffected, it may be concluded that vigabatrin did not increase the resorption of the compact bone. Vigabatrin did not affect bone mineralization (the ratio of bone mineral mass to bone mass) and calcium content. The decrease in bone mass and worsening of bone histomorphometric parameters resulted in the tendency to worsen bone mechanical properties.

However, in the present study, vigabatrin caused a significant decrease in the body mass gain, consistently with previous observations in rats (45). It is possible that the observed worsening of the bone status in comparison with the control rats was the result of the decreased body mass. Since, in humans, vigabatrin not only did not decrease body mass, but even increased it (6), it is possible that the effect of vigabatrin on the human skeletal system would be less marked than that in rats.

Nevertheless, results of the present study are consistent with the recently published work of Padmanabhan et al. (46), who reported on fetal loss, as well as fetal growth restriction and skeletal hypoplasia, induced by vigabatrin administered during gestation in mice. The decreases in maternal folate and vitamin $B_{12}$ concentrations seemed to play an important role in development of the disorders (46). The mechanisms responsible for the skeletal changes observed in immature rats need to be elucidated.

In conclusion, results of the present study demonstrate that the use of vigabatrin in growing organisms may cause impairment of the skeletal system. The effects of vigabatrin were much stronger than those of the classic antiepileptic drugs, which after 4 weeks of administration were practically negligible.

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