Bone Density and Bone Turnover Markers in Patients With Epilepsy on Chronic Antiepileptic Drug Therapy

artigo original

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ABSTRACT

In this comparative, cross-sectional study, we evaluated 55 patients with epilepsy on chronic use of antiepileptic drugs (AED); [(38 females and 17 males, 35 ± 6 years (25 to 47)] and compared to 24 healthy subjects (17 females/7 males). Laboratorial evaluation of bone and mineral metabolism including measurements of bone specific alkaline phosphatase (BALP) and carboxyterminal telopeptide of type I collagen (CTX-I) were performed. Bone mineral density (BMD) was measured by DXA. BALP and CTX-I levels did not differ significantly between the groups. CTX-I levels were significantly higher in patients who were exposed to phenobarbital (P< 0.01) than those who were not. Patients presented BMD of both sites significantly lower than the controls (0.975 ± 0.13 vs. 1.058 ± 0.1 g/cm^2; p= 0.03; 0.930 ± 0.1 vs. 0.988 ± 0.12 g/cm^2; p= 0.02, respectively). Total hip BMD (0.890 ± 0.10 vs. 0.970 ± 0.08 g/cm^2; p< 0.003) and femoral neck (0.830 ± 0.09 vs. 0.890 ± 0.09 g/cm^2; p< 0.03) were significantly lower in patients who had been exposed to phenobarbital, in comparison to the non-phenobarbital users. In conclusion, patients on AED demonstrate reduced BMD. Among the AED, phenobarbital seems to be the main mediator of low BMD and increases in CTX-I. **(Arq Bras Endocrinol Metab 2007;51/3:466-471)**

Keywords: Antiepileptic drugs; Phenobarbital; Bone turnover markers

RESUMO

Densidade Óssea e Marcadores de *Turnover* Ósseo em Pacientes com Epilepsia em Tratamento Crônico com Drogas Antiepilépticas.
Neste estudo comparativo, transversal, 55 pacientes com epilepsia [38 mulheres e 17 homens; 35 ± 6 anos (25 a 47anos)] foram comparados com 24 indivíduos normais (17 mulheres / 7 homens). Foi realizada uma avaliação laboratorial do metabolismo ósseo e mineral incluindo a dosagem de fosfatase alcalina específica óssea (BALP) e telopeptídeo carboxiterminal do colágeno tipo I (CTX-I). Densidade mineral óssea (DMO) da coluna lombar e do fêmur foi medida por DXA. BALP e CTX-I não foram diferentes entre os grupos. CTX-I foi significativamente mais elevado nos pacientes expostos ao fenobarbital do que os que não usaram essa medicação (p< 0,01). DMO de ambos os sítios foi menor no grupo de pacientes (0,975 ± 0,13 vs. 1,058 ± 0,1 g/cm^2; p= 0,03; 0,930 ± 0,1 vs. 0,988 ± 0,12 g/cm^2; p= 0,02, respectivamente). DMO do fêmur total (0,890 ± 0,10 vs. 0,970 ± 0,08 g/cm^2; p< 0,003) e colo do fêmur (0,830 ± 0,09 vs. 0,890 ± 0,09 g/cm^2; p< 0,03) foi significativamente menor nos pacientes que usaram fenobarbital. Em conclusão, pacientes portadores de epilepsia em uso crônico de drogas antiepilépticas (DAE) demonstraram uma redução da DMO. Entre as DAE, o fenobarbital parece ser o principal mediador da diminuição da DMO e do aumento do CTX-I. **(Arq Bras Endocrinol Metab 2007;51/3:466-471)**

Descritores: Drogas antiepilépticas; Fenobarbital; Marcadores de *turnover* ósseo

EPILEPSY IS A CHRONIC condition that requires lifelong treatment with antiepileptic drugs (AED) (1,2). Chronic use of AED is considered to be a risk factor for secondary osteoporosis (3). In fact, others and we have demonstrated that patients who receive AEDs have alterations in bone metabolism such as a decrease in bone mineral density (BMD) and lower levels of 25-hydroxyvitamin D (25OHD), as compared to healthy matched controls (4-8). Classical AEDs associated with abnormal bone and mineral metabolism are those that induce cytochrome P450 enzymes, such as carbamazepine, phenytoin and phenobarbital (9,10). These drugs induce hepatic microsomal enzymes that increase catabolism of 25OHD (11). Other drugs that are not enzyme inducers, such as sodium valproate, may exert indirect effects on bone metabolism by altering renal function (12). In addition, it has been reported that AEDs lead to an increase in bone turnover, as demonstrated by elevated levels of bone turnover markers (13-16).

Bone turnover is a dynamic process essential to maintaining a healthy skeleton. In both men and postmenopausal women, elevated bone turnover markers have been linked to risk of fracture, independently of BMD (17).

In the present study, we evaluated serum levels of bone-specific alkaline phosphatase (BALP), bone formation marker, and carboxyterminal telopeptide of type I collagen (CTX-I), bone reabsorption marker as well as BMD in a group of patients with epilepsy being treated with AEDs. The results were compared to values in a healthy control group matched by age, gender and body mass index (BMI).

PATIENTS AND METHODS

Study design

In this comparative, cross-sectional study, we evaluated 55 patients with epilepsy [38 females and 17 males, mean ± SD of 35 ± 6 yr (25 to 47 yr)], who were followed in the Outpatient Epilepsy Clinic of the *Hospital de Clínicas da Universidade Federal do Paraná*, in Curitiba, between May 2001 and April 2003. The control group consisted of 24 healthy subjects (17 women and 7 men), matched by age, gender and BMI.

The protocol was approved by the local Institutional Ethics Committee. Subjects were included if they lived in Curitiba or its environs, were at least 25 years old, treated with AED for at least 1 year, and voluntarily agreed to participate in the study. Only women with a history of regular menses were included. Subjects with other diseases or medications known to cause or be associated with osteoporosis were excluded. All participants were evaluated at 2 time points. The initial evaluation consisted of a complete medical evaluation including a detailed chart review in order to define the specific type of AEDs, as well as their doses, during all patients follow-up. BMD and blood samples for total calcium, albumin, phosphorus, creatinine, total alkaline phosphatase (ALP), magnesium, and liver function parameters were performed. A second evaluation was performed between February and April 2003, when blood was collected for measurements of 25OHD, total testosterone (only in men), intact PTH and the bone turnover markers, BALP and CTX-I. Intact parathyroid hormone was measured in duplicate by immunochemiluminescent assay (DPC, Los Angeles, USA). The detection limit was 1 pg/ml. Intra-assay variability was less than 5.7% within the concentrations range of 72–66.2 pg/ml (normal range, 7–53 pg/ml). The 25OHD was measured in duplicate by radioimmunoassay (RIA; DiaSorin, Minnesota, USA). The detection limit was 5 ng/ml. The intra-assay variability was less than 12.5% within the concentrations range of 8.6 to 49 ng/ml (normal range: 9 to 37 ng/ml). The total serum testosterone was measured in duplicate by eletroquimiluminescence assay (Roche Diagnostics GmbH, Mannheim, Germany). The detection limit was 0.02 ng/ml (0.069 nmol/l). The variability intra-assay is lower than 4.6% to the concentrations rate of 0.24–3.45 ng/ml. The normal range varies from 280–880 ng/dl. Serum BALP and CTX-I were measured at the Anzac Research Institute, Sydney, Australia. The bone formation marker, serum BALP, was measured using an enzyme immunoassay (METRA BAP, Quide Corp., San Diego, USA) (18). The intra-assay CV was 3.9–5.8% and the inter-assay CV was 5.2–7.6%. The normal reference range is 15.0 to 41.3 U/L for men, 11.6 to 29.6 U/L for premenopausal women and 14.2 to 42.7 U/L for postmenopausal women. The bone resorption marker, serum CTX-I, was determined using an automated immunoassay (Elecsys 170, Roche Diagnostics). Intra-assay CV was approximately 2.0%. The normal reference range was variable according to age and gender: men aged 30–50 yr: 0.300 ± 0.14 ng/ml (SD), 50–70 yr: 0.304 ± 0.20 ng/ml and > 70 yr is 0.394 ± 0.23 ng/ml; and for pre and post menopausal women 0.299 ± 0.14 ng/ml and 0.556 ± 0.23 ng/ml, respectively (19).

BMD at lumbar spine, total femur, and femoral neck was evaluated using a Hologic QDR – 1000 W (Hologic, Inc., Waltham, MA) dual energy X-ray absorptiometer and was expressed as g/cm² for patients and controls.

Statistical analysis

The continuous variables with symmetric distribution were analyzed by the parametric tests: Student's *t*-test and ANOVA. For the variables that displayed asymmetric distribution, non-parametric tests were applied: Mann-Whitney and ANOVA of Kruskal-Wallis. Fisher test was used in the categorical variables. In the multiple variables analyses, models of logistic regression were used. Significance was set at $p < 0.05$.

RESULTS

Characteristics of the study population

Table 1 summarizes the main characteristics of the studied groups. The median duration of treatment with AEDs was 6 years and varied from 2 to 21 years. At the time of the study, 17 patients (31%) were receiving single antiseizure agents, while 38 (69%) were receiving multiple antiseizure agents. The most common AED used was carbamazepine (400–1500 mg/dl), taken by 44 patients, median duration time of use was 6 years (1-17). The second most common AED was phenobarbital (50–150 mg/day) used by 28 patients for 5 years (1 to 12). Phenytoin (100–400 mg/day) was taken by 23 patients for 2 years (1 to 14) and sodium valproate (500–2250 mg/day) was used by 20 patients during 3.5 years (1 to 10).

Biochemical indices of bone metabolism

Mean results of routine biochemical testing total calcium, albumin, phosphorus, magnesium, creatinine, ALP, liver function tests were normal with no statistic difference between patients and controls. At the male group, no statistic difference of serum levels of testosterone was found (4). Levels of serum 25OHD were lower in patients than controls (27.1 ± 10.3 ng/ml vs. 34.4 ± 12.8 ng/ml; p< 0.02). Serum PTH concentrations were similar between study patients and controls, although there was a trend towards higher levels in those taking AEDs (40.09 ± 12.6 pg/ml vs. 32.54 ± 11.9 pg/ml; p= 0.062). No specific AED was associated with a higher level of serum PTH than any other AED.

Serum BALP and CTX-I levels did not differ significantly between the groups (table 1). The mean level of BALP was 16.58 ± 5.74 U/L in the group of patients and 14.96 ± 4.35 U/L in the control group (p= 0.22). The median serum CTX-I value was 0.13 ng/ml (0.01–1.32) in the study group and 0.15 ng/ml (0.04–0.31) in the control group (p= 0.28). By multiple regression analyses, the variability of serum CTX-I was positively related to levels of serum ALP (p< 0.01), phosphorus (p< 0.001), and PTH (p< 0.01) (R= 0.66). Serum BALP was positively related to total ALP (p< 0.0001) (R= 0.62) but not to any other parameters.

Serum CTX-I levels were significantly higher in the 28 patients who were exposed to phenobarbital (P< 0.01) than the 27 who had not been exposed to this drug. No other biochemical abnormality was found in subjects who had been exposed to phenobarbital in comparison to those who had not (table 2). Serum BALP was not significantly different among the AED groups, although there was a tendency to higher values in patients exposed to phenobarbital versus those never exposed (p= 0.06).

Bone mineral density

Lumbar spine BMD in AED users was significantly lower than the control group (0.975 ± 0.13 g/cm^2 vs. 1.058 ± 0.1 g/cm^2; p< 0.03). Similarly, total hip BMD (0.930 ± 0.1 g/cm^2 vs. 0.988 ± 0.12 g/cm^2; p< 0.02) (table 1). No densitometric differences were found between studied subjects and controls at the femoral neck. With regard to exposure to specific AEDs, BMD of the total hip (0.890 ± 0.10 g/cm^2 vs. 0.970 ± 0.08 g/cm^2; p< 0.003) and femoral neck (0.830 ± 0.09 g/cm^2 vs. 0.890 ± 0.09 g/cm^2; p< 0.03) were significantly lower in patients who had been exposed to phenobarbital, in comparison to those who had not been exposed (figure 1). No other significant difference was found at any BMD site when other AEDs were analyzed separately. In addition, when combinations of AEDs were evaluated, only

Table 1. Baseline characteristics of the studied subjects.

	Patients (N= 55) Mean ± SD	Controls (N= 24) Mean ± SD	p value *
Age (years)	35.2 ± 6.1	34.2 ± 5.8	NS
Gender (F/M)	38/17	17/7	NS
BMI (kg/m^2)	24.3 ± 3.5	24.4 ± 4.0	NS
Lumbar spine BMD (g/cm^2)	0.975 ± 0.13	1058 ± 0.1	< 0.03
Total hip BMD (g/cm^2)	0.930 ± 0.10	0.988 ± 0.12	< 0.02
Femoral neck BMD (g/cm^2)	0.867 ± 0.09	0.889 ± 0.12	NS
25OH vitamin D	27.1 ± 10.3	34.4 ± 12.8	< 0.02
PTH (pg/ml)	40.0.9 ± 17.6	32.5 ± 12.0	NS
CTX-I (ng/ml)**	0.13 (0.01–1.32)	0.15 (0.04–0.31)	NS
BALP (IU/L)	16.58 ± 5.74	14.96 ± 4.35	NS

* p< 0.05: significative
** median; NS: non-significative; SD: standard deviation

Table 2. Biochemical data of patients exposed and never exposed to phenobarbital.

	Patients exposed to phenobarbital (N= 28)	Patients never exposed to phenobarbital (N= 27)	* p value
BALP (U/L)	17.9 ± 6.6	15.1 ± 4.3	NS
CTX-I (ng/ml)**	0.15 (0.03–1.32)	0.08 (0.01–0.30)	< 0.01
PTH (pg/ml)	43.3 ± 18.7	36.3 ± 15.8	NS
25OHD (ng/ml)	26.4 ± 11.0	29.3 ± 9.8	NS
Total hip BMD (g/cm²)	0.890 ± 0.10	0.970 ± 0.08	< 0.003
Femoral neck (g/cm²)	0.830 ± 0.09	0.890 ± 0.09	< 0.03

* p< 0.05: significative
** median; NS: non-significative; SD: standard deviation

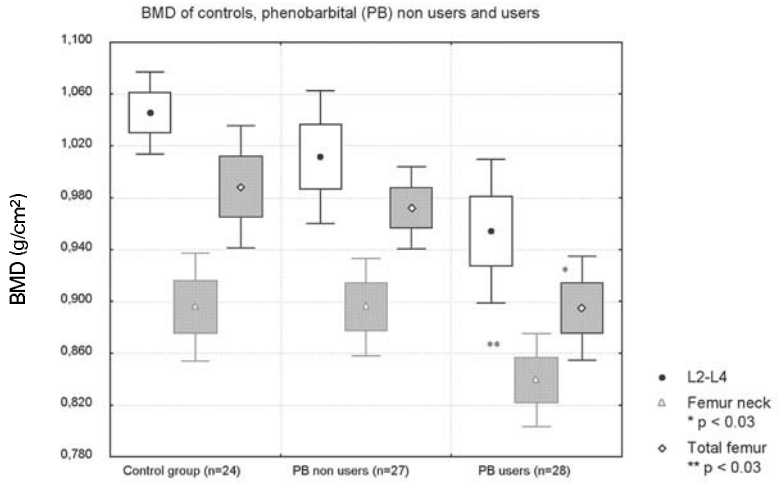

Figure 1. BMD of lumbar spine, femur neck, and total femur in controls and patients non-phenobarbital users and users.

therapeutic regimens that included phenobarbital showed a significant decrease in BMD.

By multiple regression analyses, exposure to phenobarbital accounted for variability both at the femoral neck (β= 0.27, R= 0.37, p< 0.001) and at the total hip BMD (β= 0.37, R= 0.50, p< 0.001). The relationship between phenobarbital use and BMD was also a function of time on drug (β= -0.32) and the association with other AED, such as carbamazepine (β= -0.25) and phenytoin (β= -0.21), which could explain 43% of BMD variability (R= 0.43; p< 0.001). Regarding the time on drug, greater period of exposition on phenobarbital was related to lower BMD.

DISCUSSION

This study defines further abnormalities in mineral metabolism among subjects treated with AEDs. Among users of AEDs, BMD was lower at the lumbar spine and total hip. Although there were no differences in bone turnover markers between a control group and AED users, further insight was gained when those taking phenobarbital were specifically analyzed. In subjects taking phenobarbital, serum CTX-I levels were higher along with a trend towards higher levels of serum BALP. In addition, exposure to phenobarbital was specifically associated with lower BMD at the hip. In contrast, exposure to phenytoin, carbamazepine or valproate, as mono- or polytherapy without phenobarbital, was not associated with abnormalities in bone turnover markers or BMD.

Phenobarbital is the most-widely used AED in the developing world, and because of its lower price, remains a popular choice in many industrialized countries (20). This drug is known to impair bone metabolism through several mechanisms. First, it stimulates hepatic mixed function oxidase activity, accelerating the breakdown of vitamin D metabolites (11). In addition, both phenobarbital and phenytoin interfere with active transport of calcium in the small intestine (21). These two agents have also been shown to interfere with PTH-mediated bone resorption. Both the accelerated metabolism of vitamin D and interference with

PTH action could lead to an increase in circulating PTH levels (22,23). Although the PTH levels were not significantly different between the group exposed to phenobarbital and those patients not exposed, there was a trend for PTH levels to be higher in the phenobarbital users.

Although others and we have demonstrated a decrease in 25OHD levels among chronic users of AEDs (4-6), the histomorphometric studies have established antiepileptic bone disease as a disorder of high bone turnover, more than a mineralization defect (24,25). However, there is no agreement in the literature regarding the type of the drug, as well as dose and time of exposure that might influence bone remodeling. Verrotti et al. (16) have evaluated markers of bone turnover in 60 epileptic patients in 3 different stages of pubertal growth before and after monotherapy with carbamazepine. In their study, an increase in markers of bone turnover was seen after 2 years, but vitamin D metabolism was normal in all pubertal stage groups. In contrast, 2 other studies have failed to find an association between carbamazepine and altered bone turnover (15,26). Pack et al. (15) evaluated 93 premenopausal epileptic women receiving carbamazepine, phenytoin, lamotrigine or valproate as monotherapy. Only in women treated with phenytoin were there significant elevations in serum BALP.

One of the limitations of the present study is the cross-sectional nature, where BMD was measure only once, turning it difficult to know if the low bone density observed in the patients group is specifically related to AEDs use. However, by the fact that other secondary causes were excluded and the patients presented lower BMD than the control matched group, we do believe that the use of AEDs may play a role on this issue.

Our results call attention to reduced bone mineral density among chronic users of AEDs, with a potential for major complications, such as fractures. It seems reasonable, therefore, to consider a preventative approach to maintaining skeletal health when AED therapy is initiated, especially in children and teenagers, in whom adequate calcium and vitamin D are fundamental to the establishment of bone peak mass. In addition, further studies are needed to determine appropriate therapeutic protocols for patients who present initially with low bone mass and low levels of vitamin D.

In conclusion, we have shown that patients with epilepsy on long-term AEDs demonstrate reduced BMD and lower serum levels of 25OHD. When individual effects of drugs were considered separately, phenobarbital seems to become the main mediator of low bone density and increases in serum CTX-I. Further and prospective studies with larger number of patients are needed to elucidate the influence of AED on BMD and on bone turnover.

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