ORİJİNAL ARAŞTIRMA ORIGINAL RESEARCH

DOI: 10.5336/medsci.2020-73684

The Effects of Levetiracetam as Anti-Epileptic Drug on Bone and Vitamin D Metabolism

Anti-Epileptik İlaç Olarak Levetirasetam'ın Kemik ve D Vitamini Metabolizması Üzerine Etkileri

Şebnem GARİP USTAOĞLU[a]

[a]Department of Medical Biochemistry, Altınbaş University Faculty of Medicine, İstanbul, TURKEY

ABSTRACT Objective: Levetiracetam (LEV) is one of the new generations of anti-epileptic drugs. There are controversial results reported in the literature for the effects of LEV on bone metabolism. Some studies reported low bone mineral density (BMD) and vitamin D values with high bone turnover markers, while the others reported no effect on BMD. This study is the first report on elucidation of the mechanism of LEV and seizures on vitamin D and bone metabolism. **Material and Methods:** 100 mg/kg of LEV in each day was given to rats for twelve weeks. 25-hydroxyvitamin D [25(OH)D] and 1,25-dihydroxyvitamin D [1,25(OH)2D], calcium, bone turnover markers and parathyroid hormone (PTH) were measured by Enzyme-Linked Immunosorbent Assay (ELISA) while the protein levels of anabolic and catabolic enzymes of vitamin D were measured by western blot. **Results:** The serum levels of calcium, 25(OH)D and 1,25(OH)2D were significantly reduced in all rats, while there were increased amounts of PTH in just LEV-treated rats compared to the control. High bone turnover markers were also observed for drug-treated groups. Both epileptic seizures and LEV increased the amount of 24-hydroxylase; vitamin D catabolic enzyme. **Conclusion:** Based on the findings of the current study, both the LEV treatment and seizures cause vitamin D deficiency in rats by increasing the 24-hydroxylase amount in epileptic and LEV-treated groups which resulted in the conversion of active vitamin D to an inactive form and reduction in 25(OH)D and 1,25(OH)2D amounts.

Keywords: Bone; levetiracetam; vitamin D; bone turnover markers; parathyroid hormone (PTH); epilepsy; genetically-induced absence seizures; WAG/Rij rats; calcium; 25(OH)D

ÖZET Amaç: Levetirasetam (LEV) yeni nesil anti-epileptik ilaçlardan biridir. Literatürde LEV'nin kemik metabolizması üzerindeki etkileri için tartışmalı sonuçlar vardır. Bazı çalışmalar, yüksek kemik döngüsü belirteçleri değeri ile düşük kemik mineral yoğunluğu (BMD) ve D vitamini değerleri bildirirken, diğerleri BMD üzerinde herhangi bir etki bildirmemiştir. Bu çalışma, LEV ve nöbetlerin D vitamini ve kemik metabolizması üzerindeki etki mekanizmasının aydınlatılması üzerine ilk rapordur. **Gereç ve Yöntemler:** On iki hafta boyunca sıçanlara her gün 100 mg/kg LEV verildi.25-hidroksivitamin D [25(OH)D] ve 1,25-dihidroksivitamin D [1,25(OH)2D], kalsiyum, kemik döngüsü belirteçleri ve paratiroid hormonu (PTH) Enzim bağlantılı immunosorbent testi (ELISA) ile, D vitamini anabolik ve katabolik enzimlerinin protein seviyeleri Western Blot tekniği ile ölçüldü. **Bulgular:** Serum kalsiyum, 25(OH)D ve 1,25(OH)2D seviyeleri tüm sıçanlarda önemli ölçüde azalırken, sadece LEV ile tedavi edilen sıçanlarda kontrole kıyasla artan miktarlarda PTH değeri vardı. İlaçla tedavi edilen gruplar için yüksek kemik döngüsü belirteçleri de gözlenmiştir. Hem epileptik nöbetler hem de LEV, D vitamini katabolik enzimi olan 24-hidroksilazın miktarını arttırdı. **Sonuç:** Mevcut çalışmanın bulgularına dayanarak, hem LEV tedavisi hem de nöbetler, epileptik ve LEV ile tedavi edilen gruplarda 24-hidroksilaz miktarını artırarak ve buna bağlı olarak aktif D vitamininin aktif olmayan bir forma dönüştürülmesine ve 25(OH)D ve 1,25(OH)2D miktarlarında azalmayı sağlayarak sıçanlarda D vitamini eksikliğine neden olmaktadır.

Anahtar Kelimeler: Kemik; levetiracetam; vitamin D; kemik döngüsü belirteçleri; paratiroid hormone (PTH); epilepsi; genetik olarak indüklenen absans nöbetleri; WAG/Rij sıçanlar; kalsiyum; 25(OH)D

Epilepsy which affects ~1-2 % of the population worldwide is one of the most common neurodegenerative diseases.[1] In epilepsy, excessive discharge of cerebral neurons results in recurrent seizures.[2] All treatments for epilepsy aim to make the active lives of people easier by preventing further seizures while avoiding the side effects of the treatments. Primary treatment is to use anti-epileptic drugs (AEDs). How-

Correspondence: Şebnem GARİP USTAOĞLU
Altınbaş University Faculty of Medicine, Department of Medical Biochemistry, İstanbul, TURKEY/TÜRKİYE
E-mail: sebnem.garip@altinbas.edu.tr

Peer review under responsibility of Turkiye Klinikleri Journal of Medical Sciences.

Received: 20 Jan 2020 ***Received in revised form:*** 15 Jun 2020 ***Accepted:*** 30 Jun 2020 ***Available online:*** 22 Sep 2020

ever, the side-effects of using these drugs produce other risks for epileptic individuals. Disorders of vitamin D and bone metabolism due to long term AED treatment have been reported previously.[3] Despite great numbers of reports of AED-related osteopenia and/or osteoporosis in adults and in children, the exact mechanism for AED-associated pathologies on bone tissues cannot be completely clarified.[3-9]

Levetiracetam (LEV) which is a new generation anti-epileptic drug is recently used for the inhibition of partial type seizures.[10] The treatment of adult and children epileptic patients with LEV mono- or polytherapy has been gradually increasing.[11] The most important reason for using LEV for epilepsy treatment is the little side-effects of this drug on tissues when compared to the other anti-epileptic drugs.[12,13]

The side-effects of LEV on bone tissues have not been revealed with existing studies up to now. There are no reported side-effects of Keppra (brand name of LEV) on bone tissues in the records of manufacturing company (Product Monograph, UCB Pharma, Brussels, Belgium). In spite of the clinical studies that reported no alterations in bone mineral density (BMD) for epileptic patients, there are clinical and animal studies reported low BMD values, low amounts of Vitamin D and high bone turnover markers in the literature.[6,8,12-16] There are also a clinical and an animal study which reported increased values of BMD, vitamin D and bone formation markers after treatment with LEV.[17,18] Moreover, reduced bone strength without any alteration in BMD values due to LEV treatment was also reported by Nissen-Meyer et al.[19] The study of Nissen-Meyer et al. reported the possibility of LEV to affect the collagen-rich matrix of bone independently from BMD without any significant change in the mineral structure.[19] The investigation of the side-effects of LEV on both bone mineral and matrix independently from each other would be helpful to reveal the different effects of the drug on bone structure. The exact mechanisms for possible side effects of LEV on bone tissues have not been determined yet.

In this study, our goals were to clarify the effects of LEV on bones and also elucidate the mechanism of AED and epilepsy on the metabolism of vitamin D and bone.

This study will shed light on the side effects of epileptic seizures and an anti-epileptic drug LEV, on neurological disease treatments including epilepsy.

MATERIAL AND METHODS

ANIMAL PREPARATION AND RECORDING OF ELECTROENCEPHALOGRAPHY (EEG)

Procedures of the animal studies which were performed at Kocaeli University were conducted by the protocols of "Guide for the Care and Use of the Laboratory Animals" and approved by the Animal Ethical Committee of Kocaeli University (Decision date: 10/14/2014 and number: KOÜ HADYEK 8/5-2014). This study was conducted in accordance with the Declaration of Helsinki. Wistar Albino Glaxo from Rijswijk (WAG/Rij) rats with genetically-induced epilepsy was used as the experimental group, while healthy Wistar rats were rendered as the control group. For both groups, 6-month adult male rats (250-300 g) were deliberately chosen. 100 mg/kg/day LEV was intraperitoneally administered to drug-treated groups for 12 weeks as described previously.[11]

In the present study, there were four animal groups; Group 1- control (Wistar rats + physiological saline); Group 2- epileptic (WAG/Rij-AGS rats + physiological saline); Group 3- drug-treated control (Wistar rats + LEV) and Group 4- drug-treated epileptic (WAG/Rij-AGS + LEV). There were 6 rats in each group.

The effects of disease and seizures alone on bone and vitamin D metabolism were investigated by comparing the epileptic rats (Group 2) with the control group (Group 1), while to get information about the effects of drug on bone and vitamin D metabolism in the absence of diseases, the control group (Group 1) was compared with drug-treated control group (Group 3).

Cortical electrodes were placed on frontal and parietal regions, while the reference electrodes were put on the cerebellum to record EEG of animals. After one week, the rats were started to be predisposed to "key ring" sound stimulation for once a day during twelve weeks. The EEG recording of the animals was done both during and after seizures for 30 minutes.

The blood samples of the animals were taken at the end of 12 weeks. The liver and kidney tissues were also removed after the decapitation of rats. Tissues were stored at -80 ˚C until the Western Blot studies are carried out. The centrifugation of blood samples was done using 3000 rpm for 30 minutes at + 4 ˚C to get serum samples. After centrifugation, the supernatants were kept at -80 °C until biochemical assays.

MEASUREMENT OF SERUM PARAMETERS

The measurement of serum parameters was done according to our previous report.[20] Serum calcium levels were measured with Calcium Arsenazo III Colorimetric method using Abbot Aeroset auto analyzer and Aero-set kit (Abbot Lab. Abbot Park, IL 60064, USA). In this method Calcium with Arsenazo lll (1, 8-Dihydroxy-3, 6-disulpho-2,7-naphthalenebis (azo)-dibenzenearsonic acid), at neutral pH, yields a blue colored complex. The intensity of the color formed is proportional to the calcium concentration in the sample. Osteocalcin (OC) and PTH amounts were measured with DRG ELISA kit (Cat # EIA-2095 and EIA-4935, respectively), bone specific alkaline phosphatase (BSAP) and 25(OH)D amounts were measured with Biorbyt (Cat # orb410881) and IDS (Cat #KRR1971) ELISA kit respectively, $1,25(OH)_2D$ amounts were measured with Cusabio ELISA kit (Cat# CSB-E13342r), while C telopeptide (CTX) amounts were measured with Uscn ELISA kit (Cat # CEA665Ra). The optical density (OD) values at 450 nm were read by an ELISA microplate reader (Biochrome EZ Read 400, Biochrome Ltd., UK).

MEASUREMENT OF PROTEIN LEVELS IN KIDNEY AND LIVER TISSUES

The protein amounts of 25-hydroxylase (CYP27A1) enzyme in liver, 1-α-hydroxylase (CYP27B1) and 24-hydroxylase (CYP24) enzymes in kidney were measured by Western Blot analysis. To determine the CYP enzyme protein levels, tissue lysates were prepared from kidney and liver tissues of all rats. For this aim, 1 mg of tissue was digested in 2,5 µl lysis buffer. After the centrifugation of lysates at 14000 x g for ten minutes, obtained supernatants were frozen in -20 ˚C until analysis.

The protein levels in the tissues were measured by Bradford assay. The standard samples from Bovine Serum Albumin (BSA) were used in different concentrations including 1:250, 1:500, 1:750, and 1:1000 dilutions of 2 mg/ml stock solution. 5 µl of 1:100 diluted lysate samples with 245 µl of 1x Coomassie Blue protein assay solution was added to each well of the 98-well plates. 5 µl of standard samples with 245 µl protein assay solution was also added to the other wells on the plate. The plates were read at 595 nm and a standard curve was drawn. Protein amount in tissue samples was calculated by using this curve.

For sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE) preparation procedure was executed according to Laemmli U.K.[21] 6 µg of protein, calculated according to the results of Bradford Assay, was mixed with 6x loading dye and denaturated at 95 °C for 6 minutes by using a heating dry bath (Daihan HB48, Daihan Scientific Co., Ltd., Korea). Then, 6µl of protein ladder (10-250 kDa) (Thermo Scientific, USA) and samples were put into the wells of prepared gels. The gel was run and then the protein was transferred to polyvinylidene fluoride (PVDF) membranes (Roche Applied Sciences, Germany) using electrophoresis and blotting unit, respectively (Hoefer, Inc., USA). After the protein transfer, the membrane was washed and then blocked with 5% skim milk powder. Primary antibodies used in this study are CYP27a1 (1:600) (Abcam, USA), CYP27b1 (1:750) (Santa Cruz, USA) and CYP24 (1:750) (Santa Cruz, USA). The membrane was incubated by using one of these antibodies for overnight at 4 °C. The membrane was incubated using a horseradish peroxidase-conjugated (HRP) secondary antibody (1:2000) for an hour after the washing procedure. As a loading control, Actin (1:1000) (Santa Cruz, USA) was used. The imaging of the antibody-loaded membranes was done with using Enhanced chemiluminescent (ECL) reagents (Pierce) and medical X-ray film (Kodak, USA). Image analysis and the calculation of the protein amount in the samples were done with Image J software.

STATISTICAL ANALYSIS

GraphPad Prism software version 6.01 (La Jolla, USA) was used for statistical analysis. After testing data for normal distribution and homogeneity of the

variance, statistical difference between specific groups was determined by using Kruskal Wallis H test. The coefficient of variation (standard deviation/mean) was calculated for each parameter in each animal and the data summarized as the mean and standard deviations for each group. $\alpha = 0.05$ was taken as the level of significance.

RESULTS

These studies addressed the investigation of possible side-effects of LEV on bone and vitamin D metabolism of healthy and genetically epileptic rats and elucidate the mechanism of LEV and epileptic seizures on these metabolisms.

Measurement of bone formation or resorption molecular markers allows diagnosis of bone-related disorders and the determination of drug effects on bones. In the current study, the amount of bone formation serum markers, such as BSAP, OC and bone resorption serum marker CTX, besides serum parameters including calcium, PTH, 25(OH)D and $1,25(OH)_2D$ was measured by ELISA. Thus, the levels of mentioned parameters can be helpful to clarify the effects of both LEV and seizures on bone metabolism.

Moreover, the protein amounts of vitamin D metabolism enzymes were measured using Western Blot analysis to elucidate the mechanism of AED and seizures on vitamin D and bone metabolism.

CHANGES IN SERUM PARAMETERS

The only vitamin D metabolite for the determination of vitamin D deficiency, sufficiency or intoxication is 25(OH)D.[22] $1,25(OH)_2D$ induces calcium transport from bone, intestine and kidney to the blood. The production of $1,25(OH)_2D$ is induced by PTH. Calcium decreases PTH with a negative feedback, while there is another feedback directly between $1,25(OH)_2D$ and PTH. Vitamin D deficiency results in osteoporosis, whereas its insufficiency can cause osteomalacia. The serum levels of blood parameters mentioned above are shown in Table 1. As seen from Table 1, serum amounts of 25(OH)D, $1,25(OH)_2D$ and total calcium concentration were significantly reduced in all rat groups, while there were elevated amount of PTH in just LEV-treated rats, but not in the epileptic group rats compared to the healthy controls (Table 1).

Serum calcium homeostasis is maintained by PTH and $1,25(OH)_2D$ together in intestines and kidneys. They increase bone resorption while calcium and phosphate absorptions are increased in the intestines and reabsorption of calcium with excretion of phosphate is increased in the kidneys. Decreased serum calcium amount in the LEV-treated groups induces the secretion of PTH in elevated levels.[23] Moreover, the elevated amount of PTH induces the production of $1,25(OH)_2D$.[24] Furthermore, the transcription of the PTH gene can be inhibited independently by $1,25(OH)_2D$.[25] In the present study, besides the reduced calcium and increased PTH level in LEV-treated rats, serum levels of both vitamin D molecules were decreased. Therefore, it was indicated that LEV induces reduced vitamin D amounts by affecting vitamin D metabolism. Since there were reduced amounts of serum calcium in epileptic rats, an increased PTH level was expected in this group. However, a small reduction in PTH levels of epileptic

TABLE 1: Serum calcium, 25(OH)D, 1,25(OH)2D and PTH levels of control, epileptic and LEV-treated groups.

	Calcium (mg/dl)	25-(OH) Vitamin-D (nmol/l)	1.25-(OH) Vitamin-D (ngr/ml)	PTH (pgr/ml)
Control	241.670±41.420	43.140±2.090	21.650±0.640	0.923±0.026
Control + LEV	187.830±23.800 (p=0.02)*	37.970±2.200 (p=0.005)**	18.030±0.570 (p=0.009)**	1.066±0.069 (p=0.009)**
Epileptic	194.830±37.870 (p=0.02)*	39.480±2.140 (p=0.04)*	19.670±0.900 (p=0.04)*	0.936±0.032 (p=0.09)
Epileptic + LEV	152.170±11.670 (p=0.0005)*** (p=0.04)+	33.690±1.480 (p=0.0005)*** (p=0.02)+	17.550±0.340 (p=0.0005)*** (p=0.04)+	1.228±0.240 (p=0.0005)*** (p=0.0008)+++

Kruskal Wallis H test was used for statistical analysis. (*) represents the significance compared to the control group and (+) represents the significance compared to the epileptic group. The p values less than 0.05 were considered as statistically significant (*, +p<0.05; **, ++p<0.01; ***, +++p<0.001).

group was observed instead of an increase. Thus, there may be an impaired PTH secretion in this group.

Products of collagen catabolism such as collagen telopeptides are the commonly used biomarkers for bone resorption. On the contrary, side products of collagen synthesis and/or osteoblast-related proteins including OC and BSAP are the markers of bone formation. As seen from Table 2, these bone turnover biomarkers (OC, BSAP, and CTX) showed significantly increased levels in LEV-treated rats, while a slight increase was seen in the epileptic rats.

PROTEIN LEVELS OF THE ENZYMES THAT FUNCTION IN VITAMIN D METABOLISM

In the present study, the amount of CYP27A1 enzyme in liver, CYP27B1 and CYP24 enzymes in kidney were measured using the Western Blot technique to find out if there is any effect of LEV treatment on vitamin D metabolism.

Vitamin D is transported to the liver after its absorption or production from precursors. In the liver, CYP27A1 enzyme converts vitamin D to 25(OH)D. After that 25(OH)D is converted to the active metabolite of vitamin D; $1,25(OH)_2D$ by CYP27B1 in the kidney.[26] In the kidneys, 25(OH)D is also converted to $24,25(OH)_2D$, which is the biologically-inactive version of vitamin D, by the CYP24 enzyme.

Both seizures and drug treatment increased CYP24 amount which has a role in the catabolism of vitamin D in the kidney (Figure 1). Moreover, protein levels of CYP27A1 were also stimulated in the same groups (Figure 1). The highest CYP24 enzyme

TABLE 2: Serum bone formation (BSAP and OC) and resorption (CTX) of control, epileptic and LEV-treated groups.

	Bone Specific ALP (µg/l)	Osteocalcin (ngr/ml)	C-telopeptide (ngr/ml)
Control	9.970±0.390	1.318±0.004	0.168±0.010
Control + LEV	10.530±0.140 (p=0.02)*	1.331±0.005 (p=0.04)*	0.192±0.011 (p=0.005)**
Epileptic	10.120±0.210 (p=0.08)	1.327±0.007 (p=0.04)*	0.176±0.007 (p=0.06)
Epileptic + LEV	11.020±0.600 (p=0.01)*	1.338±0.005 (p=0.005)**	0.244±0.010 (p=0.0005)*** (p=0.0005)$^{+++}$

Kruskal Wallis H test was used for statistical analysis. (*) represents the significance compared to the control group and (+) represents the significance compared to the epileptic group. The p values less than 0.05 were considered as statistically significant (*, $^{+}$p<0.05; **, $^{++}$p<0.01; ***, $^{+++}$p<0.001).

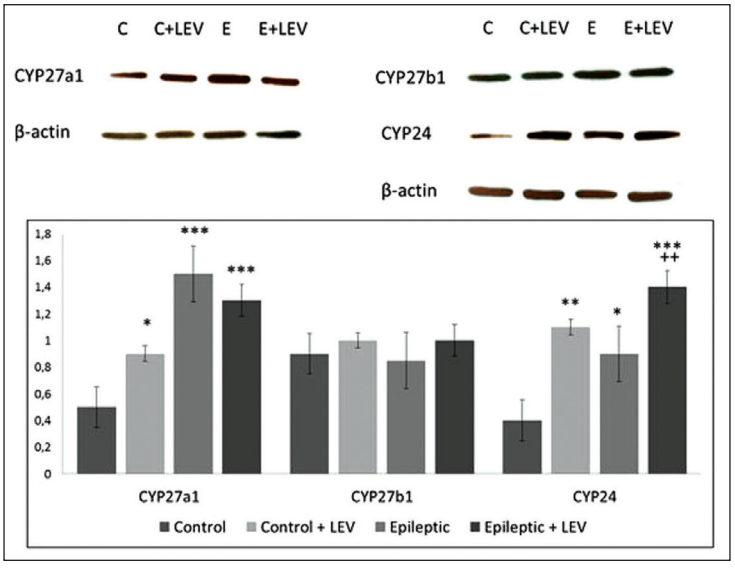

FIGURE 1: Protein levels of CYP27A1, CYP27B1 and CYP24 enzymes in the liver and kidney tissues of control, epileptic and LEV-treated groups are shown in representative Western Blot images **(A)** and histograms **(B)**. B-Actin protein was probed as a loading control.

Kruskal Wallis H test was used for statistical analysis. (*) represents the significance compared to the control group and (+) represents the significance compared to the epileptic group. The P values less than 0.05 were considered as statistically significant (*, $^{+}$p<0.05; **, $^{++}$p<0.01; ***, $^{+++}$p<0.001).

levels were observed in the drug-treated epileptic rats, while CYP27A1 enzyme levels were highest in the seizure group epileptic rats.

DISCUSSION

DIFFERENT EFFECTS OF LEV TREATMENT AND SEIZURES ON PTH AMOUNT AND BONE TURNOVER MARKERS

The serum amounts of 25(OH) D, $1,25(OH)_2D$ and total calcium concentration were significantly decreased in both epileptic and drug-treated groups implying a vitamin D deficiency in these rat groups. Durá-Travé et al. also reported vitamin D deficiency in epileptic children after LEV monotherapy by measuring 25(OH)D levels of these patients.[8] Moreover, in LEV-treated rats there were elevated amount of PTH levels while there was a small reduction in the epileptic group. Loupy et al. reported that in low calcium levels, impaired PTH secretion may be the cause of primary hypoparathyroidism.[27] Reduced vitamin D amounts might be induced by the effects of epilepsy itself and the epileptic seizures on the metabolism of vitamin D as seen in LEV-treated groups.

Both $1,25(OH)_2D$ and PTH stimulates osteoclastogenesis and osteoclastic bone resorption by increasing the amount and activity of osteoclasts through increasing the expression of RANKL (receptor for activation of the nuclear factor kappa ligand) in osteoblasts.[28] In the present study, levels of bone turnover biomarkers increased in LEV-treated rats, while there was a slight increase in the epileptic group. El-Haggar et al. measured the bone formation and bone resorption markers of LEV-treated epilepsy patients and reported that drug treatment decreased bone formation biomarkers, while bone resorption biomarkers were increased with low BMD values in these patients.[6] In contrast to our studies, Karesova et al. reported increased BSAP and CTX of type I collagen markers in Wistar rats after twelve weeks of LEV treatment.[18]

In summary, alterations in PTH and $1,25(OH)_2D$ amounts in LEV-treated rats might stimulate bone turnover by increasing bone formation and reducing bone resorption. In epileptic rat group, a low amount of $1,25(OH)_2D$ might cause reduced bone formation due to insignificant alteration in PTH amounts. PTH level is the only difference in blood parameters between LEV-treated and epileptic rats, except for the bone turnover markers. Thus, elevated PTH amount may have a role for the high bone turnover in LEV treatment. High bone turnover elucidate the structural alterations in bone mineral and matrix composition in LEV-treated healthy rat group in our previous study (unpublished data). However, these structural alterations were also found in epileptic rats compared to control rats which are reported in our previous study.[29] Thus, epilepsy and seizures may affect bone metabolism by another mechanism besides a decreased vitamin D amount. Further studies should elucidate these unknown mechanisms.

VITAMIN D CATABOLISM IS STIMULATED BY LEV AND EPILEPTIC SEIZURES THROUGH CYP24 ENZYME

The protein levels of CYP24 and CYP27A1 increased in both seizure and drug treated groups, while the highest enzyme levels were observed in the drug-treated epileptic rats for CYP24 and in the seizure group for CYP27A1. Thus, vitamin D deficiency with decreased amount of serum 25(OH)D and $1,25(OH)_2D$ in LEV-treated rats may be caused by the catabolism of active form of vitamin D to inactive by elevated CYP24 enzyme levels.

The pregnane X receptor (PXR) was supposed to be related to AED-induced deficiency of vitamin D.[30] The PXR and vitamin D receptors (VDRs) have 60% homology for DNA binding domains and have been shown to induce the cytochrome P450 enzymes that are important for the drug metabolism. Moreover, PXR can be stimulated by different anti-epileptic drugs including phenytoin (PHT) and carbamazepine (CBZ).[30] It was reported that these PXR activators may increase the expression of CYP24 which results in vitamin D deficiency and causes reduced bone density and bone loss through increasing bone turnover.[30,31]

Although, the increase in amount was observed for CYP27A1 and CYP24 enzymes of all rats in our study, PXR receptor induction cannot define vitamin D deficiency in this situation. LEV is not one of the known activators of PXR. Moreover, epileptic sys-

tems did not include any known PXR activators. However, a connection of PXR polymorphism with drug resistance in the absence form of epilepsy was previously reported in humans.[32] ABCB1 (ATP Binding Cassette Subfamily B Member 1) gene expression which is believed to have a role in drug resistance in epilepsy is under the control of PXR.[33] Therefore, PXR polymorphism might have a role in drug-resistant absence form of epilepsy for responding to AEDs in patients.[34]

In our study, one of the genetically-induced epileptic rat models that are the closest one to human genetically-induced absence form of epilepsy amongst other rat models was used.[35] They are called WAG/Rij rats. It is suggested that the sub-group of WAG/Rij rats (mixed epileptic form) used in our study were reported as mainly resistant to AED treatment.[36] In our study, rats responded to the LEV treatment and the seizures were inhibited in drug-treated epileptic rats by the drug treatment during the study (12 weeks) since an optimum dose of the drug was used. Thus, these drug-resistant epileptic rats may have ABCB1 polymorphism, and as a result, PXR polymorphism, which has a role in drug resistance. The increased amount of PXR-induced enzymes; CYP27A1 and CYP24 in the epileptic group may also be explained by this polymorphism. However, there are no reports in the literature indicating the existence of ABCB1 and/or PXR polymorphism in genetically-induced epileptic WAG/Rij rats.

Although LEV is not a known activator of PXR receptor, an increased amount of CYP27A1 and CYP24 enzymes in the drug-treated groups was observed. There should be another mechanism for the effects of LEV on the vitamin D and bone metabolism. Previous studies reported the relationship of LEV with the Wnt signalling pathway in both epileptic patients and non-epileptic rats.[16,37] Bone formation is induced by the stimulation of the Wnt signaling pathway, on the other hand, the inactivation of this pathway results in osteopenia.[38] In the osteogenesis process, activation and inactivation of the canonical Wnt signaling pathway cause sclerosteosis and osteoporosis, respectively.[39] Previous studies also reported the role of $1,25(OH)_2D$ in stimulating Wnt signaling for bone formation.[40] The extracellular endogenous regulators of the canonical Wnt signaling pathway include dickkopf-1 (Dkk1) which is an inhibitor of this pathway. Antibodies against endogenous antagonists have shown hopeful results in encouraging bone formation and fracture healing.[41] LEV is reported to increase the Dkk1 amount, Wnt signaling pathway inhibitor, in control and ovariectomized female rats which resulted in bone loss in these animals.[16] In another study, it was also reported that LEV treatment changes the serum levels of Wnt signaling inhibitors in epileptic patients.[37] These mechanisms [decrease in $1,25(OH)_2D$ amount and increase in Dkk1 amount] may explain the effects of LEV on bone metabolism and explain our results including an increased amount of catabolic enzymes of vitamin D, the elevation of PTH and bone turnover biomarkers used in the current study.

The limitations of the current study include the use of only one dose of LEV treatment (100 mg/kg/day) in a single time-point (12 weeks). Since the potential use of LEV for restraining status epilepticus (SE)-, traumatic brain injury (TBI)- or stroke-induced chronic epilepsy was also reported[42], investigation of the various LEV doses with different time points could help to further elucidate the dose-dependent effects of LEV in short- and long-term treatment in seizures caused by different diseases.

CONCLUSION

Although significantly increased PTH and bone turnover markers were observed in LEV-treated rats, which induced a deficiency of vitamin D in these rat groups, there was no elevation in PTH or bone turnover biomarkers in epileptic rats. However, in epileptic rats, there was also a vitamin D deficiency. Thus, it was suggested that epileptic seizures may induce compositional changes in bone tissue by other mechanisms rather than stimulating the bone turnover. In the present study, it was found that LEV treatment increased the amount of catabolic enzyme of vitamin D; CYP24. Moreover, an increase in the amount of CYP24 enzyme was also found in epilepsy group rats which imply almost identical effects of seizures on vitamin D metabolism with LEV.

Acknowledgement

The Wistar and WAG/Rij rat tissues were kindly provided by Dr. Gul Ilbay and Dr. Deniz Sahin (Department of Physiology, Kocaeli University Medical School, Turkey). Language Editing: We thank to Daniel Golestanijam for the language redaction of the manuscript.

Financial Support

The research work presented in this paper was financially supported by an Altinbas University research grant PB-009-2013.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

This study is entirely author's own work and no other author contribution.

REFERENCES

1. Iseman MD. [Preventive treatment in tuberculosis]. Iseman MD, ed. Özkara Ş, çeviri editörü. Klinisyenler İçin Tüberküloz Kılavuzu. 1. Baskı. İstanbul: Nobel Tıp Kitabevleri; 2002. p.355-98.
2. Özkara Ş. [Preventive treatment in tuberculosis]. Özkara Ş, Kılıçaslan Z, editörler. Tüberküloz. İstanbul: Toraks Kitapları; 2010. p.586-97.
3. Abubakar I, Chakaya J, Maeurer M, Zumla A. Towards optimal treatment for latent Mycobacterium tuberculosis infection. Lancet Respir Med. 2019;7(3):195-7. [Crossref] [PubMed]
4. Haley CA. Treatment of latent tuberculosis infection. Microbiol Spectr. 2017;5(2). [Crossref] [PubMed]
5. Yang J, Lee S, Oh S, Han S, Park SY, Kim Y, et al. The risk of active tuberculosis among individuals living in tuberculosis-affected households in the Republic of Korea, 2015. PLoS One. 2019;14(12):e0225744. [Crossref] [PubMed] [PMC]
6. World Health Organization. Guidelines on the Management of Latent Tuberculosis Infection. Geneva, Switzerland: World Health Organization; 2015. p.34.
7. Turkish Republic Ministry of Health. Tüberküloz Tanı ve Tedavi Rehberi. Yayın No: 862. Ankara: Başak Matbaacılık ve Tanıtım Hizmetleri Ltd. Şti.; 2011. p.153.
8. Turkish Institution of Statistics. [Population and demography]. Temel İstatistikler [Link]
9. Turkish Republic Ministry of Health, Tuberculosis Department, Özkara Ş, Aktaş Z, Özkan S, Ecevit H. Türkiye'de Tüberkülozun Kontrolü İçin Başvuru Kitabı. Ankara: Rekmay Ltd. Şti.; 2003. p.112.
10. Bishai WR, Chaisson RE. Short-course chemoprophylaxis for tuberculosis. Clin Chest Med. 1997;18(1):115-22. [Crossref] [PubMed]
11. Swift MD, Molella RG, Vaughn AIS, Breeher LE, Newcomb RD, Abdellatif S, et al. Determinants of latent tuberculosis treatment acceptance and completion in healthcare personnel. Clin Infect Dis. 2020;71(2):284-90. [Crossref] [PubMed]
12. Han SS, Lee SJ, Yim JJ, Song JH, Lee EH, Kang YA. Evaluation and treatment of latent tuberculosis infection among healthcare workers in Korea: a multicentre cohort analysis. PLoS One. 2019;14(9):e0222810. [Crossref] [PubMed] [PMC]
13. Lee EH, Kim SJ, Ha EJ, Park ES, Choi JY, Leem AY, et al. Treatment of latent tuberculous infection among health care workers at a tertiary hospital in Korea. Int J Tuberc Lung Dis. 2018,1;22(11):1336-43. [Crossref] [PubMed]
14. Shedrawy J, Jansson L, Röhl I, Kulane A, Bruchfeld J, Lönnroth K. Quality of life of patients on treatment for latent tuberculosis infection: a mixed-method study in Stockholm, Sweden. Health Qual Life Outcomes. 2019;17(1):158. [Crossref] [PubMed] [PMC]
15. Santos JC, Silva JB, Rangel MA, Barbosa L, Carvalho I. Preventive therapy compliance in pediatric tuberculosis - A single center experience. Pulmonology. 2020;26(2):78-83. [Crossref] [PubMed]
16. Lam CK, McGinnis Pilote K, Haque A, Burzynski J, Chuck C, Macaraig M. Using video technology to increase treatment completion for patients with latent tuberculosis infection on 3-month isoniazid and rifapentine: an implementation study. J Med Internet Res. 2018;20(11):e287. [Crossref] [PubMed] [PMC]
17. Cohen A, Mathiasen VD, Schön T, Wejse C7. The global prevalence of latent tuberculosis: a systematic review and meta-analysis. Eur Respir J. 2019;54(3):1900655. [Crossref] [PubMed]
18. Reichler MR, Khan A, Sterling TR, Zhao H, Chen B, Yuan Y, et al; Tuberculosis Epidemiologic Studies Consortium Task Order 2 Team. Risk factors for tuberculosis and effect of preventive therapy among close contacts of persons with infectious tuberculosis. Clin Infect Dis. 2020;70(8):1562-72. [PubMed]
19. Huaman MA, Sterling TR. Treatment of latent tuberculosis infection-an update. Clin Chest Med. 2019;40(4):839-48. [Crossref] [PubMed]
20. Chee CBE, Reves R, Zhang Y, Belknap R. Latent tuberculosis infection: opportunities and challenges. Respirology. 2018;23(10):893-900. [Crossref] [PubMed]
21. Kwara A, Herold JS, Machan JT, Carter EJ. Factors associated with failure to complete isoniazid treatment for latent tuberculosis infectionin Rhode Island. Chest. 2008;133(4):862-8. [Crossref] [PubMed]
22. Hirsch-Moverman Y, Daftary A, Franks J, Colson PW. Adherence to treatment for latent tuberculosis infection: systematic review of studies in the US and Canada. Int J Tuberc Lung Dis. 2008;12(11):1235-54. [PubMed]
23. Plourde PJ, Basham CA, Derksen S, Schultz J, McCulloch S, Larcombe L, et al. Latent tuberculosis treatment completion rates from prescription drug administrative data. Can J Public Health. 2019;110(6):705-13. [Crossref] [PubMed] [PMC]
24. Pease C, Zwerling A, Mallick R, Patterson M, Demaio P, Finn S, et al. The latent tuberculosis infection cascade of care in Iqaluit, Nunavut, 2012-2016. BMC Infect Dis. 2019;19(1):890. [Crossref] [PubMed] [PMC]
25. Séraphin MN, Hsu HC, Chapman HJ, de Andrade Bezerra JL, Johnston L, Yang Y, et al. Timing of treatment interruption among latently infected tuberculosis cases treated with a nine-month course of daily isoniazid: findings from a time to event analysis. BMC Public Health. 2019;19(1):1214. [Crossref] [PubMed] [PMC]
26. Kawatsu L, Uchimura K, Ohkado A. Trend and treatment status of latent tuberculosis infection patients in Japan - Analysis of Japan TB Surveillance data. PLoS One. 2017;12(11):e0186588. [Crossref] [PubMed] [PMC]

27. Sentís A, Vasconcelos P, Machado RS, Caylà JA, Guxens M, Peixoto V, et al. Failure to complete treatment for latent tuberculosis infection in Portugal, 2013-2017: geographic, sociodemographic, and medical-associated factors. Eur J Clin Microbiol Infect Dis. 2020;39(4):647-56. [Crossref] [PubMed]

28. Schein YL, Madebo T, Andersen HE, Arnesen TM, Dyrhol-Riise AM, Tveiten H, et al. Treatment completion for latent tuberculosis infection in Norway: a prospective cohort study. BMC Infect Dis. 2018;18(1):587. [Crossref] [PubMed] [PMC]

29. Noh CS, Kim HI, Choi H, Kim Y, Kim CH, Choi JH, et al. Completion rate of latent tuberculosis infection treatment in patients aged 65 years and older. Respir Med. 2019;157:52-8. [Crossref] [PubMed]

30. Kan B, Kalin M, Bruchfeld J. Completing treatment for latent tuberculosis: patient background matters. Int J Tuberc Lung Dis. 2013;17(5):597-602. [Crossref] [PubMed]

31. Kolsuz M, Ersoy M, Küçükkebapçi C, Metintaş M, Uçgun I, Erginel S. [The evaluation of close contact case of pulmonary tuberculosis patients enrolled to Eskisehir Deliklitas Tuberculosis Control Dispensary]. Tuberk Toraks. 2003;51(3):282-8. [PubMed]

32. Kaçmaz Başoğlu Ö, Atasever A. [Treatment of latent tuberculosis infection in tuberculosis dispensaries in İzmir city]. Turkiye Klinikleri Arch Lung. 2005;6(1):14-8.

33. Dundar C, Oztomurcuk D, Terzi O. Regional success on screening and chemoprophylaxis in contacts of patients with pulmonary tuberculosis in Turkey: a dispensary experience in 2016-2017. Trans R Soc Trop Med Hyg. 2019;113(6):351-5. [Crossref] [PubMed]