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A study to assess the bone remodeling effects of three AEDs in female mice

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Abstract

If you suffer from epilepsy, you will likely need antiepileptic medication for the rest of your life (AEDs). Chronic AED treatment usage has been linked to decreased bone density and an increased risk of fracture during the last four "decades (Kruse, 1968; Lee et al., 2010; Petty et al., 2007). As previously demonstrated by recent clinical data, AEDs over the long run alter bone mineral density (BMD), increase the risk of fracture, and result in overt osteomalacia (Shiek et al., 2012; Espinosa et al., 2011). AED-induced bone effects have been observed at prevalence rates of up to 50%. (Valsamis et al., 2006). Bone microarchitecture and BMD are extensively modulated not only by AEDs but also by the illness itself, increasing the risk of fractures (Valsamis et al., 2006; Pack et al., 2008). Bony changes and increased fracture risk may be due to a variety of causes, including use of AEDs, seizure-related falls, trauma, and a sedentary lifestyle (Khanna et al., 2009").

AED-related bone "fragility is likely caused by a number of mechanisms that are still poorly understood, but AEDs that have been shown in the literature to cause bone metabolism problems are those that are strong inducers of the cytochrome P450 (CYP 450) monoxygenase system (phenytoin, carbamazepine, and phenobarbitone), which affects the calcium-vitamin D axis by reducing bioavailable vitamin D and causing hypocalc (Valsamis et al., 2006; Khanna et al., 2009). Even while this has long been assumed to be the major mechanism causing AED-induced bone loss, this isn't always the case for a variety of reasons. For one, not all individuals with bony deficiency have low levels of vitamin D. (Pack et al., 2011) In the second place, enzyme inhibitor AEDs have been linked to skeletal problems (Boluk et al., 2004). Hypovitaminosis K, calcitonin insufficiency, decreased calcium absorption from the digestive tract, hyperhomocysteinemia, and low" oestrogen levels during AED medication might all have an impact on bone health (Fitzpatrick, 2004; Khanna et al., 2009, 2011).

Keywords: Epilepsy, AED therapy, BMD, hyperhomocysteinemia, hypovitaminosis

Introduction

Due to advancements in "AED technology, second and third generation AEDs are now available, although phenytoin (PHT) and sodium valproate (SVP) are remain the first-line medicines and are frequently recommended for the treatment of partial seizures and generalised tonic-clonic convulsions (Nolan et al., 2013). There is a wealth of information available on the harmful effects of various AEDs on bone (Boluk et al., 2004; Khanna et al., 2009; Lee et al., 2010; Pack et al., 2011). More research is needed to determine whether newer AEDs, such as gabapentin, lamotrigine, topiramate, or vigabatrin, alter bone metabolism in epileptic patients, however there are just a few studies" to support this theory (Khanna et al., 2009; Lee et al., 2012; Sheth and Hermann, 2007).

One of the most "commonly used medications for the treatment of both partial and generalised seizures is levetiracetam (LTM) (Lyseng-Williamson, 2011). Few studies have looked at the effects of levetiracetam on BMD in individuals with epilepsy, despite it being used more often in clinics as a monotherapy or a polytherapy. According to these research, either no effects on bone metabolism or BMD were found in epileptic patients (Koo et al., 2013) or bone biomechanical strength was lowered in animals" without substantial changes in BMD (Koo, 2013). (Nissen-Meyer et al., 2007).

Antiosteoporotic "regimens in epilepsy need to be studied for their efficacy in evaluating AED-induced bone alterations and for testing the pathophysiology behind these changes. However, there are animal models of osteoporosis in mice that employ glucocorticoids (Yang et al., 2009) or ovariectomy (Nissen-Meyer et al.,

2007; Onodera et al., 2002) to examine the effect of AEDs on bone (Bouxsein et al., 2005). In our study, we recently shown that phenytoin (PHT) treatment at 35 mg/kg po for three months results in bone demineralization in male mice (Khanna et al., 2011). Since only a few animal studies have evaluated the effect of these medications on BMD and bony markers in mice, we explored whether continuous treatment with sodium valproate (SVP) or LTM might promote bone loss. Because the majority of previous research on the effects of AED on bone were done on male animals, we focused our attention on studying the effects of" antiepileptic medications on bone in female mice in this study.

No one can argue with the fact that estrogens play a critical role in bone health. As well as lowering cytokine "levels (IL-6, IL-7, and tumour necrosis factor-) that attract osteoclasts (Vaananen and Harkonen, 1996), estrogens also oppose the calcium mobilising actions of parathyroid hormone (Marcus, 1991) and increase osteoblast differentiation primarily by regulating transforming growth factor, TGF3, a bone matrix protein with anti-osteoclastic property (Robinson et al., 1996). Osteoblasts and osteoclasts both contribute to bone density by regulating the amount of bone matrix deposited and" reabsorbed (Grainger et al., 1999). There has also been prior evidence that oestrogen deficient conditions decrease the rat bone deposition of TGF.

Few studies have looked "at the effects of various therapies for bone disease such as selective oestrogen receptor modulators (SERMs), bisphosphonates, hormone replacement therapy and calcitonin on AED-induced bone loss despite these medicines being authorised (Das and Crockett, 2013). AED-induced bony effects are well known since 1968 (Kruse, 1968), but little study has been done with authorised anti-osteoporotic medicines on the subject. This is unexpected. Chronic usage of AEDs usually necessitates the use of calcium-vitamin D (CVD) supplements. Recent research has shown that bisphosphonates can reverse PHT-induced bone loss in mice by reversing the pro-oxidant effects of PHT, which are mediated via PHT-induced hyperhomocysteinemia (Khanna et al., 2011). Earlier this year, the ADOPT" (Anti-epileptic Drug and Osteoporosis Prevention Trial) showed that risedronate plus CVD supplementation improved bone "mineral density in nearly 70% of epileptic males and prevented the development of vertebral fractures (Lazzari et al., 2013). Bisphosphonates have been linked to excessive suppression of bone turnover and osteonecrosis of the jaw in some people, thus their usage may be restricted in some cases due to side effects including nausea and vomiting (Lee et al., 2008; Khosla" et al., 2007).

To find out if "RLX has any effect on antiepileptic drug-induced bone alterations, we compared it to calcium and vitamin D3 supplementation (CVD). Additionally, researchers studied the impact of raloxifene on seizures as a whole, as well as the possibility that it may modulate the antiepileptic efficacy of PHT and SVP. AEDs" or raloxifene's bone effects may be mediated via oestrogen and TGF-3, according to the research.

Literature Review

Epilepsy is a frequent "neurological condition that affects the brain. The word "epilepsy" has been in use since 500 BC and simply means "attack" or "assault.". recurring, unprovoked epileptic seizures are a hallmark of this chronic disease [ILAE (International League against Epilepsy) commission report, Fisher et al, 2005] Seizures caused by abnormally excessive or synchronised brain neuronal activity are known as epilepsies (Fisher et al., 2005). Neurobiological, cognitive, psychological, and social repercussions are common when someone has epilepsy (EVI) (Fisher et al., 2005). There is a significant prevalence in the first year of birth and in the elderly over 65 years, although" it affects people of all ages (Hauser et al., 1993; Olafsson et al., 2005).

Although active epilepsy is rare "in affluent nations (Hauser et al., 1996; Keränen and Riekkina, 1988), it affects around one person in every 100,000 people (incidence) (Hauser et al., 1996; Olafsson et al., 2005). In the poor world, it has an impact on 50 million individuals, with 80 percent of them living in the least

developed countries (Meyer et al., 2010). There are around 5 million epileptics in India, according to current estimates, and the incidence rate ranges from 38 to 49.3 per 100,000 people per year, according to two community-based studies (Ray et al., 2002; Sridharan", 2002).

Seizures and "epilepsies were classified by the International League Against Epilepsy (ILAE) in the 1980s (Commission on Classification and Terminology of the International League Against Epilepsy, 1981) and 1989 (Commission on Classification and Terminology of the International League Against Epilepsy, 1989) based on ideas that had developed over the previous decades (Gastaut, 1969a,b). Though most of us are aware with and comfortable with the ILAE categories of seizures and epilepsies, they" are unable to take into account the huge amount of new information that is constantly being added to our knowledge and care of persons with epilepsy on a meaningful, transparent basis.

It has been observed by the "ILAE Task Force that the 1989 epilepsy classification's dichotomies of localization related vs generalised and idiopathic versus symptomatic is too simple and difficult to apply. Rather than just describing clinical behaviour and EEG, seizure type is now considered a diagnostic entity, as opposed to the previous seizure categorization system, developed in 1981 (Commission of ILAE, 1981). (Engel, 2001). When a syndromic diagnosis isn't obvious, the identification of seizure types as diagnostic entities paves the way for better patient treatment and prognosis based on a diagnosis" of a specific seizure type.

Despite the fact that the "number of antiepileptic medications (AEDs) available has grown enormously, contemporary principles regulating drug therapy are strikingly comparable to those established more than 100 years ago (Shorvon, 2009). To have long-lasting effects, AED medication must be initiated after extensive risk-benefit evaluations" have been conducted because it is generally maintained for several years and sometimes for life, especially in adults (Perucca et al., 2000).

Epilepsy "treatment's ultimate objective is seizure-free living without side effects for the patient for the long term. A patient's seizure type or epileptic syndrome and tolerability concerns should be primary factors in selecting the initial AED, with data from carefully constructed randomised controlled trials" preferred.

Distinct AEDs "have different profiles of action against various seizure types and disorders (Table 3). Certain types of epilepsy have been discovered to respond well to certain drugs. sodium valproate is an effective therapy for juvenile myoclonic epilepsy, while Vigabatrin is widely accepted as the treatment of choice for infantile spasms caused by tuberous sclerosis (Sundqvist et al., 1998). (Elterman et al., 2001). Narrow-spectrum medicines, on" the other hand, can exacerbate myoclonic jerks and absence seizures (Liporace et al., 1994; Duarte et al., 1996; Ascapone, 2000; Gelisse et al., 2004).

In addition to "efficacy and effectiveness, the choice of AEDs may be influenced by a number of other features that standard randomised controlled trials may be unable to capture. There are undesirable consequences such as rare idiosyncratic responses, teratogenic effects, and chronic side-effects to take into consideration.. There are other considerations besides enzyme-inducing effects and medication interactions, such as the availability of parenteral formulations and the ability to swiftly attain an effective target dosage in some situations. The selection of an AED must take specific patient" features into account, as stated by all extant recommendations. Seizure kinds are important, but so are the patient's age, reproductive potential, and concomitant conditions.

While "considering the risks of withholding therapy, the expected efficacy of AEDs must be balanced against any adverse effects that may occur. Patients' perspectives on seizure hazards, including the possibility of seizure-related morbidity and death, along with AED toxicity, should be considered while

evaluating these risks. In an ideal world, evaluating" the possible advantages of therapy would include knowledge about untreated epilepsy's natural course and consequences.

Research Gap

For the first time, "our study shows that raloxifene can help prevent and treat PHT and SVP-induced bone loss while still maintaining the antiepileptic properties of existing AEDs. AEDs have been shown to have a detrimental effect on bone in earlier studies, but this is the first time they have been found in Swiss albino female" mice of the Swiss strain. Because of this, LTM may be a better option for female epileptic patients or those at risk of osteoporosis than PHT or SVP in terms of bone health.

Even though screening all "patients on chronic AED therapy for early diagnosis of possible effects on bone health is recommended (Sheth and Harden, 2007), there are no consensus guidelines available for addressing the effect of AEDs on bone health. CVD supplements should be prescribed to all patients susceptible to bone loss (though latter has also been questioned in some cases). Unfortunately, there aren't many research on the use of anti-osteoporotic drugs that can be administered in conjunction with AED therapy, so doctors aren't aware of the potential interaction between these drugs and AEDs, which could alter their protective effects. Anti-osteoporotic drugs' effects" on seizures are crucial, thus research into that is critical. Prescription of an AED to an epileptic lady with osteoporosis and vice versa might benefit from knowing this information.

Due to the "skeletal changes we saw in Swiss albino female mice after four months of PHT and SVP therapy, we now have animals that may be used to study osteoporosis treatments. It can be concluded that LTM may be a safer alternative for epileptic females who are prone to osteoporosis or who have a risk factor for osteoporosis since it failed to produce any change in either histopathology, BMD" or bone turnover markers. However, further clinical studies are definitely warranted.

PHT was given for three "months in our earlier work (Khanna et al. 2011) and histological alterations in the femoral bones were seen, but not in the lumbar bones. The length of therapy was increased by one month and the alterations in the lumbar bones were also detected. SVP increased the number of osteoclasts and rarefied the bone matrix at both dosages tested, but the effects were more evident at 300 mg/kg. LTM-treated" mice, on the other hand, showed no differences in the bone morphology between the two dosages.

A decrease in bone "mineral density (BMD) was seen in histology and verified by dual energy X-ray absorptiometry (DEXA), the gold standard for osteoporosis diagnosis. PHT and SVP both decreased BMD substantially, which is in line with previous preclinical and clinical investigations that found lower BMD in patients using these AEDs (Boluk et al., 2004; Nissen-Meyer et al., 2007; Pack et al., 2008; Lee et al., 2010). Chronic" PHT therapy decreased BMC, "although SVP had a less impact on BMC than it did on BMD. Some of the earlier clinical data (Boluk et al. 2004; Guo et al. 2001) supported the negative bony consequences of SVP, whereas others did not (Triantafyllou et al. 2010). Valproate has been shown to have strain-specific effects in animals in a research conducted by Senn and colleagues (Senn et al., 2010). C3H/HeJ and Balb/c strains of mice were shown to be susceptible, whereas A/J was found to be resistant to the bone deficiencies caused by valproate. A new strain (the Swiss albino mouse) has been shown to be vulnerable to valproate-induced bone deficiencies in our research. There was no reduction in BMD after 4 months of therapy with LTM (100 and 200 mg/kg). This is in line with what Nissen- Meyer and colleagues found in rats while studying BMD (Nissen-Meyer et al. 2007). The later research, on the other hand, found that LTM decreased femoral neck biomechanical strength. Because the lumbar vertebrae (L2-L4) are primarily made up of trabecular bone, we looked at whether LTM affects them. However, we found no evidence of lumbar vertebral alterations in our research, and it's conceivable that LTM has distinct effects on the femoral neck

and lumbar regions, which would necessitate more research. There have also been conflicting findings in terms of clinical outcomes. While one year of LTM monotherapy was reported to have no harmful effects on bone strength and metabolism and no obvious secondary effect on bone mass, quality (influenced by bone micro-architecture, geometry, and bone matrix composition), and bone remodelling (Koo et al., 2013), a recent study showed that LTM compromised BMD comparable to oxcarbazepine (OXC) after two years of treatment was LTM (Beniczky et al., 2012). A bigger population or longer treatment duration may lead to detrimental effects on bone after LTM administration because the previous study (Koo et al., 2013) presented data from a single site, was done in a small number of patients, and did not account for the assessment of fracture risk. Another research on" young adult epileptic patients, however, supports our findings on LTM that patients who transitioned to LTM had greater BMD in both the lumbar spine and femur than those on enzyme inducing AEDs such as PHT).

Research Objective & Methodology

The experiment "made use of female albino Swiss strain mice weighing between 25 and 35 grammes. The animals were given a week of acclimatisation time before the trial began. The animals were kept in polypropylene cages (10 per cage) in a room with a 12 hour light–dark cycle at a temperature of 232 °C and 5515 percent humidity. To keep the mice healthy, they were provided a regular pellet diet. Six groups of ten animals each were used in this experiment. LTML (100 mg/kg), SVPH (300 mg/kg), and LTMH (200 mg/kg) were given orally once daily for four months as antiepileptic medicines to the dogs. Following a three-month treatment period and plasma levels within the therapeutic range, PHT (35 mg/kg) was previously established in our lab to cause bone loss in mice femurs (Khanna et al., 2011). To see if the lumbar bones" changed throughout the course of the therapy, we kept them in for an extra month.

Urine was collected "for assessment of urinary calcium after 4 months of therapy, and blood was drawn under ether anaesthesia to separate the serum for drug concentration estimate. A final sacrifice was made so that bone fragments from femur and low back vertebra could be obtained for histological analysis as well as biochemical" analysis.

A 24-hour urine "collection was performed for the measurement of urinary calcium at the conclusion of each therapy (preventive and therapeutic) (U-Ca). In order to estimate the serum estradiol concentration, the mice were fasted overnight (E2). To collect bones for histopathology and biochemical estimations, mice were euthanized immediately after blood was collected, and femoral and lumbar (L2-L4) bones were collected for DEXA scan BMD analysis of both femoral and lumbar bones, as well" as for alkaline phosphatase, tartrate resistant acid phosphatase, and hydroxyproline (HxP) in lumbar bones.

Data Analysis & Findings

There were more "osteoclasts and ruffled borders in the lumbar vertebrae and the femoral bone matrix after four months of therapy with PHT (35 mg/kg) and SVP (300 mg/kg) than in the control group, according to histology. Osteoclasts and a ruffled border were similarly seen in SVP (100 mg/kg), but LTM did not cause any" histological alterations in the femoral or lumbar vertebrae when used at either of the two dosages.

Dexa scan results reveal a "decline in BMD in the lumbar vertebrae (L2-L4) and the femur (Fig.17). Treatment with PHT (35 mg/kg) and SVP (300 mg/kg) for four months reduced the BMD of the lumbar vertebrae and the femoral bones in mice significantly when compared to the control. SVP (100 mg/kg) had no effect on femoral BMD, but mainly had a lumbar effect. After 4 months of therapy, LTM did not affect BMD at any of the tested" dosages (100 or 200 mg/kg).

Using PHT for four months decreased BMC by 35 mg/kg, but SVP and LTM showed no impact at 100, 300, or 200 mg/kg, respectively.

Our earlier "investigation found that administering PHT (35 mg/kg) for four months resulted in mean blood drug concentrations that were well within the therapeutic range (10-20 g/ml) (Khanna et al., 2011). Also, when administered at 100 and 300 mg/kg, SVP led to blood drug concentrations that were in the therapeutic range for humans (50-100 g/ml) (Senn et al., 2010). While the higher dosage of LTM (400 mg/kg) generated" serum concentrations within the therapeutic range of LTM (12-46 g/ml), the lower dose failed to do so (Patsalos et al., 2008).

In the lumbar "vertebrae of mice, AED administration for four months resulted in alterations in bone formation markers (alkaline phosphatase, ALP) or bone" resorption markers (TRAP, hydroxyproline, and urine calcium, or U-Ca).

When lumbar "vertebrae were treated with PHT (35 mg/kg p.o.) and SVP (300 mg/kg p.o.) but not with SVP (100 mg/kg p.o.) or LTM (100 and 200 mg/kg p.o.), bone ALP" activity was decreased substantially (p0.001).

The lumbar vertebral TRAP activity of mice treated with PHT (35 mg/kg) and SVP (300 mg/kg) was substantially increased (p0.001) compared to controls (p0.05 with SVP 100mg/kg), but LTM (100, 200 mg/kg) had no effect on bone TRAP activity in a meaningful way.

This study found "that the hydroxyproline (HxP) content of PHT's lumbar vertebra was significantly lower than that of the control group (p0.05) and that of SVP's vertebra was significantly higher than that of LTM's vertebra" (p0.001).

Conclusion

Antiepileptic "medications have the potential to have bone-related side effects (AEDs). Despite this, research on the impact of anti-osteoporotic treatments on AED-induced bone loss is sparse. Following AED medication, we speculate that oestrogen deficiency might have harmful bone consequences. AEDs block the human enzyme aromatase and accelerate the microsomal breakdown of estrogens, which is accomplished by the enzyme microsomal catabolism. A bone matrix protein called transforming growth factor- (TGF-3), which has anti-osteoclastic properties, is known to be reduced in oestrogen deficient conditions. As a result, an attempt was made to compare the effects of raloxifene (RLX), an" oestrogen receptor selective "modulator, with calcium and vitamin D3 (CVD) supplementation on AEDs-induced bone changes in mice and to discover the role of estradiol and TGF-3 in mediating bony effects by either AEDs or raloxifene. Additionally, researchers looked at the impact of raloxifene on seizure frequency and AED antiepileptic" effectiveness.

For four months, mice were "treated with PHT (35mg/kg) and SVP (300mg/kg), and bone mineral density in the femur and lumbar vertebrae was decreased. BTM changes in lumbar bones corroborated the findings, including reduced alkaline-phosphatase activity (markers of bone formation), increased TRAP activity, as well as lower HxP concentration, and increased urinary U-Ca excretion. The presence of osteoclasts, ruffled border, and rarefaction of bone matrix were also found in the histopathology of femoral and lumbar bone. These modifications were noted at plasma drug concentrations well within the approved therapeutic limits for humans, where the dosages were" administered.

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