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A study to compare the effects of three AEDs on female mice

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Abstract

Epilepsy is a common neurological condition that often necessitates the use of antiepileptic medications for the rest of one's life (AEDs). The use of prolonged AED treatment has been consistently established over the last four decades to be related with decreased bone density and a greater risk of fracture. "Recent clinical data has also confirmed prior research showing long-term use of AEDs affects bone mineral density (BMD), increases the frequency of fractures, and causes overt osteomalacia (decreased bone density). It has been observed that AED-induced effects on bone occur at rates of 50 percent or more in some cases. Not only do AEDs, but also the illness itself, has an intricate effect on bone microarchitecture and bone mineral density (BMD), increasing the likelihood of fractures. Because a variety of variables may be involved, gross bone alterations and higher risk of fractures are not simply attributed to AEDs, but also to seizure activity-associated falls, trauma, and" an inactive lifestyle.

Despite the fact that the mechanisms responsible for AED-related bone fragility are likely to be numerous and still poorly understood, the AEDs most commonly reported to cause disorders of bone "metabolism are potent inducers of the cytochrome P450 (CYP 450) monoxygenase system (phenytoin; carbamazepine; phenobarbitone) that influence the calcium-vitamin D axis by reducing bio-available vitamin D resulting in hypocalcemia and compens. Despite the fact that this has been considered to be the primary and most common mechanism for AED-induced bone loss, this is not always the case for a variety of reasons, including the fact that not all patients who develop bony deficits have deficient vitamin D levels, and the fact that AEDs that are enzyme inhibitors have also been associated with bony adverse effects. Another mechanism that has been described during AED medication includes hypovitaminosis K, calcitonin insufficiency, decreased" intestinal absorption of calcium, hyperhomocysteinemia, and low levels of oestrogen. These and other processes might all play a role in the detrimental effects on bone.

Keywords: Epilepsy, AED therapy, monoxygenase, osteomalacia

Introduction

However, despite the introduction of second and third generation antiepileptic drugs in recent decades, phenytoin (PHT) and sodium valproate (SVP) are still considered first line drugs and are widely prescribed in the management of partial seizures and generalised tonic-clonic seizures, respectively (Nolan et al., 2013). There is a substantial quantity of information available on the negative effects of various AEDs on "bone health (Boluk et al., 2004; Khanna et al., 2009; Lee et al., 2010; Pack et al., 2011). Evidence on the effects of newer AEDs on bone metabolism is limited and requires further investigation, though there have been a few reports of gabapentin, lamotrigine, topiramate, and vigabatrin affecting bone turnover in epileptic patients (Khanna et al., 2009; Lee et al., 2012; Sheth and Hermann, 2007; Khanna et al., 2009").

Levetiracetam (LTM) is a relatively new medicine that has quickly gained popularity as one of the most commonly prescribed medications for the treatment of partial and generalised seizures (Lyseng-Williamson, 2011). "Despite the increasing use of levetiracetam in clinics, both as monotherapy and as part of a polytherapy regimen, only a few studies have examined its impact on bone mineral density (BMD) in epileptic patients. These studies have produced conflicting results, with some reporting no effects on bone metabolism or bone mineral density in epileptic patients (Koo et al., 2013), while" others report reduced bone biomechanical strength without any significant changes in bone mineral density in animal studies (Koo et al., 2013).

We require appropriate animal models for evaluating the pathogenesis of AED-induced bone alterations

and for "studying the effectiveness of antiosteoporotic regimens in the treatment of epileptic patients. While the majority of the previous research utilised laboratory rats to examine the effect of AEDs on bone (Nissen-Meyer et al., 2007; Onodera et al., 2002), recent studies have used mice to study the effects of AEDs on bone using glucocorticoids (Yang et al., 2009) or ovariectomy (Yang et al., 2009). (Bouxsein et al., 2005). Using" phenytoin (PHT) treatment at 35 mg/kg po for three months, we recently showed in our laboratory a model of bone demineralization in male mice following phenytoin therapy (Khanna et al., 2011). We conducted this investigation to evaluate if bone loss may be caused in mice following continuous treatment of sodium valproate (SVP) or LTM because only a few animal studies have been conducted to establish the effect of these medications on bone mineral density (BMD) and bone markers. "Furthermore, because the majority of previous animal studies on the effects of antiepileptic medications on bone have been done in male animals, we chose female mice for this study in order to establish whether there" are any gender-specific effects of antiepileptic drugs on bone.

It is widely recognised that estrogens play a critical function in the preservation of bone health in women. The anti-osteoclastic "properties of estrogens are mediated primarily through the regulation of transforming growth factor 3, a bone matrix protein with anti-osteoclastic properties (Vaananen and Harkonen, 1996). Estrogens also inhibit the calcium mobilising actions of parathyroid hormone (Marcus, 1991) and increase the differentiation of osteoblasts (Robinson et al., 1996). In addition to managing bone density through regulating the balance between bone matrix deposition by osteoblasts and its resorption by osteoclasts, the latter also has an essential function in bone formation (Grainger et al., 1999). Aside" from that, it has been previously shown that oestrogen shortage conditions can decrease the deposition of TGF- in rat bones (Finkelman et al., 1992).

In the current investigation, "we predict that oestrogen deficiency following AED medication may result in unfavourable bone consequences in the participants. The following" are some of the probable factors that lead us to believe the same thing:

In addition, many AEDs "inhibit the human aromatase (CYP19) enzyme (Jacobsen et al., 2008), thereby inhibiting the conversion of testosterone to estradiol. AEDs also stimulate microsomal catabolism of estradiol and estrone, resulting in increased levels of sex hormone binding globulin (SHBG), which in turn lowers testosterone as well as other adrenal androgens that are aromatized to estrogens Raloxifene has been proposed as a possible treatment for AED-induced bone degeneration. For the prevention and treatment of osteoporosis in postmenopausal women, the drug raloxifene" (RLX), a benzothiophene that also functions as a Selective Estrogen Receptor Modulator (SERM), has been authorised by the FDA (Ettinger et al., 1999). "RLX inhibits osteoclastic activity and bone remodelling in a manner similar to oestrogen by interacting with the oestrogen receptor (ER) with high affinity (Bryant, 2001). When compared to bisphosphonates, it has a substantial impact in reducing the risk of vertebral fracture (Reginster, 2011). There has been evidence of the beneficial benefits of RLX on the prevention of morphometric and clinical vertebral fractures, which are the most prevalent fractures in postmenopausal osteoporosis patients (Ettinger et al., 1999; Nakamura et al., 2006). Aside" from that, it is related with an early rise in lumbar spine bone mineral density (BMD) "and has beneficial effects on biochemical markers of bone turnover and lipid profile (Morii et al., 2003). Unusual for this compound, in addition to having agonistic actions on bone, it also has antagonistic effects on the breast (Delmas et al., 1997; Cummings et al., 1999). Because RLX is also effective" at lowering the chance of getting breast cancer, the FDA authorised it for the treatment of postmenopausal women at high risk of developing breast cancer in "2007 (Lee et al., 2008; Lee et al., 2009").

Literature Review

Epilepsy is one of the most prevalent neurological diseases of the central nervous system (CNS). The name "epilepsy" has been in use since 500 BC, "and it literally means" "attack" or "assault" in the Greek language. In accordance with the International League Against Epilepsy (ILAE) "commission report [Fisher et al., 2005], it is characterised by recurring and unprovoked epileptic seizures [ILAE (International League Against Epilepsy) commission report]. In the brain, an epileptic seizure is defined as a brief occurrence" of signs and/or symptoms that are caused by abnormally excessive or synchronised neuronal activity (Fisher et al., 2005). Epilepsy is "typically associated with neurobiological, cognitive, psychological, and social ramifications (Fisher et al., 2005). Individuals of all ages are affected by it, with the largest incidence occurring in the first year of birth" and in those over the age of 65 ("Hauser et al., 1993; Olafsson et al., 2005; Hauser et al., 2006").

Activated epilepsy is found "in 0.7 percent of the population in industrialised nations (Hauser et al., 1996; Keränen and Riekkinen, 1988), with an incidence rate of around 50 per 100,000 people in developing countries (Hauser et al., 1996; Olafsson et al., 2005). It affects 50 million individuals globally, with the majority (80%) of those affected living in poor countries (Meyer et al., 2010). However, according to two community-based studies (Ray et al., 2002; Sridharan, 2002), the prevalence rate in India is approximately 5 per 1000 population (at this rate, the current estimate of the total number of epileptics" in this country is approximately 5 million), and the incidence rate ranges from 38 to 49.3 per 100,000 population per year (Ray et al., 2002; Sridharan, 2002).

The International League Against Epilepsy "Task Force has said that the 1989 seizures classification's dichotomies of localization-related vs generalised and idiopathic versus symptomatic epilepsy are excessively simple and difficult to implement. As opposed to the existing 1981 seizure classification (Commission of the International League of Epilepsy, 1981), the notion of seizure type as a diagnostic entity, rather than a description" of clinical behaviour and "electroencephalogram (EEG), is a novel concept (Engel, 2001). When seizure types are recognised as diagnostic entities, it becomes possible to infer patient treatment and prognosis from a diagnosis of a specific seizure type when a syndromic diagnosis" is not readily apparent.

Antiepileptic medicines (AEDs) " constitute the cornerstone of epilepsy treatment, and despite the fact that the number of available AEDs has increased enormously, the principles regulating drug therapy today are strikingly identical to those established more than a century ago (Shorvon, 2009). Perucca et al. (2000a) noted that while AED medication is generally maintained for several years and is frequently for life in adults, the" choice to start treatment has far-reaching effects and must be based on rigorous risk-benefit evaluations.

The ultimate objective of "epilepsy treatment is to provide patients with long-term relief from seizures while minimising side effects. As a result, the choice of the initial AED should be guided largely by evidence of efficacy for the patient's seizure type or epileptic syndrome, as well as tolerability concerns, ideally on the basis of data from well-designed" randomised controlled trials.

When it comes to AEDs, "the profile of action against distinct seizure types and disorders differs from one another (Table 3). Specialized medicines have been discovered to be particularly effective in treating various epilepsy disorders. For example, sodium valproate has been shown to be effective in the treatment of juvenile myoclonic epilepsy (Sundqvist et al., 1998), while Vigabatrin is widely considered as the therapy of choice for infantile spasms related to tuberous sclerosis (Elterman et al., 2001). On the other hand, narrow spectrum medications such as carbamazepine (Liporace et al., 1994), phenytoin (Duarte et al., 1996), gabapentin (Ascapone, 2000), and oxcarbazepine (Gelisse et al., 2004) might" exacerbate myoclonic jerks and absence seizures.

Along with "effectiveness and efficiency, there are numerous other characteristics that may influence the choice of AEDs, which the conventional randomised controlled trials may not be able to capture. Adverse effects such as rare idiosyncratic responses, teratogenic consequences, and persistent side-effects are among those to be concerned about. The presence of enzyme-inducing effects and the potential for medication interactions, as well as the availability of parenteral formulations and the possibility of achieving an effective target dosage quickly in some circumstances, are all significant considerations. As a matter" of fact, all existing guidelines highlight the need of taking unique patient features into account when selecting an AED. The presence of reproductive potential, elderly age, and comorbidities are all variables that should be considered in addition to seizure kinds.

The expected efficacy of "AEDs must be evaluated against the possibility of unwanted effects, while also taking into consideration the dangers associated with delaying therapy. These hazards should be evaluated in the broadest meaning possible, taking into consideration the patient's perspective in connection not just to the risk of additional seizures but also to the risk of seizure-related morbidity and death, as well as the risk of AED toxicity, among other things. In an ideal world, a knowledge of the normal course" of untreated epilepsy and its effects would be included in the evaluation of the possible advantages of therapy (Perucca and Tomson, 2011).

Finally, issues like as the "cost of medicines, reimbursement considerations, and the unique advantages and disadvantages associated with each drug all influence" the selection of AED treatment.

Research Gap

This work provides the first "experimental evidence for the possible involvement of raloxifene in the prevention and amelioration of PHT and SVP-induced bone loss, without interfering with the antiepileptic efficacy of these AEDs, according to the authors. It is the first time that an unfavourable impact of AEDs on bone has been reported in Swiss strain albino female mice, and this study expands prior findings of such an effect. Moreover, the absence of bony effects seen following LTM treatment implies that the same may be a superior option" to PHT or SVP in female epileptic patients or those with an increased risk of developing osteoporosis.

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.

On "electroshock-induced seizures, we studied the effects of raloxifene alone and in combination with either PHT or SVP over four months. According to our findings, prolonged raloxifene (RLX) therapy failed to alter electroshock-induced seizures' ability to cause hind limb extension (HLE). According to their findings, RLX therapy increased survival following status epilepticus in rats. However, in our study, we found no protective impact of RLX on HLE latency or duration, as Scharfman and coworkers had shown. To find out if RLX has any antiepileptic effects, it must be studied in different seizure models, such as threshold models. With PHT, SVP and LTM it generated outcomes equivalent to those of PHT, SVP and LTM on their own. To conclude, prolonged use of RLX with these AEDs did not affect their antiepileptic effectiveness (PHT, SVP, or LTM), therefore" ruling out any probable drug-drug interaction. 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. Recent research on young adult epileptic patients supports our findings on LTM, which found that patients who converted to LTM had greater BMD in the lumbar" spine and femur than those on enzyme-inducing AEDs like PHT (Phabphal et al., 2013).

Research Objective & Methodology

The mice utilised were Swiss strain female albino (25–35 g). 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. They were fed a normal pellet meal" and free access to water.

Six groups of ten animals each were "used in this experiment. Drugs used to treat epilepsy were given orally

once a day for four months, including PHT (35 mg/kg), SVPL (100 mg/kg), SVPH (300 mg/kg), LTML (100 mg/kg), and LTMH (200 mg/kg) (Table 12). 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). We kept the patient on the treatment plan for an extra month to monitor any changes in the lumbar vertebrae.

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.

Bone tissue sample preparation

We removed the muscles and tissues around the "lumbar vertebrae (L2-L4) via dissection. A 10 volume solution of 10 mM triethanolamine buffer was used to homogenise the bones, and each one was weighed separately (pH 7.5). The homogenate was centrifuged after 1.5 hours of stirring at 4°C. Bone extracts were utilised to determine the activity of alkaline phosphatases (ALP) and tartrate-resistant acid phospholases ("TRAP") after the extraction technique" had been performed twice. 105 C for 24 hr hydrolyzed the insoluble pellets, which were then tested for hydroxyproline (HxP) concentration.

Serum preparation

Each animal's blood "was taken and incubated for 45 minutes in an upright posture at room temperature (about 2.5 times the volume required for usage). The samples were centrifuged at 3,000 rpm for 10 minutes after the clot had" retreated. The resulting serum was chilled to -20°C and kept for further use.

Urine Collection

Urine was collected for "24 hours in a flask on ice, then kept at -20°C until" needed again.

Histopathological studies (Belur et al., 1990)

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). Using LTM (200 mg/kg) resulted in blood concentrations" that were within the therapeutic range (12-46 g/ml), however using a lower dose did not result in serum drug concentrations that were within the therapeutic range

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.

Researchers studied the effects on bone of "different doses of PHT, SVP, and LTM given to Swiss female mice for four months each. According to the research, the plasma levels of AEDs were found to be within clinically relevant therapeutic limits for the drugs. It was necessary to convert the doses used therapeutically in humans to generate the RLX and calcium and vitamin D supplement (CVD/CVDD) dosages. RLX or CVD was used as a preventive therapy for 4 months while AEDs were used as a therapeutic treatment for 1 month following the 4 month treatment period with AEDs. X-ray absorptiometry and histology of the femur and lumbar vertebral bones were used to examine changes in bone density and mineral content in these bones (DEXA). Researchers" also found alkaline phosphatase (ALP), tartrate resistant acid phosphatase (TRAP), and hydroxyproline (HxP) in lumbar bones, as well as calcium excretion in the urine. In addition, the researchers measured levels of serum estradiol and lumbar TGF3.

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