Antiepileptic drugs and Bone Health

Epilepsy is a common neurological disorder usually requiring lifelong treatment with antiepileptic drugs (AEDs). The last four decades has steadily demonstrated that the use of chronic AED therapy is associated with reduced bone mass and a higher risk of fracture (Kruse, 1968; Lee et al., 2010; Petty et al., 2007). Recent clinical evidence has also authenticated previous studies that long term administration of AEDs affect bone mineral density (BMD), increase the incidence of fracture and overt osteomalacia (Shiek et al., 2012; Espinosa et al., 2011). Prevalence rates of 50% or more have been reported for AED-induced effects on bone (Valsamis et al., 2006). Not only AEDs but also the disease per se intricately modulates the bone microarchitecture and BMD thereby increasing the incidence of fractures (Valsamis et al., 2006; Pack et al., 2008). Multiple factors may contribute and hence the gross bony changes and increased risk of fractures are not solely attributable to AEDs but also to seizure activity associated falls, trauma and sedentary lifestyle (Khanna et al., 2009).

While the mechanisms responsible for AED-related bone fragility are probably multiple and still inadequately understood, AEDs most commonly reported to cause disorders of bone metabolism are potent inducers of the cytochrome P450 (CYP 450) monooxygenase system (phenytoin; carbamazepine; phenobarbitone) that influences calcium-vitamin D axis by reducing bio-available vitamin D resulting in hypocalcemia and compensatory secondary hyperparathyroidism that restores calcium levels both rapidly by increasing the reabsorption of calcium by renal tubules of the kidneys and slowly by mobilizing calcium from bones thereby contributing to bone loss (Valsamis et al., 2006; Khanna et al., 2009). Though this has been considered to be the primary and the most common mechanism for AEDs-induced bone loss, the same is not always true due to various reasons; firstly, not all patients who develop bony deficits have deficient vitamin D levels (Pack et al., 2011) and secondly, AEDs that are enzyme inhibitors have also been associated with bony adverse effects (Boluk et al., 2004). Thus, other mechanisms such as hypovitaminosis K, calcitonin deficiency, reduced intestinal absorption of calcium, hyperhomocysteinemia and deprived estrogen levels reported following AED therapy may contribute to adverse effects on bone (Fitzpatrick, 2004; Khanna et al., 2009, 2011).

Despite the introduction of second and third generation AEDs in the past decades, phenytoin (PHT) and sodium valproate (SVP) are still considered to be the first line drugs and are widely prescribed in the management of partial seizures and generalized tonic-clonic
seizures (Nolan et al., 2013). Ample amount of literature is available on the adverse consequences of these AEDs on bone (Boluk et al., 2004; Khanna et al., 2009; Lee et al., 2010; Pack et al., 2011). The evidence on newer AEDs affecting bone metabolism is limited and needs to be investigated though there have been 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).

Levetiracetam (LTM) is relatively new and one of the widely prescribed drugs for the management of partial and generalized seizures (Lyseng-Williamson, 2011). Despite the increasing use of levetiracetam both as monotherapy and polytherapy in clinics, few studies have evaluated its effects on BMD in epileptic patients. These studies have yielded conflicting results reporting either no effects on bone metabolism or BMD in epileptic patients (Koo et al., 2013) or reduced bone biomechanical strength without any significant changes in BMD in animal studies (Nissen-Meyer et al., 2007).

For assessing the pathophysiology of AED-induced bony changes and for investigating the efficacy of antosteoporotic regimes in epilepsy, we need suitable animal models. Most of the earlier studies have used laboratory rat to study the effect of AEDs on bone (Nissen-Meyer et al., 2007; Onodera et al., 2002), though there are animal models of osteoporosis in mice using glucocorticoids (Yang et al., 2009) or ovariectomy (Bouxsein et al., 2005). We recently demonstrated a model of bone demineralization in male mice following phenytoin (PHT) administration at 35 mg/kg po for 3 months in our laboratory (Khanna et al., 2011). In the present study, we investigated whether bone loss can be induced following chronic administration with sodium valproate (SVP) or LTM since only few animal studies have determined the effect of these drugs on BMD and bony markers in mice. Further, since most of the earlier animal studies on AED effects on bone have been conducted in male animals, we selected female mice in this study to determine possible gender-specific effects of antiepileptic drugs on bone.

**Estrogen, TGF β3 and AED-induced effects on bone**

It is well known that estrogens play an important role in maintaining bone health. Estrogens not only reduce the level of cytokines (interleukins, IL-6, IL-7; tumor necrosis factor, TNF-α) that recruit osteoclasts (Vaanannen and Harkonen, 1996) but also oppose the calcium mobilizing actions of parathyroid hormone (Marcus, 1991) and increase the
differentiation of osteoblasts mainly through regulation of transforming growth factor, TGFβ3, a bone matrix protein having anti-osteoclastic property (Robinson et al., 1996). The latter plays an important role in controlling bone density by regulating the balance between bone matrix deposition by osteoblasts and its resorption by osteoclasts (Grainger et al., 1999).

In addition to this, estrogen deficiency states have been previously reported to reduce the deposition of TGF-β in rat bones (Finkelman et al., 1992).

We hypothesize in the present study that estrogen deprivation following AED therapy could lead to adverse bony effects. The possible reasons which led us to believe the same are as follows:

a) Many AEDs inhibit human aromatase (CYP19) enzyme (Jacobsen et al., 2008) thereby inhibiting conversion of testosterone to estradiol,

b) AEDs stimulate microsomal catabolism of estradiol and estrone resulting in increased levels of sex hormone binding globulin (SHBG) that in turn lowers testosterone and other adrenal androgens that are aromatized to estrogens (Khanna et al., 2009),

c) AEDs-induced vitamin D deficiency may reduce the expression of aromatase that could lead to bony deficits via a) described above.

**Raloxifene as a potential candidate for AED-induced bone disease**

Raloxifene (RLX), a benzothiophene and a Selective Estrogen Receptor Modulator (SERM), is approved for prevention and treatment of osteoporosis in postmenopausal women (Ettinger et al., 1999). RLX suppresses osteoclastic activity and bone remodelling in a manner similar to estrogen through high affinity interactions with ERα (Bryant, 2001). It significantly reduces vertebral fracture risk nearly as much as the bisphosphonates (Reginster, 2011). The positive effects of RLX on the prevention of morphometric and clinical vertebral fractures, the most common fractures in postmenopausal osteoporosis, have been reported (Ettinger et al., 1999; Nakamura et al., 2006). It is also associated with an early increase in the lumbar spine BMD and has favourable effects on biochemical markers of bone turnover and lipid profile (Morii et al., 2003). Interestingly, together with its agonistic effects on bone, it displays antagonistic effects on the breast (Delmas et al., 1997; Cummings et al., 1999). Given that RLX is also effective at reducing the risk of developing breast cancer, the FDA
approved it for the treatment of postmenopausal women with a high risk of breast cancer in 2007 (Lee et al., 2008).

Although multiple therapy for bone disease such as bisphosphonates, hormone replacement therapy, selective estrogen receptor modulators (SERMs) and calcitonin are approved (Das and Crockett, 2013), very few studies have evaluated the effect of these treatments in AED-induced bone loss. This is surprising as the adverse effects on bone have been reported with these AEDs since 1968 (Kruse, 1968), yet not much research has been carried out with approved anti-osteoporotic agents on AED-induced bone effects. Calcium-vitamin D (CVD) supplementation is generally recommended on chronic use with AEDs. Recently, we demonstrated reversal of PHT-induced bone loss in mice by bisphosphonates through reversal of pro-oxidant effects of PHT mediated through PHT-induced hyperhomocysteinemia (Khanna et al., 2011). More recently, the antiepileptic drug and osteoporosis prevention trial (ADOPT) also reported an improvement in BMD in about 70% of epileptic men and prevention of development of vertebral fractures following risedronate with CVD supplementation (Lazzari et al., 2013). However, the frequent gastrointestinal problems and the reports of over suppression of bone turnover and osteonecrosis of jaw following bisphosphonates may limit their use in at least some of the patients (Lee et al., 2008; Khosla et al., 2007).

The present study is an attempt to investigate whether RLX, a SERM and an approved drug for osteoporosis in woman, is effective in preventing or ameliorating AEDs-induced bony deficits in female mice. We selected raloxifene due to multiple reasons:

a) Firstly, women may be particularly susceptible to enzyme-inducing AED-induced bone loss because of an independent risk of osteoporosis and fractures that occur in early menopause and in postmenopausal state (Sanders and Geraci, 2013);

b) Secondly, most AEDs including PHT and SVP could lead to estrogen deprivation-induced bony deficits via mechanisms described in the preceding paragraphs;

c) Thirdly, RLX may increase the threshold for seizures based on some reports on the antiepileptic effects of SERMs in animal models (Borowicz et al., 2002; Scharfman et al., 2009).
Thus, we investigated the effects of RLX (in comparison with calcium and vitamin D3 (CVD) supplementation) on antiepileptic drugs-induced bony changes. Further, the effect of raloxifene on seizures *per se* and on the possible modulating effect on the antiepileptic efficacy of PHT and SVP was determined. The probable role of estrogen and TGF-β3 in mediation of bony effects by either AEDs or raloxifene was also investigated.

**AIMS AND OBJECTIVES**

This study was designed with the following aims and objectives:

- To evaluate the comparative effect of three AEDs (phenytoin, sodium valproate and levetiracetam) on bony alterations in female mice
- To investigate the effect of raloxifene (in comparison with calcium and vitamin D3 supplementation) on AEDs-induced alterations in bone mineral density and bone turnover markers
- To study the effect of raloxifene on the antiepileptic efficacy of AEDs in electroshock-induced seizures
- To assess the probable role of estrogen and TGF-β3 in mediation of bony effects by either AEDs or raloxifene