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Short communication

Anti-epileptic drugs and bone loss: Phenytoin reduces pro-collagen I and alters the electrophoretic mobility of osteonectin in cultured bone cells

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ABSTRACT

Phenytoin is an antiepileptic drug used in the management of partial and tonic-clonic seizures. In previous studies we have shown that valproate, another antiepileptic drug, reduced the amount of two key bone proteins, pro-collagen I and osteonectin (SPARC, BM-40), in both skin fibroblasts and cultured osteoblast-like cells. Here we show that phenytoin also reduces pro-collagen I production in osteoblast-like cells, but does not appear to cause a decrease in osteonectin message or protein production. Instead, a 24 h exposure to a clinically relevant concentration of phenytoin resulted in a dose-dependent change in electrophoretic mobility of osteonectin, which was suggestive of a change in post-translational modification status. The perturbation of these important bone proteins could be one of the mechanisms to explain the bone loss that has been reported following long-term treatment with phenytoin.

1. Introduction

Many of the most commonly used anti-epileptic drugs (AEDs) are associated with bone disease, as evidenced by biochemical abnormalities, increased fracture risk and decreased bone mineral density (reviewed by Nakken and Taubøll (2010) and Lee et al., 2010). AEDs that are implicated in hepatic cytochrome p450 dysregulation leading to vitamin D deficiency with subsequent bone loss appear to have the strongest association with bone abnormalities (Välimäki et al., 1994; Pack 2003). This association does not fully explain the mechanism(s) of AED-induced bone loss however, since an increase in bone turnover with AEDs can occur independently of vitamin D deficiency (Välimäki et al., 1994; Weinstein et al., 1984).

Despite the clear body of evidence that describes the effects of AEDs on fracture risk and bone mass, few studies have investigated the direct effect of AEDs on bone cells. In a previous study, we examined the effect of the AED, valproate, on an established cell-based model of long bone-derived osteoblasts (hFOB1.19) and found for the first time that valproate reduced the amount of two key bone proteins, collagen I and osteonectin (Humphrey et al., 2013). Collagen I is the main protein component of bone matrix and osteonectin has a major role in bone development and mineralisation (Delany et al., 2003), so reduced levels may contribute to bone loss following long-term treatment with valproate. The aim of this study was to determine whether other commonly used AEDs also reduce levels of these important bone proteins in osteoblast-like cells.

2. Materials and methods

2.1. AED compounds

AEDs were tested at a range of concentrations that were as close as possible to clinically relevant serum concentration (i.e. phenytoin (5–40 μg/mL, Gallagher and Sheehy, 2000); topiramate (5–40 μg/mL, Huh et al., 2013); levetiracetam (5–40 μg/mL, Bobustuc et al., 2010), lamotrigine (2.5–20 μg/mL, Johannessen and Tomson, 2006) and carbamazepine (5–40 μg/mL, Gao and Chuang, 1991) (all from Sigma-Aldrich, UK). AEDs were solubilized in DMSO and stored as 2000-fold stock solutions.

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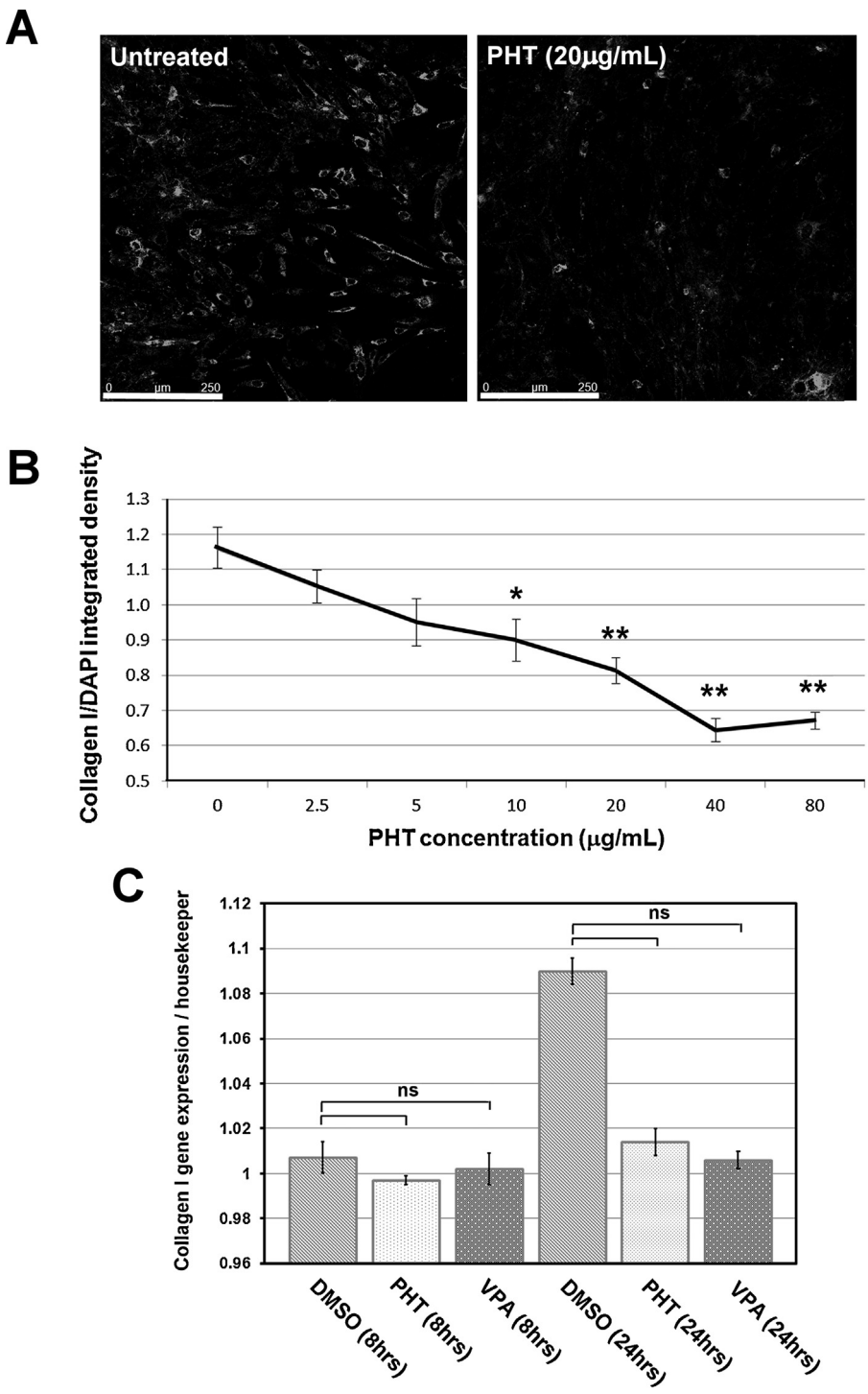

Fig. 1. Pro-collagen I protein, but not gene expression levels, is reduced in osteoblast-like cells after treatment with phenytoin. Acetone/methanol fixed cells were incubated with the anti-pro-collagen I monoclonal antibody (M-38) and visualised using a goat anti-mouse ALEXA 488. Bar = 100 μm. Ten fields of view were chosen at random from each slide using DAPI view to avoid bias and include at least 200 cells over 10 images. The integrated density for each confocal microscope image was measured using ImageJ software and normalised to DAPI staining for each image. A representative image (A) and quantitative measurements from the dose response of phenytoin (PHT) on collagen protein (B) are shown. Collagen I gene expression was measured in cells treated with valproate (VPA) or PHT for 8 or 24 h. No significant change in gene expression could be detected with either AED (C).

2.2. Western blotting

Human foetal hFOB1.19 osteoprogenitor cells (hFOBs) were cultured as described previously (Humphrey et al., 2013). After establishing that parallel differentiated cultures were producing and mineralising a matrix in culture (Supplementary Fig. 1), the hFOBs were treated with vehicle control (i.e. DMSO) or AEDs. Triplicate cultures of control and AED-treated hFOBs were harvested by trypsination after 24 h of treatment and analysed by western blotting using an antibody against osteonectin (Santa Cruz Biotechnology), as described previously (Fuller et al., 2010).