REVIEW ARTICLE

An Update on the Mechanisms of Phenytoin Induced Gingival Overgrowth

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Abstract:
Background: Phenytoin induced gingival overgrowth, a side effect with multifactorial aetiology, is characterized by an increase in the volume of extracellular tissues, particularly collagenous components, with varying degrees of inflammation.

Objective: The aim of this paper is to review the available literature regarding the pathophysiological mechanisms of phenytoin induced gingival overgrowth.

Methods: A thorough literature search of the PubMed/ Embase/ Web of science/ Cochrane central database was conducted to identify the mechanisms involved in the process of phenytoin-induced gingival overgrowth using the following keywords: Phenytoin; Anticonvulsant; Gingival Overgrowth; Gingival Enlargement, Gingival Hyperplasia; Drug Induced Gingival Enlargement; Drug Induced Gingival Overgrowth

Results: According to the available evidence, several mechanisms have been proposed addressing the pathophysiological mechanism of phenytoin induced gingival overgrowth both at a cellular and molecular level. Evidence suggests that the inflammatory changes in the gingival tissues orchestrate the interaction between phenytoin and fibroblasts particularly resulting in an increase in the extracellular matrix content.

Conclusion: However, the mechanism of production of inflammatory mediators is not fully understood. This, together with the high prevalence of Phenytoin induced gingival overgrowth, warrants further research in this area in order to develop treatment and preventive strategies for the management of this condition.

Keywords: Anticonvulsant, Gingival overgrowth, Gingival enlargement, Phenytoin, Drug induced gingival enlargement, Drug induced gingival overgrowth.

1. INTRODUCTION

Gingival Enlargement (GE) or Gingival Overgrowth (GO) is a clinical condition that alters the position of the gingival margin and comprises an increase in the size of the gingiva [1]. GO was previously described as hypertrophic gingivitis or gingival hyperplasia. Nevertheless, “gingival hyperplasia” is a misnomer as enlargement is rather an increase in extracellular tissue volume and not an increase in the number of cells [2]. It may be caused due to plaque induced inflammatory conditions, medications [3, 4], hereditary causes and systemic diseases [5 - 7].

Medication induced gingival overgrowth, is an unwanted side effect of systemic medication on periodontal tissues [7]. It was reported in the early 1960’s in dental literature and is sometimes referred to as “Drug Induced Gingival enlargement or Overgrowth” or “DIGO” [7, 8]. The three drugs most frequently implicated are phenytoin, calcium channel blockers such as nifedipine [9], amlodipine, and verapamil [10] and lastly, an immune-suppressant, cyclosporine [11, 12]. Gingival overgrowth is a well-known and established side effect of phenytoin [13 - 15]. Other drugs for example sodium valproate
[16] and erythromycin [17] have been implicated in case reports, but the occurrence is rare [18].

In terms of prevalence, phenytoin, cyclosporine and calcium channel blockers account for 50%, 30% and 10-20% of drug induced gingival overgrowth, respectively [13, 19, 20]. However, the prevalence varies significantly and depends upon the population being investigated [21]. The stated figures do not take into account the severity of the overgrowth. Various risk factors have been identified for drug induced gingival overgrowth, such as age (children and teenagers) [15], demographic variables, drug variables, periodontal variables [22], genetic factors and concomitant medication [23].

Phenytoin, introduced in 1938, is now an established and effective treatment of acute repetitive seizures, partial-onset and generalized tonic-clonic seizures and status epilepticus [24]. Phenytoin was later used as an antiarrhythmic drug in cardiology [25]. However, currently, its usage as antiarrhythmic purpose is abandoned, but still reserves their importance in the treatment of epilepsy [24, 26].

Considering the fact that it is a major first-line antiepileptic drug (AED) in the treatment of partial and secondarily generalized seizures, it is important to know the mechanisms of phenytoin induced gingival overgrowth in order to minimize the occurrence of this adverse effect. A comprehensive understanding of the pathogenesis of this unwanted side effect is mandatory to develop suitable regimens for its management [23, 27].

As of now, in the literature several studies have aimed to ascertain the pathogenesis of drug-induced GO. However, the trigger mechanisms for such conditions are yet inconclusive. Therefore, the aim of this work was to revisit the most relevant studies published about phenytoin-induced gingival overgrowth and outline the possible mechanisms associated with this condition.

A literature search of the PubMed/ Embase/ Web of science/ Cochrane central database was conducted to identify the mechanisms involved in the process of phenytoin-induced gingival overgrowth with no time or language restriction.

2. MECHANISMS OF PHENYTOIN INDUCED GINGIVAL OVERGROWTH

Gingival overgrowth, the enlargement of gingival tissues, is due to an increase in the Extracellular Matrix (ECM) content or increase in the number of cells or both. Increase in the ECM content could be due to increased production or reduced degradation or a combination. An increase in the number of cells may be due to increased proliferation or reduced apoptosis or both. In gingival overgrowth, the expression of genes related to these mechanisms are either increased or decreased. Inflammatory mediators play a major role in altering the expression of the genes related to gingival overgrowth [4]. Some studies have shown that phenytoin decreases the production of ECM and therefore decreases collagen synthesis [28], confirmed by a reduction in mRNA expression of type I and type III collagen. It is evident that gingival overgrowth is due to the impairment in the balance between synthesis and degradation of ECM [20, 28]. Phenytoin enhances the production of inflammatory mediators [29]. Salivary secretion of unbound phenytoin is 10% of the level in the blood. In addition, dental plaque contains phenytoin and its primary metabolite, the para-hydroxyl form [2]. A high concentration of phenytoin and lower tissue levels of the para-hydroxyl metabolite increases the severity of the gingival overgrowth [2].

Phenytoin in the saliva is absorbed or diffused via the sulcular epithelium into the serum causing a double exposure to phenytoin via two pathways, mainly from the systemic circulation and partly from the reabsorption pathway of the saliva. Thus, gingival sulcular tissue is more susceptible to the effects of phenytoin causing gingival overgrowth [30]. However, currently, the sources and the mechanisms of the production of inflammatory mediators in the gingival overgrowth tissues are unclear [30].

Gingival tissue is in a constant state of injury and repair. This results in an increase in chemical mediators such as cytokines, chemokines and an abundance of inflammatory cells [28, 29]. Another possible mechanism is phenytoin related stimulation in the growth of microorganisms such as Bacteroides, actinomyces, Prevotella intermedia, Porphyromonas gingivalis and Treponema denticola and fusiform bacteria [28]. These organisms produce established dental plaque biofilms aggravating the inflammatory process [28, 29]. The abundant inflammatory mediators contribute to the gingival overgrowth. Phenytoin is known to cause folate deficiencies locally or systemically. Folate is necessary for many vital cellular functions such as nucleic acid metabolism and cell proliferation. As a result, phenytoin induced folate deficiency can cause degenerative changes in gingival epithelium. Degenerative materials induce an inflammatory response [4].

The immune system could be stimulated by the drug phenytoin via mechanisms including the induction of lymphoid overgrowth and cell-mediated immunological reaction via interleukin-1β (IL-1β) and tumor necrosis factor alpha (TNF-α), IL-6 and IL-8 and also medullasin [31]. The mediator medullasin activates the inflammatory response via modulating cytokines [31].

IL-13 induces the expression of TGF-β, which is a cytokine found in high concentrations in platelets, macrophages, neutrophils, and fibroblasts [32, 33]. It acts mainly on fibroblasts and endothelial cells and results in collagen and matrix synthesis [33]. This cytokine is maintained in the inactive form by binding non-covalently to a protein called Latency-Associated Protein (LAP) and stored within the cell as a homodimer [34]. Cathepsins and Matrix Metalloproteinases (MMPs) release the LAP from TGF-β and activate TGF-β [35]. IL-13 also increases the expression of MMP-2, MMP-9 and cathepsins [38]. Phenytoin enhances IL-13 secretion, thus TGF-β expression and produces its active form.

The occurrence of fibrosis requires both TGF-β and Connective Tissue Growth Factor (CTGF). In the gingival tissue, the effects of TGF-β are mainly via CTGF. However, in the gingival overgrowth tissue, the TGF-β is in a higher concentration in the initial period whereas the concentration of
CTGF remains high at a constant level [36]. This implicates that TGF-β induces the expression of CTGF through a cascade effect. CTGF is a known producer of fibrosis. It also increases the proliferation of fibroblasts due to its mitogenic, angiogenic and chemotactic activities [36].

The degradation of collagen occurs via two mechanisms. One is extracellularly, by collagenases and the second intracellularly following collagen phagocytosis [37]. MMPs are a family with 25 enzymes that are endopeptidases [38]. The action of MMPs depends on Calcium and Zinc; MMPs can be secreted or are membrane associated and vital in ECM metabolism [39]. As, MMPs are collagenases, stromelysins, and gelatinases, they are involved in tissue remodeling of ECM especially tissue degradation [38, 39]. MMP-1, also known as collagenase-1, degrades the fibrillar collagen through activating the inactive collagenase [39]. Any altered balance due to either activation or inhibition results in a change in the amount of ECM. Thus, inhibition of MMPs causes excessive accumulation of connective tissues in the ECM [40 - 42].

Natural MMP inhibitors exist in all animal tissues [43]. One of the main MMP inhibitor families is Tissue Inhibitors of Metalloproteinases (TIMPs) consisting of four types. MMP-1 mediated ECM remodeling, the degradation of ECM, is inhibited by TIMP-1, resulting in excessive accumulation of connective tissue in ECM. In addition, TIMP-1 has growth-promoting activity in several types of cells [44].

Phenytoin causes the inhibition of collagen degradation via MMPs/TIMP-1 [44, 45]. Phenytoin reduces intracellular folic acid and induces the production of mediators such as TGF-β and TNF-α [45]. The three main drug categories (phenytoin, cyclosporine and calcium channel blockers) causing drug induced gingival overgrowth have an inhibitory action on cation channels. Based on the available evidence, a unified theory has been proposed [4]. The antiepileptic action of phenytoin is mainly through the inhibition of sodium channels. However, phenytoin also inhibits the calcium channels, including in the gingival fibroblasts [46, 47]. Folic acid requirement depends on the type of tissue and is higher in some tissues including gingival tissues. This can result in local folate deficiency despite normal serum folate levels [4]. Cellular uptake of folic acid occurs through two mechanisms. The first is passive diffusion and the second cation dependent active transport [4, 48]. The cation dependent active cellular intake of folic acid is impaired by phenytoin [4]. This can result in a systemic and localized folate deficiency in the gingival tissue.

Decreased cellular folic acid leads to reduced expression of E-cadherin and SMAD (SMAD proteins are signal transducers and transcriptional modulators that mediate multiple signaling pathways) which reduces the expression of the AP-1 gene. Reduced AP-1 activates the TIMP-1 gene expression. The result is that phenytoin decreases the MMP-1 by increasing the expression of TIMP-1. As a result of this, collagen accumulates in ECM and causes gingival overgrowth [44, 49, 50].

Cathepsin degrades ECM components such as type 1 collagen, laminin and proteoglycans.

As phenytoin inhibits cathepsin, the result is the accumulation of ECM components [42, 51, 52].

Integrins are a large family of heterodimeric transmembrane receptors. Each heterodimer consists of α and β subunits. In mammals, there are 17α and 8β subunits that can form 40 different integrins through different combinations. The α1β1 integrin recognizes type IV collagen preferentially and the α2β1 integrin recognizes type I collagen preferentially [53]. Studies have indicated that α2β1 integrins serve as specific receptors of type I collagen in fibroblasts. For collagen phagocytosis, collagen should adhere to the fibroblast as the first step. It has been also shown that α2 integrin plays a critical role in the phagocytic regulation of collagen internalization by adhering to collagen [53]. Phenytoin reduces the collagen internalization, therefore causing degradation either by reducing the affinity of integrins to collagens or reducing the expression of integrins. A proposed mechanism for the reduced expression of integrins is diminished intracellular calcium due to the calcium channel antagonist effect of phenytoin. Hence, collagen accumulation in the ECM will result in gingival overgrowth [28].

Another mechanism through which phenytoin induces gingival overgrowth is by the conversion of androgens to their active metabolites. Studies have shown that fibroblasts of the gingiva have the ability to metabolize testosterone to its active metabolite 5α-dihydrotestosterone, enhanced by the addition of phenytoin to the cultured fibroblasts [54]. ECM synthesis could be enhanced by the stimulatory effect of phenytoin on the type 2 isoenzyme of 5α-reductase activity. This action takes place in either a ligand-dependent manner through the stimulation of their own receptors, or a ligand-independent manner, through the direct stimulation of the androgen receptor. Type 2 reductase of 5α-reductase, via the production of 5α-dihydrotestosterone, has an anabolic role in gingiva by activating the fibroblast cells to produce more collagen fibers or decrease collagenase activity [54]. Some authors have suggested that a trigger of alkaline phosphatase may mediate some of the matrix stimulatory actions of androgen metabolites [55]. Others have mentioned the role of oestrogens to increase the local level of dihydrotestosterone and thereby stimulating collagen formation. Oestrogen enhances the incorporation of proline in collagen molecules being synthesized in the gingival fibroblasts [56].

Phenytoin acts on macrophages, enhancing the production of IL-1β, which enhances the expression of Cyclooxygenase 2 (COX-2) through the transcription factor (nuclear factor-kappaB [NF-κB]). COX-2 is the main enzyme in the production of Prostaglandins (PGs) from arachidonic acid. One such important prostaglandin is PGE2 [30]. Transcription of TGF-β and its receptors are enhanced by PGE-2 [57]. EP-3 receptors are found in gingival fibroblasts but not in lung or kidney fibroblasts. PGE-2 acts on EP-3 receptors in gingival fibroblasts and enhances the TGF-β1 stimulation of JNK-MAP kinase pathway of CTGF expression [30].

Platelet Derived Growth Factor (PDGF), produced by platelets, macrophages, endothelial cells, and fibroblasts, is believed to enhance mitogenic activity and chemotaxis [58]. Phenytoin is also known to increase the production of PDGF. PDGF activates the cytosolic phospholipase A2 enzyme which releases arachidonic acid from the cell membrane by increasing
intracellular calcium. Apart from the indirect effect of PDGF, there is a direct effect by increasing the expression of COX-2 which has been proven in some studies [59, 60].

Epithelial Mesenchymal Transition (EMT) is a biological process of acquisition of the mesenchymal phenotype by the epithelial cells where it acquires an increased migratory capacity, invasiveness and importantly inhibition to apoptosis and enhanced ECM production. Normally, a polarized epithelial cell interacts with the basement membrane via its basal surface [61 - 63]. In EMT, there is a degradation of the basement membrane at the rete ridges and transformed epithelial cells migrate to the connective tissue. EMT is involved in several processes such as embryonic development, cancer progression, and epithelial injury [64]. The process of EMT involves the loss of the polarity of epithelial cells, cell-cell and cell matrix adhesion, remodeling and rearrangement to gain the features of mesenchymal cells [64].

E-cadherin is important in epithelial cell contact which is essential for the barrier function of epithelial tissue [65, 66]. In EMT, there is a reduction in the levels of E-cadherin expression which results in the loss of integrity and barrier function of the epithelial cells [66]. In EMT, a specific marker for fibroblast, Fibroblast Specific Protein-1 (FSP-1) and also fibronectin, and an alternatively spliced form of fibronectin (ED-A isoform), 19 are secreted and found in elevated levels [66 - 68].

Transforming Growth Factor (TGF-β) down regulates E-cadherin and potently stimulates the epithelial mesenchymal transition and at the same time, TGF-β increases the expression of MMP-9 and MMP-2 which degrade type IV collagen in the basement membrane and allow migration of epithelial cells and interact with connective tissue [69 - 71]. Some types of integrins activate the latent TGF-β and can promote EMT.

Forkhead box transcription factors (FOXO) proteins belong to a Forkhead family of transcription factors. It is involved in the activation or inhibition of many genes related to important cellular functions [59]. Importantly, it reduces anabolic metabolism, increases apoptosis and arrests the cell cycle. FOXO-1 enhances the production of TGF-β contributing to gingival overgrowth. Phenytoin is known to inhibit FOXO-1 [72, 73].

A recent study has revealed that Transient Receptor Potential Ankyrin (TRPA1) channels have an important role to play in the pathophysiologic mechanism of phenytoin-induced GE. The calcium-permeable ion channels TRPA1 [74], Transient receptor potential channels, of the vanilloid subtype (TRPV1), and its capsaicin-insensitive isoform TRPV1b are expressed in Human Gingival Fibroblasts (HGFs), in which phenytoin increase the intracellular calcium levels by acting on the mentioned ion channels [72]. Further, phenytoin did not augment the proliferative rate of HGFs whereas it induced extracellular matrix accumulation of collagen [72].

The role of phenytoin in death receptor-induced apoptosis of gingival fibroblasts has been explored in a laboratory study suggesting that phenytoin treatment decreases the proportion of apoptotic cells in gingival fibroblasts compared to a serum-free control culture. This is in response to the upregulation of cellular FLICE-Like Inhibitory Protein (c-FLIP), the cellular inhibitor of apoptosis 2 (cIAP2) and downregulation of Fas-Associated Protein with Death Domain (FADD), caspase-3, caspase-8, caspase-9 and TNF Receptor Associated Factor 2 (TRAF2) by the effect of phenytoin on Receptor-Interacting serine/threonine-Protein Kinase 1 (RIPK1) activity and (B-cell lymphoma 2) Bcl-2 activity [75].

3. SUMMARY FINDING(S) FROM THE STUDY

Although several mechanisms, both at a cellular and molecular level, have been proposed for phenytoin induced gingival overgrowth, there is a lack of understanding in these mechanisms of phenytoin induced gingival overgrowth. Hence, there is no treatment or prevention for the management of this condition. This prompts the necessity of future research in this field, particularly at a molecular level.

CONCLUSION

Phenytoin induced gingival overgrowth is a side effect with multifactorial etiology. Several mechanisms have been proposed addressing the pathophysiological mechanism of phenytoin induced gingival overgrowth both at a cellular and molecular level. Evidence suggests that the inflammatory changes in the gingival tissues orchestrate the interaction between phenytoin and fibroblasts particularly resulting in an increase in the ECM content. However, the mechanism of the production of inflammatory mediators is not fully understood. This, together with the high prevalence of phenytoin induced gingival overgrowth, warrants further research in this area in order to develop treatment and preventive strategies for the management of this condition.

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CONFICT OF INTEREST

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