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Mechanism of drug-induced gingival overgrowth revisited: a unifying hypothesis

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Abstract

Drug-induced gingival overgrowth (DIGO) is a disfiguring side effect of anti-convulsants, calcineurin inhibitors, and calcium channel blocking agents. A unifying hypothesis has been constructed which begins with cation flux inhibition induced by all three of these drug categories. Decreased cation influx of folic acid active transport within gingival fibroblasts leads to decreased cellular folate uptake, which in turn leads to changes in matrix metalloproteinases metabolism and the failure to activate collagenase. Decreased availability of activated collagenase results in decreased degradation of accumulated connective tissue which presents as DIGO. Studies supporting this hypothesis are discussed.

Keywords

drug-induced gingival overgrowth; folic acid; matrix metalloproteins; calcineurin inhibitors; calcium channel blocking agents; phenytoin

Introduction

Drug-induced gingival overgrowth (DIGO), also referred to as drug-induced gingival enlargement, and previously referred to as drug-induced gingival hyperplasia, is a noted side effect of calcineurin inhibitors such as cyclosporine (CsA) and tacrolimus (TAC), anti-convulsant/anti-seizure drugs such as phenytoin (PHT), and calcium channel blocking agents (CCBAs) such as amlodipine and nifedipine. This drug side effect of PHT was first reported by Kimball (1939). DIGO impedes oral hygiene procedures which tend to increase gingival and periodontal inflammation (Hassell and Hefti, 1991; Seymour, 1993; Hood, 2002;

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Trackman and Kantarci, 2004; Kataoka *et al*, 2005; Correa *et al*, 2011). The drug categories, representative drugs, DIGO incidence, and drug mechanisms are listed below in Table 1.

Successful therapeutic management of DIGO includes dental home care/plaque control, topical folic acid (FA), and topical azithromycin therapies. Gingival recontouring with blade or laser has been the two accepted surgical approaches (Rossmann *et al*, 1994; Clementini *et al* 2008). Brown *et al* (1990) discussed a biochemical mechanism of this drug side effect pointing to decreased cellular folate uptake, leading to decreased catabolism, due to an insufficient amount of activated collagenase. Brown *et al* (1991a) further enlarged upon this mechanism discussion regarding a biochemical pathway hypothesis which included the following: (i) increased connective tissue production secondary to bacterial inflammation, (ii) possible increased gingival fibroblast proliferation and/or connective tissue production secondary to the inducing drugs, (iii) the biochemical commonalities of the inducing drugs which all appear to have an inhibiting effect upon cation channels, (iv) research which characterized cellular folate uptake as dependent upon both active transport through cation channels and passive diffusion, (v) possible decreased folate uptake within gingival fibroblasts due to an inhibitory effect of the inducing drugs with regard to cellular folate active transport, (vi) research which demonstrated that DIGO appears to be related to an increased amount of connective tissue (and therefore not hyperplastic), (vii) research which demonstrated that the activation of collagenase appears to be complicated and involves matrix metalloproteinases (MMPs) biochemistry, (viii) research which demonstrated that folate is necessary for amino acid and protein synthesis, (ix) the possibility that DIGO may be related to insufficient activation of collagenase necessary to degrade excess connective tissue and (x) the possibility that DIGO may be secondary to insufficient degradation of excess connective tissue (Figure 1).

This hypothesis purports that the biochemical pathway for DIGO is influenced by bacterial plaque which causes gingival inflammation which increases the buildup of gingival connective tissue (glycosaminoglycans—GAGs). The inducing drugs (anti-convulsants, CCBAAs, and calcineurin inhibitor/immunosuppressive drugs) decrease folate cellular uptake in gingival fibroblast cells. The secondary effect of decreased cellular folate is that the synthesis and/or activation of a particular MMP (or MMPs) is/are decreased and as that (a) particular MMP(s) is/are necessary to convert inactive collagenase to active collagenase within the gingiva; therefore, there is an insufficient amount of active collagenase necessary to breakdown excess gingival connective tissues (built up secondary to inflammation) resulting in the side effect of DIGO (Brown *et al*, 1991a).

The biochemical hypothetical pathway was determined based on several known concepts at the time (1991) and is demonstrated in the Table 2 below.

The formation of the hypothesis was based upon several issues which came to light at the time. As the side effect of DIGO appeared to be relatively the same side effect of three very different drug categories, it stood to reason that these different drug categories had one particular commonality (Brown *et al*, 1991a). All of the DIGO drugs appeared to influence cation flux. Several studies reported that FA cellular uptake was dependent upon both an active transport cation regulated channel and passive diffusion (Ariel *et al*, 1978, 1982). The

Drew *et al* (1987) study reported efficacy in the reversal of DIGO with the utilization of topical FA, while all of the other systemic folate DIGO studies were not nearly as dramatic (Brown *et al*, 1991b). Furthermore, there were inferences that DIGO was the result of the accumulation of connective tissue due to a failure of connective tissue degradation (Kantor and Hassell, 1983) secondary to decreased activated collagenase (Tipton *et al*, 1991, 1994). As bacterial plaque is known to cause increased connective tissue buildup and plaque control demonstrated efficacy in the treatment of DIGO (Modeer *et al*, 1986), a type of 'Rube Goldberg' conceptual biochemical fabrication connecting the dots was constructed. What is particularly important with regard to this hypothesis, with reference to therapy, is that the proposed initial etiology is secondary to an inhibition of the cellular active transport of the FA uptake system which can conceivably be countered. The literature supports that an increased concentration of FA adjacent to the gingival fibroblasts would tend to produce an increased cellular uptake through passive diffusion due to an increased concentration gradient. Therefore, a therapeutic remedy is feasible. In the 20 plus years since the publication of this purported biochemical mechanistic unifying hypothesis, a number of studies have been published which add to the support this unifying hypothesis.

Na+/Ca++ ion flux drug mechanisms

Brown *et al* (1990, 1991a) suggested that it is reasonable to assume that all the three drug categories of DIGO-inducing drugs (anti-seizure, CCBAs, and immuno-suppressive drugs) possess a particular commonality with regard to an inhibitory influence upon cation channels.

Jones and Wimbish (1985) reported that PHT decreases resting fluxes of Na⁺ ions as well as Na⁺⁺ currents with respect to action potentials or chemically induced depolarizations. Colombani *et al* (1985) reported that CsA may interfere with Ca⁺⁺ and calmodulin interactions.

Dretchen *et al* (1986) reported that both PHT and CCBAs decrease the lethality of diisopropylfluorophosphate (DFP) due to an inhibition of Ca⁺⁺ flux into excitable membranes.

Fugii and Kobayashi (1990) reported that both PHT and several CCBAs inhibited Ca⁺⁺ uptake within gingival fibroblast. Modeer *et al* (1991) evaluated cell lines of DIGO responders and non-responders. They reported that in a study of gingival fibroblasts from PHT-IGO responders compared to those of non-responders, demonstrated a basal level of calcium ions which was significantly decreased. They concluded that there is a relationship between PHT changes in calcium ions within gingival fibroblasts and that this relationship corresponded with the clinical development of DIGO. Marche and Stepien (2000) reported that the anti-hypertensive mechanism of CCBAs is due to inhibition of the L-type Ca⁺⁺ channel. Oliveria *et al* (2012) reported, with regard to anti-calcineurin drugs, that calcineurin permits Ca⁺⁺-dependent inactivation of neuronal L-type Ca⁺⁺ channels, and as excitation-driven entry of Ca⁺⁺ through L-type voltage-gated Ca⁺⁺ channels controls gene expression in neurons, a variety of biochemical events are promoted resulting in a limitation of Ca⁺⁺ entry. Thomas and Petrou (2013) reported that anti-seizure drugs reduced sodium channel availability and secondarily produced a reduction in the action potential amplitude. A

downstream event which followed was reduced calcium entry and a reduction of calcium-activated potassium channels. These studies appear to demonstrate that all three categories of DIGO-inducing drugs all effect Ca^{++} flux in similar manner.

A summary Table 3 is provided below.

Plaque control

Since 1991, a number of studies have backed up the previous studies regarding the efficacy of plaque control in the treatment and prevention of DIGO. Pilatti and Sampaio (1997) evaluated the effectiveness of 0.12% chlorhexidine (CHX) in the treatment of CsA-IGO in a rat study. The study included a control group and three active drug groups with CsA or CHX each alone or in combination. The combined CsA and CHX group exhibited significantly lower GO compared to the CsA alone treatment group. Ilgeni *et al* (1999) evaluated the effectiveness of periodontal therapy in CsA- and nifedipine-induced GO. They reported severe GO regression in 13 of the 38 patients, 18 months following periodontal surgery. Multiple regression analysis indicated that age, gingival inflammation, and attendance a recall appointments were significant factors regarding the recurrence of severe DIGO. Aimetti *et al* (2005) evaluated non-surgical and supportive periodontal treatment on transplant patients with CsA-induced GO. They concluded that plaque control appeared to be effective in controlling GO over an extended time period. Dannewitz *et al* (2010) evaluated non-surgical disinfection DIGO therapy. They reported that all clinical parameters improved significantly after therapy. From the above studies, it can be concluded that inflammation secondary to dental plaque is a factor in pathogenesis of DIGO and plaque control should be utilized in both DIGO prevention and therapy.

Increased production of GAGs

There are a great many studies reporting increased connective tissue and proliferation of gingival fibroblasts secondary to the inducing drugs as the primary causation of DIGO (Brown *et al*, 1991a; Seymour *et al*, 1996). Recently, Salman *et al* (2013) reported that CsA-induced fibroblast overgrowth involved IL-6, IL-8, IL-1beta, TGF-beta1, and PGE2. Subramani *et al* (2013) also reported upon recent observations noting TGF-beta, CTGF, IGF, PDGF, ET-1, Ang II, mast cell chymase, and tryptase enzymes, with regard to fibroblast activation and increased connective tissue production. Cotrim *et al* (2003) also reported increased TGF-beta1 stimulation of CsA-treated gingival fibroblasts. However, as noted by Brown *et al* (1991a), there are many conflicts with regard to increased GAG production being the primary rationale for DIGO due to the heterogeneity of gingival fibroblasts, (Tipton *et al*, 1991, 1994) although increased gingival fibroblast production of GAGs is certainly a possibility. However, as treatment with anti-plaque modalities has demonstrated efficacy and more importance with regard to the treatment of DIGO, increased connective tissue production secondary to bacterial-induced production of connective tissue appears to be the more important concern.

Increased GAGs

Past research to determine the pathogenesis of DIGO has largely focused upon the view that this side effect was secondary to hyperplasia or the increased production of connective tissue

secondary to a drug-induced stimulation of gingival fibroblasts. Hassell *et al* (1976) reported that PHT-induced GO tissue overgrowth is made up predominantly of collagen, and therefore, the etiology appeared to be related to fibrosis and a connective tissue abnormality, although they also reported that DIGO fibroblasts appeared to demonstrate increased protein synthesis. However, Hassell (1982) reported that PHT-induced gingival fibroblasts compared to normal gingival fibroblasts secreted increased amounts of an inactive collagenase. Therefore, he proposed that the cause of DIGO may be related to a failure in catabolizing connective tissues. Kantor and Hassell (1983) reported an increased accumulation of sulfated GAGs and noted that this finding could be related either to increased synthesis or decreased catabolism. Furthermore, they reported major differences in gingival fibroblasts subpopulations confirming the heterogeneity of gingival fibroblasts. Goultschin and Shoskan (1980) also noted that PHT inhibited collagen breakdown. Bonnaure-Mallet *et al* (1995), reported that the drug side effect of DIGO is secondary to an increased amount of gingival extracellular macromolecules with an inflammatory infiltrate. Nares *et al* (1996) and Seymour *et al* (1996) also suggested a defect in collagen breakdown as a possible mechanistic issue with regard to the pathogenesis of DIGO. Mariani *et al* (1996), in an ultra-structural and histochemical evaluation of DIGO, concluded that that CsA caused an increased amount of GAG deposition and that therefore, increased GAGs appeared to be the main cause of DIGO. Arora *et al* (2001) reported that CsA markedly inhibits collagen degradation due to a severe decrease in the function of an intracellular phagocytic pathway leading to DIGO. They also reported a net increase in matrix proteins. Kato *et al* (2005) noted that collagen fibers are degraded through either extracellular means (collagenase) or phagocytosis. They evaluated the human gingival fibroblasts in various concentrations of PHT with regard to any potential increase in cell number and the changes in the amount of collagen. Furthermore, gene and protein expression of MMP and tissue inhibitors (TIMP) were quantified by reverse transcription-polymerase chain reaction (RT-PCR) analyses and Western blotting. They determined that the proliferation of human gingival fibroblasts was not significantly affected by PHT, but noted a significant increase in collagen. Furthermore, the expression of MMP-1, MMP-2, and MMP-3 was suppressed by PHT, while TIMP-1 was induced. PHT also prevented collagen endocytosis by gingival fibroblasts. They concluded that PHT causes an impaired degradation of collagen secondary to decreased degradation of TIMP and that the result was collagen accumulation leading to DIGO. Kataoka *et al* (2005) suggested that DIGO is due to a disruption of homeostasis of collagen synthesis and degradation, predominantly through the inhibition of gingival fibroblastic collagen phagocytosis. They concluded that DIGO is caused by reduced gingival fibroblastic collagen phagocytosis through an alpha-2 beta-1 cell surface integrin. They also noted that the actin-binding protein, gelsolin, may be an important factor, because of it maintains normal tissue integrity through integrin binding affinity to collagens, an important factor for the regulation of collagen phagocytosis. Kanno *et al* (2008) evaluated collagen fiber distribution histologically after PHT, CsA, and CCBA therapy to correlate it with collagen I and MMP-1 and MMP-2 gene expression levels. They concluded that these drugs led to phased and drug-related gene expression patterns which resulted in impaired collagen metabolism. Although much of the DIGO literature focuses upon connective tissue production, it is certainly reasonable to believe that a drug-induced defect in catabolism rather than increased anabolism is responsible for the drug side effect of DIGO.

Folate cellular uptake

Vogel (1977) proposed that DIGO may be secondary to a localized FA deficiency. Since 1991, a number of studies increased our known information regarding cellular folate uptake. Heimburger (1992) noted that it is possible that the requirements for FA may be higher in some tissues compared to other tissues, resulting in a localized deficiencies, even though serum folate may be within normal ranges. Furthermore, Heimburger noted that such localized FA deficiencies may occur due to decreased tissue uptake due to an inborn error. Kumar *et al* (1997) concluded that cellular FA uptake appears to involve a carrier-mediated system which is temperature, pH, and energy-dependent and appears to be under the regulation of cAMP and protein tyrosine kinase. Fowler (1998) reported that conversion of a dietary vitamin into an intracellular active co-enzyme is often complex and may require several physiological and biochemical processes, including intestinal uptake, carriers, transporters, cellular release, and intracellular compartmentalization. Fowler noted that genetic folate disorders include tetrahydrofolate reductase deficiency and glutamate formiminotransferase deficiency and a hereditary intestinal folate transport disorder. Sierra and Goldman (1999) reported that the transport of FA in most cells is mediated by a reduced folate carrier (RFCI), an anion exchanging concentrative process which is opposed by independent exit pump(s) within an active transport system. Purine and pyrimidine synthesis may be inhibited by anti-folates which are also transported by the transport system by blocking polyglutamylation to active derivatives (Timmis, 1961). Balamurugan and Said (2006) reported that a reduced folate carrier (RFC) system is the major folate uptake system that is functional in intestinal epithelial cells.

Opladen *et al* (2010) recently reported upon the effect of anti-convulsant drugs upon the folate receptor 1 (FOLR1)-dependent 5-methyltetrahydrofolate (MTHF) transport. They reported that the metabolic breakdown of such anti-convulsants as valproate, carbamazepine, and PHT generates reactive oxygen species (ROS). They studied the effect of these drugs and secondary ROS upon FOLR1 dependent 5-MTHF uptake and reported that MTHF uptake is time and concentration dependent and demonstrates saturation kinetics. At physiologic MTHF concentrations, the high-affinity FOLR1 represented the predominant mechanism for cellular uptake. Exposure to PHT could lead to higher MTHF uptake; however, exposure to superoxide and hydrogen peroxide radicals significantly decreased cellular MTHF uptake. Therefore, it appears that the FOLR1-dependent 5-MTHF transport could also be involved with regard to inhibited folate transport and decreased folate uptake in gingival fibro-blasts.

Successful folate therapy

Drew *et al* (1987) reported a successful efficacy study. Further DIGO folate efficacy studies have followed. Prasad *et al* (2004) in a study of adolescent epileptics receiving PHT concluded that systemic FA delayed the onset and reduced the incidence and severity of gingival overgrowth. Arya *et al* (2011) evaluated systemic FA administration at the initiation of PHT anti-epileptic therapy on adolescent patients and determined that FA was significantly associated with the prevention of gingival overgrowth. Studies reported by Backman *et al* (1989), Poppell *et al* (1991), Prasad *et al* (2004), and Arya *et al* (2011), utilizing systemic therapy, while demonstrating efficacy were not nearly as dramatic. The

systemic folate study by Brown *et al* (1991b) did not demonstrate efficacy and utilized a study population of intellectually impaired adults with problematic oral hygiene compared to the other studies. These studies do tend to demonstrate that topical administration may be more efficacious compared to systemic administration, although it appears that systemic administration may be effective with regard to the prevention of DIGO. Although there was only one reversal efficacy study with topical FA, the success of the Drew *et al* (1987) study supports previously noted evidence which determined that if epithelial cellular FA active transport is shut down, an available high concentration of FA could result in concentration-dependent gradients which would allow for resumed FA cellular uptake. As such, this would support Vogel's (1977) hypothesis that DIGO is an end-organ folate deficiency (Table 4).

Matrix metalloproteinases

Matrix metalloproteinases are a family of more than twenty enzymes that are involved in tissue remodeling with regard to the degradation of extracellular matrix (connective tissue). They include collagenases, stromelysins, and gelatinases. Imbalances in inhibition and activation may result in excessive degradation or accumulation of connective tissues. Taylor *et al* (1996) reported on the mechanism of TIMP-1, the collagenase inhibitor of fibroblastic collagenase. They evaluated time-course inhibition experiments. They described a two-step mechanism concerning the formation of an inactive reversible complex. Collagenase without mini-collagenase (the carboxyl terminal domain) is inhibited by TIMP-1. The mini-collagenase of fibroblastic collagenase is important for the initial, rapid binding of TIMP-1, and the initial complex contributes to the overall binding.

Domeij *et al* (2004) reported regarding MMP-1 and TIMP-1 production. They suggested that there was a different regulatory process for interleukin-1b and calcium in human gingival fibroblasts, and this difference is markedly amplified in the presence of the PKC-activator and proposed that the discrepancy in MMP-1 and TIMP-1 production in gingival fibroblasts may contribute to connective tissue destruction with regard to periodontal disease. Furthermore, in a subsequent study, Domeij *et al* (2005) suggested that co-culture with monocytes enhances cellular expression of MMP-1 and TIMP-1 in gingival fibroblasts. They noted that the increased MMP-1 expression, in contrast to TIMP-1, was partly mediated by the adhesion molecule ICAM-1 and the p38 MAPK signal pathway.

Kato *et al* (2005) examined the effect of PHT administration upon collagen degradation. They evaluated gene and protein expressions of MMPs. They concluded that PHT causes impaired collagen degradation through MMPs/TIMP-1 through particular cellular signaling pathways of ERK1/2 and nuclear factor kappaB, possibly leading to collagen accumulation resulting in GO. Kato *et al* (2006) investigated TNF-alpha (associated with gingival inflammation) and PHT with regard to mRNA levels for collagen and MMPs, and TIMPs. They concluded that together TNF-alpha and PHT synergistically caused impaired collagen metabolism by suppression of enzymatic degradation with MMPs/TIMP-1 resulting in GO. Guo *et al* (2006) investigated whether homocysteine increases the production of MMP-2 and whether either Mg++ or FA alters MMP-2 secretion. They concluded that extracellular FA and Mg++ decreased homocysteine-induced MMP-2 secretion. Dannewitz *et al* (2006) explored the status of MMP-1 and MMP-10 along with tissue inhibitor TIMP-1 in evaluating

gene expression in comparing GO tissues and normal tissues. They reported particularly increased levels of TIMP-1 and moderate increase of MMP-1 and MMP-10 which they concluded contributed to extracellular matrix and GO. Sukkar *et al* (2007) investigated the interaction between IL-1, oncostatin M, CsA, and nifedipine in promoting upregulation of MMP-1 and TIMP-1 in gingival fibroblasts. They reported that IL-1 and oncostatin M caused a significant increase in the upregulation of MMP-1 which was reversed with CsA and nifedipine onboard. They concluded that proinflammatory cytokines significantly upregulate MMP-1 in cultured gingival fibroblasts and that the upregulation was reversed by both CsA and nifedipine and that this interaction could account for DIGO. Sonmez *et al* (2008) compared healthy gingiva with CsA-induced GO gingiva with respect to MMP-1 levels. They concluded that CsA therapy did not significantly effect the MMP-1 levels. Chiu *et al* (2009) examined the expression of MMP-1 and MMP-2 along with TIMP-2 in CsA-treated rat gingiva *in vitro*. They reported a significantly decreased expression of mRNA for MMP-1 but not for TIMP-2. In fibroblast culture medium, CsA induced a decrease in MMP-2 in a dose-dependent manner. Sorensen *et al* (2007) evaluated TIMP-1 tumor tissue levels in cancer patients treated with a drug combination including folinic acid. They concluded that decreased TIMP-1 levels were significantly and independently associated with a positive therapeutic response. Kuo *et al* (2010) investigated the effect on CsA on MMPs from a co-culture of human gingival fibroblasts and U937 macrophages in the presence or absence of Porphyromonas gingivalis lipopolysaccharide (LPS). They evaluated the activities of pro-MMP-2, MMP-2, and pro-MMP-9 and the expression of mRNA for membrane type-1 MMP in the co-cultures. They concluded that CsA could inhibit MMP activities in the presence of P gingivalis LPS. Serra *et al* (2010) evaluated the effects of PHT and its metabolite, HPPH on LPS-elicited MMP, TIMP, TNF-alpha and IL-6 levels in macrophages. They concluded that the production of MMPs is compromised both by PHT and by HPPH in a dose-dependent fashion facilitating collagen accumulation. Jotwani *et al* (2010) investigated the effect of P gingivalis and E coli on the induction of MMP-9 and TIMP-1. They reported that when MMP-9 is unregulated by tissue inhibitor TIMP-1, tissue destruction may ensue and that P gingivalis may be involving in inducing dendritic cells of MMP-9, potentially causing collagen breakdown and local tissue destruction. Williams *et al* (2011) noted that in addition to the prevention of neural tube genetic defects, FA has many other physiologic functions which include cell proliferation, DNA replication, and anti-oxidant protection. They evaluated placental cultured explants with regard to various FA concentrations. They determined that FA was important in a number of crucial early states of placental development including the secretion of MMP-2, MMP-3, and MMP-9. Vahabi *et al* (2013) recently reported that at least with regard to children, an impaired collagenase activation in DIGO was due to an MMP-1/TIMP pathway.

E-cadherin, Smad, AP-1, TIMP-1, MMP-1, inactive to active collagenase pathway

The activation of collagenase is a complicated process dependent upon multiple biochemical pathways and the interplay among these pathways which include TGF, TIMP-1, MMP-1, Smad, E-cadherin, and activator protein 1 (AP1). The mechanisms with which TGF induces TIMP-1 and decreases MMP-1 production are currently not understood (Hall *et al*, 2003). It would be helpful to be able define the biochemical pathway necessary for collagenase activation. There is the possibility that a particular protein is necessary for the inhibition of

TIMP-1, such as AP1 (Hall *et al*, 2003). It is suggested that without sufficient synthesis of this protein (or other biochemicals), the MMP collagenase activation pathway is impaired, and thus, connective tissue degradation is impaired. Hall *et al* (2003) investigated the mechanisms by which TGF-1 induces the expression of the TIMP-1 gene. TGF-1 repression of PMA-induced MMP-1 expression was compared with TGF- β 1 induction of the expression of the TIMP-1 gene. They reported that the promoter proximal AP1 site is essential for the response of both TIMP-1 and MMP-1 to TGF. The intracellular signaling pathways by which TGF- β mediates actions are multiple. The Smad pathway which is specific to the TGF- β family appears to be of prime importance, although there are certainly other viable pathways to consider, and there is also the issue of cross talk between signaling pathways. Hall *et al* (2003) reported an overall increase in AP1 binding upon TGF- β treatment compared with control. Lee *et al* (2006) reported that another biochemical, emodin, suppresses TNF-alpha-induced MMP-1 promotion through the inhibition of the AP-1 signaling pathway. Sugano *et al* (1998) reported that CsA treatment caused activation of AP-1 which may contribute to the mechanism of DIGO. Bostrom *et al* (2005) reported that CsA affects signaling molecules in gingival fibroblasts and induces an increase in AP-1 activity which may be part of the mechanism related to DIGO. Sume *et al* (2010) investigated epithelial to mesenchymal transition (EMT) with regard to PHT, CsA, and nifedipine and discovered that all three inducing drugs resulted in overgrowth tissues with diminished E-cadherin expression. They concluded that EMT likely occurs in DIGO. Tu *et al* (2006) examined the role of E-cadherin in CsA-IGO. They reported that CsA therapy may downregulate E-cadherin gene expression, leading to (cell proliferation) gingival overgrowth. Crott *et al* (2008) reported that low folate levels resulted in decreased expression of both E-cadherin and SMAD-4. These changes appeared to be consistent with cellular adaptation to folate depletion. Furthermore, low folate in particular cell lines resulted in decreased intestinal folate uptake.

The above reports support an inactive to active collagenase pathway composed of E-cadherin and SMAD activating AP-1 which inhibits TIMP-1 causing decreased inhibition of MMP-1 which is necessary for the activation of collagenase. Therefore, decreased cellular folate decreases E-cadherin and SMAD resulting in less activation of AP-1, resulting in less inhibition of TIMP-1, resulting in greater inhibition of MMP-1, which is necessary for the conversion of inactive collagenase to activated collagenase, and thus less collagenase activation (Figure 2).

Discussion

Seymour *et al* (1996) proposed that genetics plays a central role within the etiopathogenesis of DIGO. Certainly, it is noted that some patients are prone to DIGO, and some are not, and it would be reasonable to assume that genetics may play an important role within the pathophysiology of DIGO. Although the keys to the pathogenesis of DIGO may be determined through discovering an underlying genetic component, it may be more likely that it will be necessary to first discern the etiopathologic biochemical pathway. Another difficulty in regard to the understanding of any genetic pathophysiologic component of DIGO is the heterogenetic nature of gingival fibroblasts with respect to responses to inducing drugs (Hassell and Gilbert, 1983; Hassell and Stanek, 1983). There is certainly the

possibility genetics may eventually be plugged into this mechanistic puzzle. However, many researchers have investigated a genetic etiology for DIGO for some time, but as yet there appears to be no coherent genetic etiological concept (Nares *et al*, 1996; Soga *et al*, 2004; Kusztal *et al*, 2007).

Recently, several reports and studies have commented upon the efficacy of azithromycin (Azi) therapy for DIGO. Clementini *et al* (2008) performed a systemic review of studies evaluating the efficacy of and the mechanism of action of Azi treatment for CsA-induced GO. They retrieved 24 articles of which five were randomized controlled trials. They concluded that there was insufficient evidence for systemic Azi as an efficacious therapy for CsA-induced GO and that future studies are necessary.

Folic acid has been added to the food supply for more than 20 years to aid in the prevention of neural tube birth defects and other congenital anomalies. Folate supplementation to the food supplies in many nations has successfully decreased the incidence of such birth defects and congenital anomalies. Increased folate in the food supply in the last 20 years would support a decreased prevalence of DIGO. Ellis *et al* (1999) reported a prevalence of 6.3% of significant DIGO in patients taking nifedipine compared to incidence/prevalence DIGO nifedipine literature data of between 20% and 83%. In a recent study, Karnik *et al* (2012) reported a prevalence of only eight (5%) of a total of 157 dentate patients taking amlodipine had the side effect of DIGO. However, there is insufficient evidence at the present time to document that there is a cause and effect relationship with regard to increased public health folate intake and decreased incidence of DIGO, or even that the incidence of DIGO is less than years ago. There were concerns regarding toxicity with regard to the possibility of increasing the prevalence of certain cancers. However, increasing folate intake on such populations has not influenced an increase in cancers. FA is a relatively benign and safe supplement with very limited toxicity issues (Willard *et al*, 2003; Burdge and Lillycrop, 2012; Strickland *et al*, 2012; Vollset *et al*, 2013).

The characterization of gingival fibroblast cellular uptake would allow the determination of the dose-response dynamics of both the active transport and passive diffusion uptake legs. Such characterization would allow a comparison of the inhibition of the three inducing drug categories. Furthermore, it may be possible to also investigate the mechanisms of the inducing drugs' inhibition of cation flux, besides the aforementioned issue of the mechanisms of collagenase activation.

Since 1991, a great many studies as quoted above have tended to support the unifying hypothesis. Studies are still presently lacking with regard to characterization of folate uptake in gingival fibroblasts. Furthermore, there are only a limited number of studies demonstrating the efficacy of folate in reversing and preventing the occurrence of gingival overgrowth, and all studies have not universally demonstrated the efficacy of systemic folate (Drew *et al*, 1987; Backman *et al*, 1989; Brown *et al*, 1991b; Poppell *et al*, 1991; Prasad *et al*, 2004; Arya and Gulati, 2012). There have been a great number of studies which document increased fibroblastic synthesis of connective tissue in DIGO patients and animal models and various hypotheses based on a connective tissue accumulation model (Subramani *et al*, 2013). However, there is presently a more comprehensive etiopathologic

concept. A feasible suggested relationship between FA, cation flux, cellular folate uptake, collagenase activation, and dysfunctional connective tissue degradation is presented within a unifying hypothesis.

In conclusion, the unifying hypothesis proposes a connection between a particular drug action (decreased cation flux) common to all three categories of DIGO-inducing drugs, secondary decreased cellular folate uptake within gingival fibroblasts, and resulting dysfunctional connective tissue degradation. This conceptual biochemical basis for DIGO suggests the utilization of topical FA therapy for both the reversal and prevention of DIGO. As folate is a relatively innocuous pharmacotherapeutic, and FA appears to be viable therapy for DIGO, it is reasonable to consider randomized clinical therapeutic trials both with regard to treatment of DIGO and prevention of DIGO after initiating drug therapy with an inducing drug and particularly with relevance to CsA and transplant patients.

References

- Aimetti M, Romano F, Debernardi C. Effectiveness of periodontal therapy on the severity of cyclosporin A-induced gingival overgrowth. *J Clin Periodontol*. 2005; 32:846–850.
- Antman EM, Stone PH, Muller JE, Braunwald E. Calcium channel blocking agents in the treatment of cardiovascular disorders. Part 1. Basic and clinical electrophysiologic effects. *Ann Intern Med*. 1980; 93:875–885. [PubMed: 6255848]
- Ariel M, Eilam Y, Jablonska M, Grossowicz N. Effect of phenytoin on folic acid uptake in isolated intestinal epithelial cells. *Am J Physiol*. 1978; 235:E678–E685. [PubMed: 736128]
- Ariel M, Eilam Y, Jablonska M, Grossowicz N. On the mechanism of folate transport in isolated intestinal epithelial cells. *J Pharmacol Exp Ther*. 1982; 223:224–246. [PubMed: 7120120]
- Arora PD, Silvestri L, Ganss Sodek J, McCulloch CAG. Mechanism of cyclosporin-induced inhibition of intracellular collagen degradation. *J Biol Chem*. 2001; 276:14100–14109. [PubMed: 11278659]
- Arya R, Gulati S. Phenytoin-induced gingival overgrowth. *Acta Neurol Scand*. 2012; 125:149–155. [PubMed: 21651505]
- Arya R, Gulati S, Kabra M, Sahu JK, Kaira V. Folic Acid supplementation prevents phenytoin-induced gingival overgrowth in children. *Neurology*. 2011; 76:1338–1343. [PubMed: 21482950]
- Backman N, Holm AK, Hanstrom L, Blomquist HK, Heijbel J, Safstrom G. Folate treatment of dephenyhydantoin-induced gingival hyperplasia. *Scand J Dent Res*. 1989; 97:222–232. [PubMed: 2740833]
- Balamurugan K, Said HM. Role of reduced folate carrier in intestinal folate uptake. *Am J Physiol Cell Physiol*. 2006; 291:189–193.
- Beagrie G, Skougard M. Observation on the life cycle of gingival epithelial cells of mice as revealed by autoradiography. *Acta Odontol Scand*. 1962; 20:17–31.
- Bonnaure-Mallet M, Tricot-Doleux S, Godeau GJ. Changes in extracellular matrix macromolecules in human gingival after treatment with drugs inducing gingival overgrowth. *Arch Oral Biol*. 1995; 40:393–400. [PubMed: 7639642]
- Bostrom A, Bharath Saulewicz A, Narayanan AS. Cyclosporin A affects signaling events differentially in human gingival fibroblasts. *J Dent Res*. 2005; 84:532–536. [PubMed: 15914590]
- Bowman JM, Levy BA, Grubb RV. Gingival overgrowth induced by diltiazem. A case report. *Oral Surg Oral Med Oral Pathol*. 1988; 65:183–185. [PubMed: 3422722]
- Brown RS, Sein P, Corio R, Bottomley WK. Nitrendipine-induced gingival hyperplasia: first case report. *Oral Surg Oral Med Oral Pathol*. 1990; 70:593–596. [PubMed: 2234880]
- Brown RS, Beaver WT, Bottomley WK. On the mechanism of drug-induced gingival hyperplasia. *J Oral Pathol Med*. 1991a; 20:201–209. [PubMed: 1648612]

- Brown RS, Di Stanislaio P, Beaver WT, Bottomley WK. Administration of folic acid to humans with phenytoin-induced gingival hyperplasia: a double-blind, randomized, placebo controlled, parallel study. *Oral Surg Oral Med Oral Pathol.* 1991b; 71:565–568. [PubMed: 1828561]
- Burdge GC, Lillycrop KA. Folic acid supplementation in pregnancy: are there devils in the detail? *Br J Nutr.* 2012; 108:1924–1930. [PubMed: 23046900]
- Burka ER, Marks PA. Protein synthesis in human reticulocytes induced by therapy of megaloblastic anemia. *Blood.* 1967; 30:405–416. [PubMed: 6054659]
- Chiu HC, Lu YT, Chin YT, et al. Cyclosporin A inhibits the expression of membrane type-I matrix metalloproteinase in gingiva. *J Periodontal Res.* 2009; 44:338–347. [PubMed: 19210333]
- Ciavarella D, Guiglia R, Campisi G, et al. Update on gingival overgrowth by cyclosporine A in renal transplants. *Med Oral Patol Oral Cir Bucal.* 2007; 12:E19–E25. [PubMed: 17195822]
- Clementini M, Vittorini G, Crea A, et al. Efficacy of AZM therapy in patients with gingival overgrowth induced by Cyclosporine A: A systemic review. *BMC Oral Health.* 2008; 8:34. [PubMed: 19087331]
- Colombani PM, Robb A, Hess AD. Cyclosporin A binding to calmodulin: a possible site of action on T-lymphocytes. *Science.* 1985; 228:337–339. [PubMed: 3885394]
- Correa JD, Queiroz-Junior CM, Costa JE, Teixeira AL, Silva TA. Phenytoin-induced gingival overgrowth: a review of the molecular, immune, and inflammatory features. *ISRN Dent.* 2011; 2011:497850.
- Cotrim P, Martelli-Junior H, Graner E, Sauk JJ, Colette RD. Cyclosporin A induces proliferation in human gingival fibroblasts via induction of transforming growth factor-beta1. *J Periodontal.* 2003; 74:1625–1633.
- Crott HW, Liu Z, Keyes MK, et al. Moderate folate depletion modulates the expression of selected genes involved in cell cycle, intracellular signaling and folate uptake in human colonic epithelial cell lines. *J Nutr Biochem.* 2008; 19:328–335. [PubMed: 17681772]
- Dahllof G, Modeer T. Development of phenytoin-induced gingival overgrowth in non-institutionalized epileptic children subjected to different plaque control programs. *J Clin Periodontal.* 1986; 13:845–849.
- Dahllof G, Modeer T, Reinholt FP, Wikstrom B, Hjerpe A. Proteoglycans and glycosaminoglycans in phenytoin-induced gingival overgrowth. *J Periodontal Res.* 1986; 21:13–21. [PubMed: 2937892]
- Daley TD, Wysocki GP. Cyclosporine therapy. Its significance to the periodontist. *J Periodontal.* 1984; 55:708–712.
- Daley TD, Wysocki GP, Day C. Clinical and pharmacologic correlations in cyclosporine-induced gingival hyperplasia. *Oral Surg Oral Med Oral Pathol.* 1986; 62:417–421. [PubMed: 3464914]
- Dannewitz B, Edrich C, Tomakidi P, et al. Elevated gene expression of MMP-1, MMP-10, and TEVIP-1 reveal changes of molecules involved in turn-over of extracellular matrix. *Cell Tissue Res.* 2006; 325:513–522. [PubMed: 16670920]
- Dannewitz B, Krieger JK, Simon I, Dreyhaupt J, Staehle HJ, Eikholz P. Full-mouth disinfection as a nonsurgical treatment approach for drug-induced gingival overgrowth: a series of 11 cases. *Int J Periodontics Restorative Dent.* 2010; 30:63–71. [PubMed: 20224832]
- Delilieri GL, Santoro F, Polli N, Bruno E, Fumagalli L, Risciotti E. Light and electron microscopic study of Cyclosporin A-induced gingival hyperplasia. *J Periodontal.* 1986; 57:771–775.
- DeLorenzo RJ. Phenytoin: calcium- and calmodulin dependent protein phosphorylation and neurotransmitter release. *Adv Neurol.* 1980; 27:319–414.
- Domeij H, Modeer T, Yucel-Lindberg T. Matrix metallo-proteinase-1 and tissue inhibitor of metalloproteinase-1 production in human gingival fibroblasts: the role of protein kinase C. *J Periodont Res.* 2004; 39:308–314. [PubMed: 15324351]
- Domeij H, Modeer T, Quezada HC, Yucel-Lindberg T. Cell expression of MMP-1 and TEVIP-1 in co-cultures of human gingival fibroblasts and monocytes: the involvement of ICAM-1. *Biochem Biophys Res Com.* 2005; 338:1825–1833. [PubMed: 16288711]
- Dretchen KL, Bowles AM, Raines A. Protection by phenytoin and calcium channel blocking agents against the toxicity of diisopropylfluorophosphate. *Toxicol Appl Pharmacol.* 1986; 88:584–589.
- Drew HJ, Vogel RI, Molofsky W, Baker H, Frank O. Effect of folate on phenytoin hyperplasia. *J Clin Periodontal.* 1987; 14:350–356.

- Eilam Y, Ariel M, Jablonska M, Grossowicz N. On the mechanism of folate transport in isolated intestinal epithelial cells. *Am J Physiol.* 1981; 240:G170–G175. [PubMed: 7468807]
- Ellis JS, Seymour RA, Steele JG, Robertson P, Butler TJ, Thomason JM. Prevalence of gingival overgrowth induced by calcium channel blockers: a community-based study. *J Periodontol.* 1999; 70:63–67.
- Engler WO, Ramfjord S, Hinkler J. Development of epithelial and gingival sulcus in rhesus monkeys. *J Periodontol.* 1965; 36:44–56.
- Fitchie JG, Comer RW, Hanes PJ, Reeves GW. The reduction of Phenytoin-induced gingival overgrowth in a severely disabled patient: a case report. *Compendium.* 1989; 10:314–319. [PubMed: 2605605]
- Fowler B. Genetic defects of folate and cobalamin metabolism. *Eur J Pediatr.* 1998; 157(Suppl 2):S60–S66. [PubMed: 9587028]
- Francetti L, Maggiore E, Marchesi A, Ronchi G, Romeo E. Oral hygiene in subjects treated with diphenylhydantoin: effects of a professional program. *Prev Assist Dent.* 1991; 17:40–43.
- Fugii A, Kobayashi S. Nifedipine inhibits calcium uptake of nifedipine-sensitive gingival fibroblast. *J Dent Res.* 1990; 67:332. (Abstr).
- Goultchin J, Shoskan S. Inhibition of collagen breakdown by diphenylhydantoin. *Biochim Biophys Acta.* 1980; 631:188–191. [PubMed: 7397245]
- Guo H, Lee J-D, Uzui H, et al. Effects of folic acid and magnesium on the production of homocysteine-induced extracellular matrix metalloproteinase-2 in cultured rat fascular smooth muscle cells. *Circ J.* 2006; 70:141–146. [PubMed: 16377938]
- Hall M-C, Young DA, Waters JG, et al. The comparative role of activator protein 1 and Smad factors in the regulation of Timp-1 and MMP-1 gene expression by transforming growth factor- β . *J Biol Chem.* 2003; 278:10304–10313. [PubMed: 12525489]
- Hassell TM. Evidence for the production of an inactive collagenase by fibroblasts from phenytoin-enlarged human gingivae. *J Oral Pathol.* 1982; 11:310–317. [PubMed: 6286912]
- Hassell TM, Gilbert GH. Phenytoin sensitivity of fibroblasts as the basis for susceptibility to gingival enlargement. *Am J Pathol.* 1983; 112:218–223. [PubMed: 6881288]
- Hassell TM, Hefti AF. Drug-induced gingival overgrowth: old problem, new problem. *Crit Rev Oral Biol Med.* 1991; 2:103–137. [PubMed: 1912141]
- Hassell TM, Stanek EJ 3rd. Evidence that healthy human gingiva contains functionally heterogeneous fibroblast subpopulations. *Arch Oral Biol.* 1983; 28:617–625. [PubMed: 6579893]
- Hassell TM, Page RC, Narayanan Cooper CG. Diphenylhydantoin (Dilantin) gingival hyperplasia: drug-induced abnormality of connective tissue. *Proc Natl Acad Sci.* 1976; 73:2909–2912. [PubMed: 1066702]
- Heimbürger DC. Local deficiencies of folic acid in aero-digestive tissues. *Ann N Y Acad Sci.* 1992; 669:87–95. [PubMed: 1444061]
- Hood KA. Drug-induced gingival hyperplasia in transplant recipients. *Prog Transplant.* 2002; 12:17–23. [PubMed: 11993065]
- Ilgeni T, Atilla G, Baylas H. Effectiveness of periodontal therapy in patients with drug-induced gingival overgrowth. Long-term results. *J Periodontol.* 1999; 79:967–972.
- Jiang L, Gao MJ, Zhou J, Zhao J, Zhao SW, Zhao YF. Serum cyclophilin A concentrations in renal transplant recipients receiving cyclosporine A: clinical implications for gingival overgrowth. *Oral Surg Oral Med Oral Pathol Oral Radiol.* 2013; 116:447–454. [PubMed: 24035111]
- Jones, G.; Wimbish, G. Hydantoins. Antiepileptic drugs. In: Frey, H.; Janz, D., editors. *Handbook of experimental pharmacology.* Vol. 74. Berlin: Springer-Verlag; 1985. p. 351–419.
- Jotwani R, Eswaran SVK, Moonga S, Cutler CW. MMP-TEVIP-1 imbalance induced in human dendritic cells by *Porphyromonas gingivalis*. *FEMS Immunol Med Microbiol.* 2010; 58:314–321. [PubMed: 20030715]
- Kanno CM, Oliveira JA, Garcia JF, Castro AL, Crivellini MM. Effects of cyclosporin, phenytoin, and nifedipine on the synthesis and degradation of gingival collagen in tufted Capuchin monkeys (*Cebus apella*): histochemical and MMP-1 and -2 and collagen I gene expression analyses. *J Periodontol.* 2008; 79:114–122.

- Kantor M, Hassell T. Increased accumulation of sulfated glycosaminoglycans in cultures of human fibroblasts from phenytoin-induced gingival overgrowth. *J Dent Res*. 1983; 62:383–387.
- Karnik R, Bhat KM, Bhat GS. Prevalence of gingival overgrowth among elderly patients under amlodipine therapy at a large Indian teaching hospital. *Gerodontology*. 2012; 29:209–213. [PubMed: 22506838]
- Kataoka M, Kido J, Shinohara Y, Nagata T. Drug-induced gingival overgrowth - a review. *Biol Pharm Bull*. 2005; 28:1817–18121. [PubMed: 16204928]
- Kato T, Okahashi N, Kawai S, et al. Impaired degradation of matrix collagen in human gingival fibroblasts by the antiepileptic drug phenytoin. *J Periodontol*. 2005; 76:941–950.
- Kato T, Okahashi N, Ohno T, Inaba H, Kawai S, Amano A. Effect of phenytoin on collagen accumulation by human gingival fibroblasts exposed to TNF-alpha in vitro. *Oral Dis*. 2006; 12:156–162. [PubMed: 16476037]
- Kimball OP. The treatment of epilepsy with sodium diphenyl hydantoinate. *J Am Med Assoc*. 1939; 112:1244–1245.
- Kumar CK, Moyer MP, Dudeja PK, Said HM. A protein tyrosine kinase regulated pH dependent, carrier mediated uptake system for folate in human normal colonic epithelial cell line NCM460. *J Biol Chem*. 1997; 272:6226–6231. [PubMed: 9045638]
- Kuo PJ, Tu HP, Chin VT, et al. Cyclosporine-A inhibits MMP-2 and -9 activities in the presence of *Porphyromonas gingivalis* lipopolysaccharide: an experiment in human gingival fibroblast and U937 macrophage co-culture. *J Periodontol Res*. 2010; 47:431–438.
- Kusztal M, Radwan-Oczko M, Koscielska-Kasprzak K, Boratynska M, Patrzalek D, Klinger M. Possible association of CTLA-4 gene polymorphism with cyclosporine-induced gingival overgrowth in kidney transplant recipients. *Transplant Proc*. 2007; 39:2763–2765. [PubMed: 18021981]
- Lee J, Jung E, Lee J, et al. Emodin inhibits TNF alpha-induced MMP-1 expression through suppression of activator protein-1 (AP-1). *Life Sci*. 2006; 79:2480–2485. [PubMed: 16959273]
- Marche P, Stepien O. Calcium antagonists and vascular smooth muscle cell reactivity. *Z Kardiol*. 2000; 89:140–144. [PubMed: 10769418]
- Mariani G, Calastrini C, Carinci F, Bergamini L, Calastrini F, Stabellini G. Ultrastructural and histochemical features of the ground substance in cyclosporin A-induced gingival overgrowth. *J Periodontol*. 1996; 67:21–27.
- McCulloch CA, Bordin S. Role of fibroblast subpopulations in periodontal physiology and pathology. *J Periodontol Res*. 1991; 26:144–154. [PubMed: 1830616]
- Meikle MC, Atkinson SJ, Ward RV, Murphy G, Reynolds JJ. Gingival fibroblasts degrade type I collagen films when stimulated with tumor necrosis factor and interleukin 1: evidence that breakdown is mediated by metalloproteinases. *J Periodont Res*. 1989; 24:207–213. [PubMed: 2550604]
- Meisel P, Schwann C, John U, Kroemer HK, Kocher T. Calcium antagonists and deep gingival pockets in the population-based SHIP study. *Br J Clin Pharmacol*. 2005; 60:552–559. [PubMed: 16236046]
- Messing RO, Carpenter CL, Greenberg DA. Mechanism of calcium channel inhibition by phenytoin: comparison with classical calcium channel antagonists. *J Pharmacol Exp Ther*. 1985; 235:407–411. [PubMed: 2414431]
- Modeer T, Dahllof G. Development of phenytoin-induced gingival overgrowth in non-institutionalized epileptic children subject to different plaque control programs. *Acta Odontol Scand*. 1987; 45:81–85. [PubMed: 2955633]
- Modeer T, Dahllof G, Theorell K. Oral health in non-institutionalized epileptic children with special reference to phenytoin medication. *Community Dent Oral Epidemiol*. 1986; 14:165–168. [PubMed: 3459615]
- Modeer T, Brunius G, Mendez C, Juntti-Berggren L, Berggren PO. Influence of phenytoin on cytoplasmic free Ca²⁺ level in human gingival fibroblasts. *Scand J Dent Res*. 1991; 99:310–315. [PubMed: 1771377]

- Moy LS, Tan EML, Holness R, Uitto J. Phenytoin modulates connective tissue metabolism and cell proliferation in human skin fibroblast cultures. *Arch Dermatol*. 1985; 121:79–83. [PubMed: 2981518]
- Murphy G, Reynolds JJ, Bretz U, Baggiolini M. Partial purification of collagenase and gelatinase from polymorphonuclear leukocytes. Analysis of their actions on soluble and insoluble collagens. *Biochem J*. 1982; 203:209–221. [PubMed: 6285893]
- Murphy G, Hembray RM, Reynolds JJ. Characterization of specific antiserum to rabbit stromelysin and demonstration of the synthesis of collagenase and stromelysin by stimulated rabbit articular chondrocytes. *Coll Relat Res*. 1986; 6:351–363. [PubMed: 3028709]
- Murphy G, Cockett MI, Stephens PE, Smith BJ, Docherty AJP. Stromelysin is an activator of procollagenase. A study with natural and recombinant enzymes. *Biochem J*. 1987; 248:265–268. [PubMed: 2829822]
- Nares S, Ng MC, Dill RE, Park B, Cutler CW, Iacopino AM. Cyclosporine A upregulates platelet-derived growth factor B chain in hyperplastic human gingiva. *J Periodontol*. 1996; 67:271–278.
- Nuki I, Cooper SH. The role of inflammation in the pathogenesis of gingival enlargement during the administration of Dph in cats. *J Periodont Res*. 1972; 7:102–110. [PubMed: 4272036]
- Oliveria SF, Dittmer PJ, Youn D, Dell'Acqua ML, Sather WA. Localized calcineurin confers Ca^{2+} -dependent inactivation upon neuronal L-type Ca^{2+} channels. *J Neurosci*. 2012; 32:15328–15337. [PubMed: 23115171]
- O'Neil T, Figures K. The effects of chlorhexidine and mechanical methods of plaque control on recurrence of gingival hyperplasia in young patients taking phenytoin. *Br Dent J*. 1982; 152:130–133. [PubMed: 6462158]
- Opladen T, Blau N, Ramaekers VT. Effect of antiepileptic drugs and reactive oxygen species on folate receptor 1 (FOLR1): dependent 5-methyltetrahydrofolate transport. *Mol Genet Metab*. 2010; 101:48–51. [PubMed: 20619709]
- Pilatti GL, Sampaio JE. The influence of chlorhexidine on the severity of cyclosporin A-induced gingival overgrowth. *J Periodontol*. 1997; 68:900–904.
- Poppell TD, Keeling SD, Collins JF, Hassell TM. Effect of folic acid on recurrence of phenytoin-induced gingival overgrowth following gingivectomy. *J Clin Periodontol*. 1991; 18:134–139.
- Prasad VN, Chawta HS, Goyal A, Gauba K, Singhi P. Folic acid and phenytoin induced gingival overgrowth - is there a preventive effect. *J Indian Soc Pedod Prev Dent*. 2004; 22:82–91. [PubMed: 15491092]
- Rose RC, Koch MJ, Nahrwold DL. Folic acid transport by mammalian small intestine. *Am J Physiol*. 1978; 235:E678–E685. [PubMed: 736128]
- Rosenberg IH, Zimmerman J, Selhub J. Folate transport. *Chemioterapia*. 1985; 4:354–358. [PubMed: 3907863]
- Rossmann JA, Ingles E, Brown RS. Multimodal treatment of drug-induced gingival hyperplasia in a kidney transplant patient. *Compendium*. 1994; 15:1266. 68–70, 72–74, 76. [PubMed: 7987892]
- Russell BG, Bay LM. Oral use of chlorhexidine gluconate toothpaste in epileptic children. *Scand J Dent Res*. 1978; 86:52–57. [PubMed: 345408]
- Salman BN, Valhabi S, Movaghar, Mahjour F. Proliferative and inductive effects of Cyclosporine a on gingival fibroblast of child and adult. *Dent Res J (Isfahan)*. 2013; 10:52–58. [PubMed: 23878564]
- Serra R, Al-Saidi AG, Angelov N, Nares S. Suppression of LPS-induced matrix-metalloproteinase responses in macrophages exposed to phenytoin and its metabolite, 5-[phydroxyphenyl]-5-phenylhydantoin. *J Inflamm*. 2010; 7:48.
- Seymour RA. Drug-induced gingival overgrowth. *Adverse Drug React Toxicol Rev*. 1993; 12:215–232. [PubMed: 8142576]
- Seymour RA, Thomason JM, Ellis JS. The pathogenesis of drug induced gingival overgrowth. *J Clin Periodontol*. 1996; 23:165–175.
- Sierra EE, Goldman ID. Recent advances in the understanding of the mechanism of membrane transport of folates and antifolates. *Semin Oncol*. 1999; 26:11–23.
- Slavin J, Taylor J. Cyclosporin, nifedipine, and gingival hyperplasia. *Lancet*. 1987; 26:739.

- Soga Y, Nishimura F, Ohtsuka Y, et al. CYP2C polymorphisms, phenytoin metabolism and gingival overgrowth in epileptic subjects. *Life Sci.* 2004; 74:827–834. [PubMed: 14659971]
- Somacarrera ML, Hernandez G, Acero J, Moskow BS. Factors related to the incidence and severity of cyclosporin-induced gingival overgrowth in transplant patients. A longitudinal study. *J Periodontol.* 1994; 65:671–675.
- Sonmez S, Cavdar C, Gunduz C, et al. Do MMP-1 levels of gingival fibroblasts have a role in the gingival overgrowth of Cyclosporine-treated patients. *Transplant Proc.* 2008; 40:181–183. [PubMed: 18261580]
- Sorensen NM, Bystrom P, Christensen IJ, et al. TEVIP-1 is significantly associated with objective response and survival in metastatic colorectal cancer patients receiving combination of irinotecan, 5-fluorouracil, and folinic acid. *Clin Cancer Res.* 2007; 13:4117–4122. [PubMed: 17634538]
- Staple PH, Reed MJ, Mashimo PA, Sedransk N, Umenmoto T. Diphenylhydantoin gingival hyperplasia in *Macaca arctoides*: prevention by inhibition of dental plaque deposition. *J Periodontol.* 1978; 49:310–325.
- Strickland KC, Krupenko NI, Krupenko SA. Molecular mechanisms underlying the potentially adverse effects of folate. *Clin Chem Lab Med.* 2012; 51:607–616.
- Subramani T, Rathnavelu V, Alitheen NB. The possible potential therapeutic targets for drug induced gingival overgrowth. *Mediators Inflamm.* 2013; 2013:639468. [PubMed: 23690667]
- Sugano N, Ito K, Murai S. Cyclosporin A inhibits collagenase gene expression via AP-1 and JNK suppression in human gingival fibroblasts. *J Periodont Res.* 1998; 33:448–452. [PubMed: 9879517]
- Sukkar TZ, Thomason JM, Cawston TE, et al. Gingival fibroblasts grown from cyclosporin-treated patients show a reduced production of matrix metalloproteinase-1 (MMP-1) compared with normal gingival fibroblasts, and cyclosporin down-regulates the production of MMP-1 stimulated by proinflammatory cytokines. *J Periodont Res.* 2007; 42:580–588. [PubMed: 17956473]
- Sume SS, Kantarci A, Lee A, Hasturk H, Trackman PC. Epithelial to mesenchymal transition in gingival overgrowth. *Am J Pathol.* 2010; 177:208–218. [PubMed: 20489142]
- Taheri MR, Wickremasinghe RG, Jackson BFA, Hoffbrand AV. The effect of folate analogues and vitamin B12 on provision of thymine nucleotides for DNA synthesis in megaloblastic anemia. *Blood.* 1982; 59:634–640. [PubMed: 6977386]
- Taylor KB, Windsor LJ, Caterina NC, Bodden MK, Engler JA. The mechanism of inhibition of collagenase by TIMP-1. *J Biol Chem.* 1996; 271:23938–23945. [PubMed: 8798626]
- Thomas EA, Petrou S. Network-specific mechanisms may explain the paradoxical effects of carbamazepine and phenytoin. *Epilepsia.* 2013; 54:1195–1202. [PubMed: 23566163]
- Timmis GM. Antagonists of purine and pyrimidine metabolites and of folic acid. *Adv Cancer Res.* 1961; 6:369–401. [PubMed: 13921293]
- Tipton DA, Stricklin GP, Dabbous MK. Fibroblast heterogeneity in collagenolytic response to cyclosporine. *J Cell Biochem.* 1991; 46:152–165. [PubMed: 1655819]
- Tipton DA, Fry HR, Dabbous MK. Altered collagen metabolism in nifedipine-induced gingival overgrowth. *J Periodontal Res.* 1994; 29:401–409. [PubMed: 7877076]
- Trackman PC, Kantarci A. Connective tissue metabolism and gingival overgrowth. *Crit Rev Oral Biol Med.* 2004; 15:165–175. [PubMed: 15187034]
- Tu H-P, Chen Y-T, Shieh Y-S, et al. Cyclosporin-induced downregulation of expression of E-cadherin during proliferation of edentulous gingival epithelium in rats. *J Periodontol.* 2006; 77:832–839.
- Vahabi S, Salman BN, Rezazadeh F, Namdari M. Effects of cyclosporine and phenytoin on biomarker expressions in gingival fibroblasts of children and adults: an in vitro study. *J Basic Clin Physiol Pharmacol.* 2013; 25:167–173.
- Vogel RI. Gingival hyperplasia and folic acid deficiency from anticonvulsive drug therapy: a theoretical relationship. *J Theor Biol.* 1977; 67:269–278. [PubMed: 895164]
- Vollset SE, Clarke R, Lewington S, et al. Effects of folic acid supplementation on overall and site-specific cancer incidence during the randomised trials: meta-analyses of data on 50,000 individuals. *Lancet.* 2013; 381:1029–1036. [PubMed: 23352552]

- Willard RD, Davies G, Desilets V, et al. The use of folic acid for the prevention of neural tube defects and other congenital anomalies. *J Obstet Gynaecol Can.* 2003; 25:959–973. [PubMed: 14608448]
- Williams PJ, Bulmer JN, Innes BA, Pipkin FB. Possible roles for folic acid in the regulation of trophoblast invasion and placental development in normal early human pregnancy. *Biol Reprod.* 2011; 84:1148–1153. [PubMed: 21349824]
- Zimmerman J. Folic acid transport in organ-cultured mucosa of human intestine. Evidence for distinct carriers. *Gastroenterology.* 1990; 99:964–972. [PubMed: 2394350]
- Zimmerman J, Selhub J, Rosenberg IH. Role of sodium ion in transport of folic acid in the small intestine. *Am J Physiol.* 1986; 251:G218–G222. [PubMed: 2426969]

Figure 1.
Drug-induced gingival overgrowth mechanism

Figure 2.

Decreased cellular folic acid leads to decreased E-cadherin and SMAD, which leads to decreased AP-1. Decreased AP-1 results in increased TIMP-1 which leads to decreased MMP-1. As MMP-1 is necessary for the activation of inactivated collagenase to activated collagenase, the result is a decreased amount of activated collagenase

Table 1

DIGO-inducing drugs

Drug category	Anti-convulsants	Anti-calcineurins (and immunosuppressants)	CCBAs
Drugs	PHT, carbamazepine, valproic acid	CsA, TAC (azathioprine, mycophenolate mofetil)	Amlodipine, nifedipine, felodipine, nitrendipine, verapamil, diltiazem
Therapies	Treatment of epilepsy	Immunosuppression	Anti-hypertensive
Incidence of DIGO	PHT – 10–83%	CsA – 7–80%	CCBAs – 30–50%
Mechanism	Blocking Na ⁺ channels	Binding a protein in the cytosol (cyclophilin) through calmodulin	Blocking calcium channels
References	Daley <i>et al</i> (1986), Slavin and Taylor (1987), Brown <i>et al</i> (1991a), Somacarrera <i>et al</i> (1994), Kataoka <i>et al</i> (2005), Meisel <i>et al</i> (2005), Ciavarella <i>et al</i> (2007), Arya and Gulati (2012) and Jiang <i>et al</i> (2013)		

CCBAs, calcium channel blocking agents; CsA, cyclosporine; DIGO, drug-induced gingival overgrowth; PHT, phenytoin; TAC, tacrolimus.

Table 2

Support for the unifying drug-induced gingival overgrowth (DIGO) hypothesis previous to 1992

Concept	References
The research evidence regarding an anabolic hypothesis for causation for DIGO was consistently contradictory and at best confusing, and without any unifying conceptual basis	Brown <i>et al</i> (1991a) and McCulloch and Bordin (1991)
All of the inducing drugs are known to effect cation (Na ⁺ and Ca ⁺) channels	Antman <i>et al</i> (1980), DeLorenzo (1980), Colombani <i>et al</i> (1985), Jones and Wimbish (1985), Messing <i>et al</i> (1985), Dretchen <i>et al</i> (1986) and Fugii and Kobayashi (1990)
DIGO appears to be the result of a defect in catabolism due to increased amounts sulfated glycosaminoglycans (GAGs/connective tissue) within DIGO gingival tissues	Hassell (1982), Kantor and Hassell (1983), Dahllof <i>et al</i> (1986), Deliliers <i>et al</i> (1986) and Bowman <i>et al</i> (1988)
Topical folate demonstrated clinical efficacy in the treatment of DIGO (and systemic folate was not as efficacious)	Drew <i>et al</i> (1987), Backman <i>et al</i> (1989), Brown <i>et al</i> (1991b) and Poppell <i>et al</i> (1991)
Folate cellular uptake is due to both a cation regulated channel, and by passive diffusion	Ariel <i>et al</i> (1978, 1982), Rose <i>et al</i> (1978), Eilam <i>et al</i> (1981), Rosenberg <i>et al</i> (1985), Zimmerman <i>et al</i> (1986) and Zimmerman (1990)
Folate is necessary for protein synthesis and the conversion of DNA base-pairs necessary for DNA synthesis	Burka and Marks (1967) and Taheri <i>et al</i> (1982)
Plaque control decreases the incidence, recurrence, and severity of DIGO	Nuki and Cooper (1972), Russell and Bay (1978), Staple <i>et al</i> (1978), O'Neil and Figures (1982), Daley and Wysocki (1984), Dahllof <i>et al</i> (1986), Dahllof and Modeer (1986), Modeer <i>et al</i> (1986), Modeer and Dahllof (1987), Fitchie <i>et al</i> (1989) and Francetti <i>et al</i> (1991)
Collagenase is necessary for the tissue degradation of gingival connective tissue and is an inactive enzyme which requires activation with a matrix metalloproteinases	Murphy <i>et al</i> (1982, 1986, 1987), Moy <i>et al</i> (1985) and Meikle <i>et al</i> (1989)
Gingival sulcular epithelium has a relatively high turnover rate which requires protein synthesis	Beagrie and Skougaard (1962) and Engler <i>et al</i> (1965)

Table 3Na⁺/Ca⁺⁺ ion flux drug mechanisms table

Study	Drug	Mechanism
Jones and Wimbish (1985)	PHT	Decreases resting fluxes of Na ⁺ ions as well as Na ⁺ currents with respect to action potentials or chemically induced depolarizations
Colombani <i>et al</i> (1985)	CsA	Interferes with Ca ⁺⁺ and calmodulin interactions
Dretchen <i>et al</i> (1986)	PHT and CCBAs	Decreased lethality of DFP due to an inhibition of Ca ⁺⁺ flux into excitable membranes
Fugii and Kobayashi (1990)	PHT and CCBAs	Inhibition of Ca ⁺⁺ uptake within gingival fibroblasts
Modeer <i>et al</i> (1990)	PHT	The basal level of Ca ⁺⁺ was significantly decreased in gingival fibroblasts in DIGO responders compared to non-responders
Marche and Stepien (2000)	CCBAs	Reported that the anti-hypertensive mechanism of CCBAs is due to inhibition of the L-type Ca ⁺⁺ channel
Oliveria <i>et al</i> (2012)	Anti-calcineurin drugs	Calcineurin permits Ca ⁺⁺ -dependent inactivation of neuronal L-type Ca ⁺⁺ channels and as excitation-driven entry of Ca ⁺⁺ through L-type voltage-gated Ca ⁺⁺ channels controls gene expression in neurons, a variety of biochemical events are promoted resulting in a limitation of Ca ⁺⁺ entry
Thomas and Petrou (2013)	Anti-seizure drugs	Reduced sodium channel availability, and secondarily produced a reduction in the action potential amplitude. A downstream event which followed was reduced calcium entry, and a reduction of calcium-activated potassium channels.

CCBAs, calcium channel blocking agents; CsA, cyclosporine; DFP, diisopropylfluorophosphate; DIGO, drug-induced gingival overgrowth; PHT, phenytoin.

Table 4

Drug-induced gingival overgrowth folic acid efficacy studies

Topical or systemic	Result	Reversal or prevention	Study
Topical	Efficacy	Reversal	Drew <i>et al</i> (1987)
Systemic	Efficacy	Reversal	Backman <i>et al</i> (1989)
Systemic	Negative	Reversal	Brown <i>et al</i> (1991b)
Systemic	Efficacy	Prevention	Poppell <i>et al</i> (1991)
Systemic	Efficacy	Prevention	Prasad <i>et al</i> (2004)
Systemic	Efficacy	Prevention	Arya <i>et al</i> (2011)