Drug-Induced Gingival Overgrowth—a Review

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Drug-induced gingival overgrowth is a side effect associated with 3 types of drugs: anticonvulsants (phenytoin), immunosuppressive agents (cyclosporine A), and various calcium channel blockers for cardiovascular diseases. Gingival overgrowth is characterized by the accumulation of extracellular matrix in gingival connective tissues, particularly collagenous components with various degrees of inflammation. Although the mechanisms of these disorders have not been elucidated, recent studies suggest that similar disorders may be induced by the disruption of homeostasis of collagen synthesis and degradation in gingival connective tissue, predominantly through the inhibition of collagen phagocytosis of gingival fibroblasts. The integrins are a large family of heterodimeric transmembrane receptors for extracellular matrix molecules. α2β1 integrin serves as a specific receptor for type I collagen on fibroblasts, and α2 integrin has been shown to play a crucial role in collagen phagocytosis. Actin filaments, which are assembled from monomers and oligomers, are involved in collagen internalization after binding to integrins. Furthermore, the implication of intracellular calcium in the regulation of integrin-mediated binding activity and gelsolin activity, known as a calcium-dependent actin-severing protein, is also described. In this review, we focus on collagen metabolism in drug-induced gingival overgrowth, focusing on the regulation of collagen phagocytosis in fibroblasts.

Key words drug-induced gingival overgrowth; collagen phagocytosis; α2β1 integrin; type I collagen; fibroblast

INTRODUCTION

Drug-induced gingival overgrowth is known as an adverse effect with three types of drug: phenytoin, an antiepileptic; cyclosporine A, an immunosuppressant; and calcium channel blockers, such as dihydropyridines (nifedipine), diltiazem, and verapamil, which are widely prescribed for the treatment of various cardiovascular diseases.1) This is characterized by an accumulation of extracellular matrix within the gingival connective tissue, particularly the collagenous component, with various degrees of chronic inflammatory inflammation.2) An estimated 5% of elderly outpatients in the U.S.A. are taking these medications.3) The prevalence rate of this disorder has been reported to vary: 10% to 50% for phenytoin,4—7) 8% to 70% for cyclosporine A,8—12) and 0.5% to 83% for nifedipine,13—16) The accurate determination of the prevalence rate in each drug category is difficult. These differences in the reported prevalence may be due to the differing indices of gingival overgrowth. Furthermore, the presence of various degrees of gingival inflammation is one reason for difficulty in the accurate assessment of drug-induced gingival overgrowth, because inflammation acts as an exacerbation factor of gingival overgrowth.17—19) The most effective treatment for drug-induced gingival overgrowth is the withdrawal or substitution of medication.20)

Since the first report of phenytoin-induced gingival overgrowth,21) many clinical and investigative approaches have been carried out to determine the pathogenesis of this disorder. Although these studies yielded various pathogenetic data, it remains unknown why drugs with such different pharmacological actions induce similar gingival overgrowth. The genetically determined capacity of the host to deal metabolically with chronically administered drugs, the responsiveness of gingival tissue to the drugs, and preexisting gingival condition, including inflammation may, differ among individuals. Moreover, calcium channel blockers are mainly prescribed for post-middle-aged patients for control of hypertension22) whereas phenytoin and cyclosporin A are prescribed for a wide range of patients due to their wide spectrum of efficiency.23) These factors could make it difficult to elucidate the pathology of drug-induced gingival overgrowth. Although the pathology of drug-induced gingival overgrowth is not definitively known, these disorders seem to be induced through the disruption of the homeostasis of collagen synthesis and degradation in gingival connective tissues.23—27)

In this review, we focused on excess collagen fibers, especially type I collagen, the most abundant protein in mammals,28) in gingival connective tissue, and discussed the role of collagen phagocytosis of gingival fibroblasts for the induction of drug-induced gingival overgrowth.

1. ACCUMULATION OF TYPE I COLLAGEN IN GINGIVAL CONNECTIVE TISSUE

Although the pharmaceutical effect and primary target tissues of an antiepileptic, an immunosuppressant, and calcium channel blocker are different, they act similarly on gingival connective tissue, causing fibrous gingival overgrowth (Fig. 1). Drug-induced gingival overgrowth is previously termed as gingival hypertrophy or gingival hyperplasia by finding an increased number of fibroblasts in gingival connective tissue with histological analysis.29,30) However, these earlier terms, “hypertrophy” or “hyperplasia” did not accurately reflect the histologic composition of enlarged gingiva. Not increase pro-
liferation of gingival fibroblasts, but the severe accumulation of extracellular matrix within the gingival connective tissue, particularly collagenous components, was observed in human gingival overgrowth.\textsuperscript{23,31,32} These discrepancies may be due to various degrees of gingival inflammation in human subjects because of the production of inflammatory cytokines, such as interleukin 1g, which is known to stimulate the gingival fibroblast proliferation\textsuperscript{33} and has a potential influence on collagen metabolism of fibroblasts,\textsuperscript{34,35} and the situation of overgrown gingiva is complicated. With rat experimental models, the accumulation of extracellular matrix in gingival connective tissues with few inflammatory cells were also observed by the administration of nifedipine, phenytoin, and cyclosporin A.\textsuperscript{36–39} These drug-induced gingival overgrowth is shown to be induced by the excessive accumulation of type I collagen in gingival connective tissue by immunohistochemical analysis with rat experimental models.\textsuperscript{25,26} These disorders are therefore suitable to be considered as fibrosis in gingival connective tissue. “Gingival overgrowth” or “gingival enlargement” is the preferred term for all drug-related gingival lesions previously termed “gingival hypertrophy” or “gingival hyperplasia.”\textsuperscript{20}

2. SYNTHESIS AND DEGRADATION OF TYPE I COLLAGEN IN DRUG-INDUCED GINGIVAL OVERGROWTH

The metabolism of collagen, the most abundant protein in mammals,\textsuperscript{29} is precisely balanced by collagen synthesis and degradation to maintain tissue volume. Generally, fibrosis is caused by the loss of homeostasis of the synthesis and degradation of collagen fibers, especially type I collagen, resulting in the excess accumulation of collagen fibers.\textsuperscript{40} The cell proliferation and collagen synthesis rates of gingival fibroblasts isolated from human drug-induced overgrown gingiva tended to be greater than those of gingival fibroblasts isolated from non-responder exposed to nifedipine or phenytoin \textit{in vitro}.\textsuperscript{41,42} Furthermore, the stimulating effect of cyclosporin A on type I collagen synthesis in human gingival fibroblasts has been reported\textsuperscript{45}, however, there are conflicting results of these drugs on cell proliferation and/or collagen synthesis \textit{in vitro} study.\textsuperscript{44–47} Cell proliferation is not affected by nifedipine or phenytoin treatment.\textsuperscript{44,45} but collagen synthesis is inhibited by these drugs.\textsuperscript{40} Some researchers have shown that collagen synthesis in human gingival fibroblasts is not affected or inhibited by cyclosporine A treatment.\textsuperscript{46,47} These discrepancies may originate from the culture conditions (cells grown on a plastic dish or in collagen gel), dosage, and the duration of drug treatment. It has been proposed that the decreased collagen degradation caused by phenytoin may contribute to the appearance of gingival overgrowth.\textsuperscript{40} Collagen may be degraded via an extracellular pathway involving the secretion of collagenase\textsuperscript{49} and via an intracellular pathway involving phagocytosis by fibroblasts.\textsuperscript{50} The collagenase-mediated route is accompanied by a loss of tissue architecture \textit{e.g.} inflammation, while the collagenase-independent intracellular route is important during normal turnover.\textsuperscript{51} The physiological conditions, collagen fibers in gingival connective tissue, undergo rapid collagen turnover to maintain homeostasis. Due to morphological studies of human cyclosporin A-induced gingival overgrowth, the decrease of phagocytosed collagen by fibroblasts has been reported.\textsuperscript{23} McCulloch and Knowles showed decreased collagen phagocytosis of fibroblast isolated from human phenytoin-induced gingival overgrowth than healthy gingiva, and direct inhibitory effects of nifedipine and phenytoin were also shown on the collagen phagocytosis of fibroblasts.\textsuperscript{24} Furthermore, we examined the phagocytic activity in gingival fibroblasts with a rat experimental model (Fig. 2), and severe inhibition was observed in cyclosporine A-induced gingival overgrowth.\textsuperscript{25,26} Interestingly, type I collagen and collagenase mRNA expressions were significantly suppressed by cyclosporin A and nifedipine administration in these rat experimental models.\textsuperscript{25,26} From these results, drug-induced gingival overgrowth is not due to the increased synthesis of type I collagen but the decreased degradation of type I collagen in gingival connective tissue through the reduction of collagen phagocytosis of fibroblasts.

3. ROLE OF $\alpha_2$ INTEGRIN IN DRUG-INDUCED GINGIVAL OVERGROWTH

Integrins are a large family of heterodimeric transmembrane receptors for extracellular matrix molecules, and acts
as the principle mediators of the molecular dialogue between a cell and its extracellular matrix environment. Each heterodimer consists of an $\alpha$ and $\beta$ subunit. There are approximately $17\alpha$ and $8\beta$ subunits in mammals, and through different combinations, they can form approximately 40 different integrins. Unique combinations of integrin subunits determine which extracellular matrix molecules will be recognized by a cell. Both $\alpha_1\beta_1$ and $\alpha_2\beta_1$ integrins are cell surface receptors for collagen, and cells expressing the $\alpha_1\beta_1$ integrin preferentially adhere to type IV collagen, whereas cells expressing $\alpha_2\beta_1$ preferentially adhere to type I collagen, and $\alpha_2\beta_1$ integrins have been shown to serve as specific receptors of type I collagen in fibroblasts. Lee et al. reported that the initial binding step of collagen phagocytosis relies on adhesive interaction between fibroblasts and collagen, and that $\alpha_2$ integrin plays a critical role in the phagocytic regulation of collagen internalization. Systemic scleroderma is a disease characterized by an excessive deposition of collagens in the skin and various organs, and the low expression of $\alpha_2\beta_1$ integrin on fibroblasts isolated from systemic scleroderma patients has been reported. We showed significantly decreased collagen phagocytosis in fibroblasts in rat overgrown gingiva induced by cyclosporin A, and $\alpha_2$ integrin expression suppressed in fibroblasts isolated from overgrown gingiva compared to the control. Furthermore, Chou et al. showed a reduction in collagen phagocytosis of gingival fibroblasts by TNF-$\alpha$ treatment through the inhibition of collagen binding to cells by the inactivation of $\alpha_2\beta_1$ integrin. These findings indicate that one ethiological factor of drug-induced gingival overgrowth may be the inhibition of collagen phagocytosis by reducing $\alpha_2$ integrin expression or decrease of the binding activity in gingival fibroblasts. In a rat experimental model, the mRNA expression of both type I collagen and collagenase in vivo is significantly lower in overgrown gingiva than in control gingiva. Interestingly, $\alpha_2\beta_1$ integrin acts as positive regulator of type I collagen and collagenase gene expression. So, the decreased collagen and collagenase gene expression in drug-induced gingival overgrowth in rat may be due to the reduction of $\alpha_2$ integrin expression on fibroblasts.

The function of $\alpha_2\beta_1$ integrin on platelet has been well studied, and the availability of $\alpha_2\beta_1$ integrin on the platelet surface plays an essential role in platelet adhesion to the collagen vessel wall. There is, on average, a 4-fold range in platelet $\alpha_2\beta_1$ integrin density among randomly selected individuals, and these differences correlate directly with platelet adhesiveness to type I or type III collagens. Furthermore, an 807 C to T single nucleotide exchange polymorphism in the gene encoding the $\alpha_2$ subunit of $\alpha_2\beta_1$ integrin, is shown to be associated with the increased density of $\alpha_2\beta_1$ integrin on the platelet surface and with increased platelet adhesion to type I collagen, and this polymorphism is considered a genetic risk factor for arteriothrombotic diseases. These reports have led to the hypothesis that subjects with the 807 C genotype of $\alpha_2$ integrin express less $\alpha_2\beta_1$ integrin on the gingival fibroblasts surface, leading to the decreased potential of fibroblast binding to type I collagen and collagen phagocytosis by administrated drugs, and, hence, an increased the risk of drug-induced gingival overgrowth. We investigated whether $\alpha_2$ integrin polymorphism is associated with calcium channel blocker-induced gingival overgrowth. A case-control study comparing subjects taking calcium channel blockers (with or without drug-induced gingival overgrowth) demonstrated that the frequency of the +807 C allele was significantly higher in the case group than in control (submission data). These results suggested that the $\alpha_2$ integrin +807 C allele might be a genetic risk factor for drug-induced gingival overgrowth.

4. ROLE OF CALCIUM IN COLLAGEN PHAGOCYTOSIS

Although the pharmacological actions and primary target tissues of calcium channel blockers, phenytoin, and cyclosporin A are quite different, these drug are known as calcium antagonists. Calcium channel blockers are able to block the influx of calcium ions into cells and to reduce oxygen consumption. Phenytoin is known to act as a calcium channel antagonist and inhibit calcium ion flux. Cyclosporin A is reported to inhibit the release of calcium from intracellular stores, including endoplasmic reticulum and mitochondria. It is thought that integrins transduce information from the extracellular matrix to the inside of the cell by triggering intracellular signaling pathways. Integrin dependent signals have been shown to be essential for cell proliferation, cell response to growth factors, and prevention of cell death. Many signal transduction pathways downstream from the integrins, including intracellular calcium signaling, have been elucidated. Furthermore, intracellular calcium signaling participate in a positive feedback loop that enables integrin-mediated cell adhesion by altering integrin-binding affinity. In $\alpha_2\beta_1$, integrin-mediated collagen phagocytosis in gingival fibroblasts is regulated by intracellular calcium, and cyclosporin A inhibits the $\alpha_2\beta_1$ integrin-binding activity of collagen phagocytosis through a calcium-regulated pathway involving ER and mitochondrial stores. These results suggest that drug-induced gingival overgrowth may be induced through the reduction of $\alpha_2\beta_1$ integrin-binding affinity in collagen phagocytosis in fibroblasts by disturbing the intracellular calcium flux.

Actin is the most abundant protein in various types of eukaryotic cells and is involved in a variety of processes, including cell locomotion, contraction and phagocytosis. In phagocytosis, particle internalization is initiated by the interaction of receptors with ligands, and this leads to the polymerization of actin at the ingestion site, and internalization of the particle via an actin-based mechanism. After internalization, actin shed from the phagosome. Segal et al. have shown the implication of the actin filament in the regulation of collagen phagocytosis. By treating fibroblasts with latrunculin B (actin monomer sequester), the disengagement of actin from $\beta_1$ integrin receptors, increased collagen binding, and enhancement of collagen receptor mobility are observed. Furthermore, the apparently enhancement of collagen phagocytosis is shown by the disengagement of actin from collagen receptors on fibroblasts. Actin rearrangements depend on actin-serving and capping proteins. Recently, Arora et al. reported the interesting role of gelsolin, a calcium-dependent actin-severing protein, in collagen phagocytosis with fibroblasts isolated from wild-type and gelsolin knockout (Gsn-/-) mice. They showed a more significant decrease of collagen binding and internalization in Gsn-/- than in wild-type cells.
Furthermore, very limited responses of rac activation and increase of intracellular calcium in Gsn−/− cells than that of wild-type cells were observed. These phenomena in Gsn−/− cells were restored by transfecting with gelsolin. They concluded that the ability of gelsolin to remodel actin filaments is important for collagen-induced calcium entry and calcium is in turn required for rac activation, which subsequently enhances collagen binding to α2β1 integrin.27,84 These reports suggest that the possibility that drug-induced gingival overgrowth is induced by the inhibition of gelsolin activity through disturbance of the elevation of the intracellular calcium level, and thereby the reduction of collagen phagocytosis.

5. CONCLUSION

Drug-induced gingival overgrowth is induced by reduced collagen phagocytosis in gingival fibroblasts via α2β1 integrin on cell surface. These drugs are known to act as calcium antagonists. Intracellular calcium implicates in the regulation of α2β1 integrin-mediated collagen phagocytosis by altering integrin affinity. Furthermore, actin binding protein, gelsolin, is considered an important factor for this disorder because of the maintenance of normal tissue integrity by regulating collagen phagocytosis through the integrin-binding affinity to collagens. Further studies of the overgrown gingiva should provide more insight into the molecular pathogenesis of drug-induced gingival overgrowth.

REFERENCES