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Scientific Research

Phenytoin Effects on Proliferation and Induction of IL1β and PGE2 in Pediatric and Adults' Gingival Fibroblasts

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Abstract

Background: Gingival Overgrowth (GO) is a well documented and unwanted side effect that occurs mainly as a result of certain antiseizure, phenytoin. The aim of this study was to compare the effect of phenytoin on proliferation and production of IL1β and PGE2 in cultured human gingival fibroblasts (HGF) of children and adults. Materials and Methods: Normal HGFs were obtained from 4 healthy children and 4 adult and then were cultured with phenytoin (20 mg/ml). MTT test was used to evaluate the proliferation and ELISA to determine the level of IL1β and PGE2 production by HGFs. Analysis of proliferation were assessed by Independent T-Test and ANOVA analysis was used to assess the level of IL1β and PGE2 production with an a error level less than 0.05. Results: The proliferation of HGF was not affected significantly by phenytoin in both cultured fibroblast sources ($P > 0.05$). Phenytoin induced a significantly higher formation of IL1β and PGE2 in child's HGFs as compared to adult's HGFs ($P < 0.05$). Conclusion: The results suggest that different inflammatory responses and cytokine formation by child's and adult's HGFs are the probable key elements that cause different reactions of phenytoin therapy. More advanced and systematic studies are needed to verify these findings.

Keywords

Phenytoin, Gingival Overgrowth, Fibroblast, Interleukin, Children

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1. Introduction

Medication-induced gingival overgrowth (GO) is a common subsequent of consuming anti-epileptic drug, immunosuppressive drug, and calcium-channel-blockers [1]. This side-effect may cause delayed eruption of teeth and disturbances in speech, mastication, and aesthetic [2]. The highest prevalence of gingival overgrowth has been reported to be occurred in the anti-epileptic drugs group [3], which is one of the most influent drugs on central nervous system, and other than seizure, is widely prescribed in cases of psychiatric disorders, migraine prophylaxis, and neuropathic pain [4]. Phenytoin is an anti-epileptic drug, which is vastly used solely or in combination with other drugs. Some advantages include a maximum anti-epileptic effect without suppressing central nervous system, availability, low cost and periodic use [5]. Nevertheless, skeletal, endocrine, immunological and connective tissue disturbances are some of reported side-effects of phenytoin [6]. Gingival overgrowth, first reported in 1939 [7], is one of the most known side-effects of phenytoin that occurs in nearly 50% of consumers [8]. According to epidemiologic studies, it is more prevalent in male children [9]-[11] and adolescents [12]-[14]. Use of alternative drugs such as valproic acid and carbamazepine [14] [15], proper oral hygine [16]-[18], gingivectomy and periodontal flap are the common methods of prevention and treatment of gingival overgrowth [19]. However, phenytoin-induced pathogenesis is unknown, and it might recur 3 to 6 months following periodontal surgery [20].

Several mechanisms such as functional heterogeneity of the human gingival fibroblast, [21] accumulation of extracellular matrix (ECM) proteins such us collagen, [21]-[23] impaired homeostasis in the connective tissue, increase production of glycosaminoglycans, [3] disturbances in secretion of ECM enzymes such as Matrix Metallo Proteinases (MMPs), and tissue inhibitors [24]-[26] have been recognized for this overgrowth in previous studies. Recent studies have suggested that impaired balance of cytokines plays a major role in gingival overgrowth. This impaired balance has been attributed to local alterations of fibrogenic cytokines such as Tumor Necrosis Factor-α (TNF-α) and various interleukins such as Interleukin-1 (IL-1) in response to phenytoin [3] [14] [27]. IL-1 is a part of cytokine family in inflammatory lesions, which is capable of stimulating bone resorption as well as biosynthesis of Prostanoids and enhancing extracellular matrix synthesis and collagenase in human gingival fibroblasts [28].

Brunius *et al.* have showed that phenytoin increases the production of interleukin-1β (IL-1β), and Prostaglandin E2 (PGE2) which has been induced by TNF-α; and have suggested that PGE2 and IL-1β play a key role in pathogenesis of phenytoin-induced gingival overgrowth [29]. Tumor necrosis factor (TNF-α), an inflammatory cytokine, induces cellular proliferation and inhibits synthesis and phagocytosis of collagen by human gingival fibroblasts [30]. This factor has been shown to stimulate IL-1α production. Researchers have suggested that these cytokines might induce the production and secretion of collagenase, which is a necessary enzyme in ECM breakdown [31]. It is still unknown that how TNF-α and IL-1 manage such contrasting functions in breaking down and producing gingival matrix and collagen metabolism in drug-induced overgrowth [29]. Moreover, the nature of inflammatory lesions in pediatrics is different from that in adults [32]-[34]. According to Gillet, B-small lymphocytes do not change form, and constitute the majority of inflammatory infiltrates. In result, clinical lesions remain unaggressive and undeveloped. It is a phenomenon that might be the combination in cell infiltrate in latent lesions of adults [35] [36]. According to Longhorst *et al.* [37] and Seymour *et al.* [38], cell infiltrations is predominantly constituted of T cells, and when the lesions become aggressive, the infiltration of B cells increases.

There is little known about the reasons for the differences in gingival overgrowth prevalence in pediatrics and adults, and also about the details of phenytoin and inflammatory mediators in these two groups. According to this lack of knowledge, prevalent usage of phenytoin in treatment of central nervous system diseases [39] and seizure, particularly in pediatrics, and with regard to the main role of fibroblasts in phenytoin-induced gingival overgrowth and in producing inflammatory mediators and ECM and in regulating collagen metabolism [19], this study was performed to investigate the influence of phenytoin on proliferation of fibroblasts and production of inflammatory mediators, IL1β and PGE2, by gingival fibroblasts in adults and pediatrics.

2. Materials and Methods

2.1. Cell Culture

The pediatric samples were obtained from 4 healthy children, aging from 4 to 11 years, during a procedure of the mesiodense or impacted canine teeth whom have been referred due to orthodontic treatments. In addition,

adult fibroblast samples were derived from 4 healthy adults who were submitted to crown lengthening surgery. The age range was 35 - 42 years. All of the persons were in good periodontal, oral and systemic health situation without any signs of inflammations in the site of biopsies. Pregnant women, addicted persons, systemic drug users and any person with systemic diseases which might have any effects on periodontium were excluded from the study. Due to natural constraints in primary cell culture, some pediatric samples were lost during in the experiment, so that 22 adult samples and 19 pediatric samples were obtained in total. In both cases, a fragment of excess tissue was removed under local anesthesia at the moment of the surgery. Informed consent was obtained from each donor prior to the taking of samples and parent of each child was informed about the study and consent forms were signed by them. Experimental protocol was approved by the Ethics Committee in Shahid Beheshti Medical University. The tissues were rinsed three times in sterile normal saline solution and transported in complete media including: Dulbecco's Modified Eagle's Medium (DMEM; Gibco USA) supplemented with 10% fetal bovine serum (FBS; Gibco USA), 100 µg/ml streptomycin, 100 U/ml penicillin and 0.25 µg/ml Amphotericin B. Minced pieces of the tissue were explanted to 4 cm^2 plates, and incubated at room temperature for 10 - 15 minutes and then the culture plate was flooded with complete media. Then samples were incubated at 37˚C in a humidified incubator containing 5% CO_2. Samples were regularly controlled for contamination and cell growth, and were fed with fresh medium if necessary. When fibroblasts grew out from the explants, they were trypsinized and shifted to 25 cm^2 flasks (Nunc, Copenhagen, Denmark) for secondary culture. Fourth passages of the cells were used for the experiments [29] [30].

2.2. Enzyme-Linked Immunosorbent Assay (ELISA)

Gingival fibroblasts were seeded into 24-well plates (Nunc, Copenhagen, Denmark) at a density of 60×10^3 cells/well. For every individual fibroblast derived samples 6 wells were cultured but due to natural constraints in primary cell culture, several pediatric samples were lost during in the experiment, so that 22 adult samples and 19 pediatric samples were obtained in total. Wells were divided into control and experimental groups (3 wells for each group or triplicate). After 48 hours, the media of cell cultures was exchanged and Phenytoin (Sigma-Aldrich, St. Louis, MO, USA) (20 µg/ml) was added to the experimental wells and in control groups only complete media was added. Samples were then incubated at 37˚C in 95% humidified atmosphere containing CO_2 for 48 hours.

In order to assess the amount of inflammatory mediators produced by gingival fibroblasts, supernatant fluid of each control and experimental well was collected. The concentration of PGE_2, IL-1β was determined by Enzyme-Linked Immunosorbent assay (ELISA) using the ELISA kits [PGE_2 (R & D systems, Minneapolis, MN, USA Cat No. KGE004B)]. IL-1β (R & D systems, Minneapolis, MN, USA Cat No. DLB50). ELISA assay was performed according to the manufacturer's instructions. Sample absorbances were analyzed using an ELISA reader for IL-1β at 450 nm and PGE2 at 405, and the concentration of each sample determined by comparing with a standard related curve.

2.3. MTT Assay

Gingival fibroblasts were seeded into 96-well plates (Nunc, Copenhagen, Denmark) at a density of 5×10^3 cells/well, and were cultured in a 200 µl medium. For every individual fibroblast derived samples 6 wells were cultured. After 48-hour incubation, the samples were divided into control and experimental groups (3 wells for each group or triplicate). Phenytoin was added to the experimental wells. MTT stock solution [tetrazolium salt 3-[4, 5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT; Merk, Darmstadt, Germany)] was prepared in phosphate-buffer saline (PBS; Sigma-Aldrich, St. Louis, MO, USA) in proportion to 5 mg/ml. After 48 hours of incubation, the medium was replaced with 200 µl of a fresh medium containing a final concentration of MTT salt as 0.5 mg/ml. Cells were then incubated at 37˚C in 95% humidified atmosphere containing CO_2 for 4 hours. Acidic Isopropanol (0.04 M Hal in absolute Isopropanol) was used to dissolve the crystals of formazan produced by the living cells. The quantity of color related to dissolve formazan crystals was then measured at a wavelength of 570 nm with ELISA plate reader (Anthos 2020 Australia). Optical density is directly proportional to the number of living cells.

2.4. Statistical Analysis

Data which has been released by ELISA were then tested by Kolmogrov Simonov test to evaluate normal dis-

tribution of adult and pediatric gingival cell population. (Mean value of triplicate was calculated and every individual experiment and related control data were compared). ANOVA was used for statistical evaluation. Results between the two groups were compared by student's T-test. p value < 0.05 was considered statistically significant.

3. Results

3.1. Rate of Fibroblast Proliferation in Adults and Pediatrics

Rate of fibroblast proliferation in both adults and pediatrics groups increased in the presence of phenytoin. However, these changes did not reach a significant level. The difference between phenytoin-induced proliferation in adults group and in pediatrics group was not significant (**Graph 1**).

3.2. Synthesis of IL1β by Adults and Pediatrics Fibroblasts

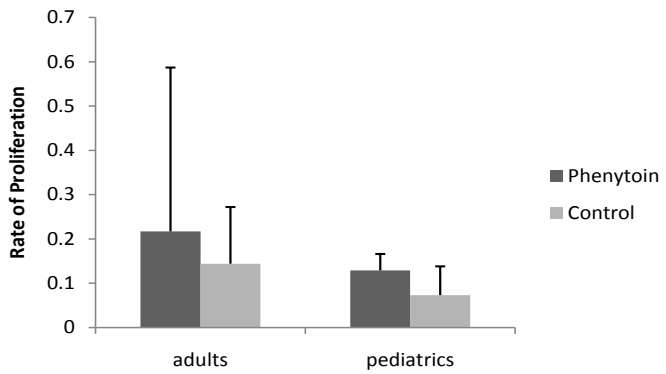
Phenytoin did not cause a significant raise in synthesis of IL-1β in adult fibroblasts. In contrast, the phenytoin-induced synthesis of IL-1β significantly increased in pediatric fibroblasts ($p < 0.05$). The change in production of IL-1β caused by presence of phenytoin was significantly higher in pediatrics than that in adults group ($p < 0.05$) (**Graph 2**).

3.3. Synthesis of PGE2 by Adults and Pediatrics Fibroblasts

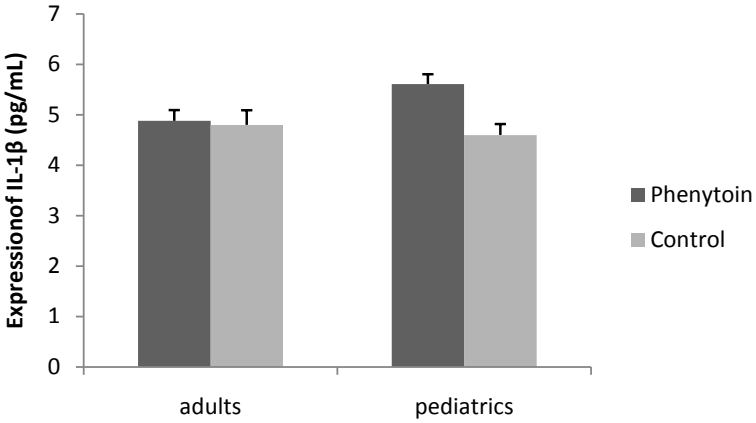
In the adults group, the level of synthesized PGE_2 decreased in presence of phenytoin, but this reduction did not reach a significant level. The production of PGE_2 in pediatrics group significantly increased ($p < 0.05$). The change in levels of synthesized PGE_2 in pediatrics was significantly higher than that in adults ($p < 0.05$) (**Graph 3**).

4. Discussion

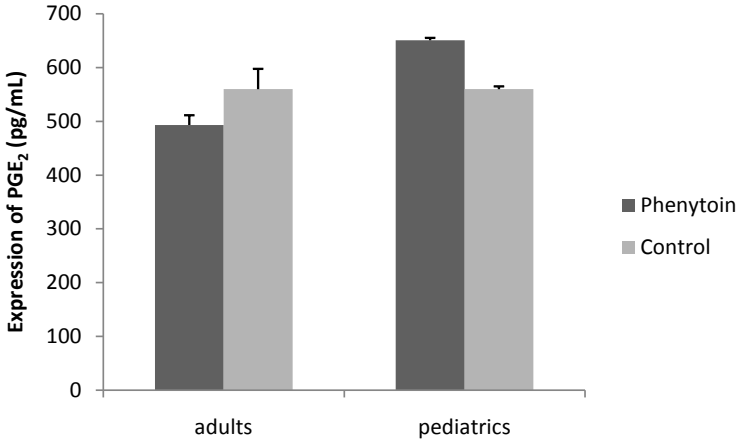
In this study, no significant increase was perceived in the rate of proliferation of pediatrics and adults fibroblasts after exposure to phenytoin. Also, the rate of proliferation was not significantly different between adults and pediatrics. In agreement with our results, Hassel *et al.* [21] and Vamada *et al.* [22] have also reported that no significant increase is perceived in levels of hyperplasia in treated fibroblasts. In contrast, Al-Ubaidy *et al.* [40] have shown that phenytoin significantly increases the mitotic activity and mitotic index of gingival fibroblasts. Another study performed on Wister mouse, has reported that phenytoin stimulates the proliferation of interdental gingival fibroblasts and of fibroblasts of periodontal ligaments. The ECM produced in this overgrowth can facilitate the fibroblast spreading and provide a better condition for growth and hyperplasia of fibroblasts [41]. In Seymour *et al.* study [41], it has been shown that phenytoin is capable of influencing the cell cycle, and that phenytoin inhibits the process of Ca^{2+} absorption in fibroblasts and also causes cell hyperplasia. Some researchers have expressed that rat gingival fibroblasts show resistance against phenytoin-induced overgrowth, probably because of their functional heterogeneity [42]. However, Pour Abbas *et al.*, by investigating metabolic activity of proliferation in rat fibroblasts, have reached to contrary results [41].



Graph 1. Rate of proliferation (mean + standard deviation) in adult and pediatric fibroblasts in both control group and phenytoin-treated group.



Graph 2. The expression of released IL-1β (mean + standard deviation) in adult and pediatric fibroblasts in both control group and phenytoin-treated group.



Graph 3. The expression of released PGE_2 (mean + standard deviation) in adult and pediatric fibroblasts in both control group and phenytoin-treated group.

Phenytoin stimulated pediatrics fibroblasts to produce more levels of $IL_{1\beta}$. However, in the adults group, no significant change was perceived. The study of Modeer *et al.* [30] agrees with our results, and also shows that the inhibitive effect of IL-1β on expression of collagen genes increases in presence of phenytoin. One other study has showed that the simultaneous presence of TNF-α and phenytoin stimulates the production of IL-1α by fibroblasts and suggested that the simultaneous presence of three inflammatory mediators, TNF-α, IL-1α, and PGE_2, could raise the effects of collagenase enzyme [43]. Brunius *et al.* [29] have proposed that phenytoin cannot stimulate the IL-1β production solely, and that the concurrent presence of TNF-α is necessary for inducing IL-1β and PGE_2 production.

In this study, after exposure to phenytoin, pediatric fibroblasts produced higher levels of PGE_2; while in adults group, the PGE_2 production decreased. Levels of produced PGE_2 by treated pediatric fibroblasts were significantly higher than those by treated adult's fibroblasts. These results are consistent with Modeer *et al.*, who have shown the increase in PGE2 production of treated fibroblasts in both *in vitro* [44] and *in vivo* [45] studies. In the *in vitro* study, the concurrent presence of IL-1β and TNF-α was necessary for PGE_2 production by treated fibroblasts [46]; however, in an *in vivo* case, the PGE_2 production occurred with or without existence of IL-1β and TNF-α [43]. Since phenytoin is capable of regulating intracellular Ca^{2+}, and since phospholipase A2 is a Ca^{2+} related enzyme, the higher levels of PGE_2 production has been attributed to the increased activity of A2 in phenytoin-induced fibroblasts. Modeer *et al.* [45] have also shown that adding exogene Arashidonik acid could result in higher activity levels of A2, and so could cause the treated fibroblasts to produce PGE_2 4 times more

[43] [45].

It seems that several mechanisms such as increased inflammatory activities and altered immunological processes and disturbed homeostasis of cytokines, as well as secretion of inflammatory mediators as TNF-α, PGE_2; and IL-1β, could affect the gingival fibroblasts and cause some disturbances in ECM homeostasis, in cell proliferation, and in homeostasis of connective tissue proteins. Studies have reported that phenytoin causes disturbance in cell population, which subsequently results in alternations in production of growth factors and receptors such as EGF and PDGF, and stimulates production of IL-1β and PGE_2. These phenomena, in turn, causes gingival overgrowth [3] [29].

IL-1β, a member of pleiotropic cytokines, is known to be influent on immunological processes, fibroblast's growth, and inflammatory lesions, and to be capable of stimulating collagenase and prostaglandins such as hyaluronic acid [46]. This cytokine probably causes a decrease in the expression of procollagen and an increase in expression of procollagenase, and so could be known as a stimulator for destroying collagen. Noguchi [47] showed that at the presence of phenytoin, the number of IL-1 β receptors increase, by the way, the IL-1β which has been produced by local immune cells has more paracrine attachment.

These results support the hypothesis that IL-1β plays an important role in regulating ECM in gingival tissue. However, the reports on this issue are inconsistent, e.g. Gonzales *et al.* have shown that phenytoin raises the inhibitive effect of IL-1β [3].

PGE2 is another inflammatory mediator that takes part in regulating the production of collagen and in ECM turn over. Yucel *et al.* [29] have shown that the PGE_2 produced by phenytoin-treated gingival fibroblasts in presence of TNF-α, causes a reduction in IL-1β production. Moreover, adding exogenous PGE_2, dose-dependently decreases the production of IL-1β. In a similar study, Brunius *et al.* have shown that the PGE_2 that is produced by stimulus of phenytoin and IL-1β is influent on synthesis of glucose amino glycan's and on inhibition of procollagen α production. They have also reported that adding exogene PGE_2 to fibroblast culture results in reduced levels of collagen type I production [29].

Recent studies have proposed different processes for drug-induced inflammatory trends. For example, one possible explanation is the role of pathogen-sensitive sensors such as Toll-like receptors (TLR) [48] [49], that are continually present on fibroblasts and are capable of responding to bacterial components. No study has yet reported the probable effects of phenytoin on TLR-related signals. However, based on similar pharmacologic function of phenytoin and Lignocaine, which is a Na^+ channel-blocker that inhibits the effects of lipopolysaccharides on mitoionic activator proteins in macrophages, one might propose that phenytoin reduces the fibroblasts-related signals [50]. By considering the hyperplasia of phenytoin-treated fibroblasts, it might be concluded that this inhibitive influence is restricted to inflammatory responses and is not related to the drug cytotoxicity. Unlike cyclosporine, phenytoin decreases the production of cytokines and the expression of CD54 as one major marker in immigrating and persistence of inflammatory cells in periodontal diseases [44]. The different functionality of cyclosporine and phenytoin could be attributed to the role of phenytoin in reducing the infiltration of inflammatory cells, an effect that is perceived not only in gingival overgrowth but also in under treatment ancephalomilites [51]. Nevertheless, some studies have reported that phenytoin, solely or in association with IL-1, can raise the production of Interleukin-6 (IL-6) and Interleukin-8 (IL-8) in cultured fibroblasts. This phenomenon can be attributed to the inhibitive impacts of phenytoin in cultures containing serum protein [52]. Modeer *et al.* [45] have also suggested that phenytoin is responsible for semi-inflammatory changes in gingival overgrowth, and that phenytoin stimulates the secretion of factors such as IL-1β and PGE_2. These factors take the latent fibroblasts to the s stage or synthesis of mitosis cycle, and cause an increase in noncollogenous ECM production. Evidences confirm that in the treatment process, the number of mononuclear cells, especially T lymphocytes, increases in gingival tissue and in not inflammatory overgrown sites, and with the increased expression of CD4, the medial marker between T lymphocytes and fibroblasts, more cytokines can emerge. Eventually, activity and interaction between these cells and the periodontal tissue cells causes the gingival overgrowth [53].

According to our literature review, this study compared the effect of phenytoin on fibroblasts is investigated through two age groups for the first time. According to epidemiological studies, age is a determinant factor in the occurrence of gingival overgrowth. Doufexi *et al.* [8] have reported that the gingival overgrowth, especially in anterior regions, is more common in pediatrics and teenagers. Also, Seymour *et al.* [54] have shown that teenagers are more susceptible to gingival hyperplasia. The prevalence of cyclosporine-induced gingival overgrowth is 52% in pediatrics in comparison to that of 30% in adults [54]. Moreover, the prevalence of phenytoin-induced gingival overgrowth is reported 67% in pediatrics, while it is 50% in adults [55]. In Majola *et al.* [56]

study, no linear relationship was perceived between the side-effects of phenytoin and length of drug consumption, but the lesions were more prevalent in younger patients. Although animal studies have confirmed such results, no specific *in vitro* study has been conducted on this issue yet, and the reduced synthesis of protein and collagen by fibroblasts in the phenytoin- or cyclosporine-induced gingival overgrowth has not shown to be correlated with age [57] [58].

Regarding to our results, the levels of two important inflammatory mediators was increased in pediatric group, and no significant difference was perceived in the cell proliferation. These results suggest that more prevalence of gingival overgrowth in pediatrics in comparison with adults might be attributed to the different immunological trends in pediatrics, as well as levels and responses of inflammatory cytokines. Unlike this study, the study of Sooriyamorthy did not resulted in a relationship between age and clinical features, a phenomenon which was attributed to the unique phonotype of fibroblasts or to androgynous metabolism influences. Monolayer cultures from gingival fibroblasts can clearly metabolize labeled testosterone into its active metabolite, 5α-dihydrotestosteron. Levels of this metabolite increases in presence of phenytoin [59]. Similarly, an increased androgenic metabolism is perceived in parted tissues from Niphedipine- or Cyclosporine-induced overgrown gingiva [60]. On the basis of these results, Sooriyamorthy suggested that the increase in androgenic metabolism is effective in pathogenesis drug-induced gingival overgrowth. Seymour *et al.* [14] have suggested that active androgenic metabolite can target a specific subgroup of gingival fibroblasts and causes an increase in collagen production or a decrease in collagenase enzyme activity. In fact, 5α-dihydrotestostron (DHT) increases in the inflammation and overgrowth process, and stimulates the production of connective tissue [61]. Soory *et al.* study [61] showed that in presence of IL-1 and phenytoin, the DHT production by fibroblasts doubles. Considering these studies and the present study, phenytoin is probably related to androgenic metabolism in fibroblasts. The increase in production of DHT is one of possible mechanisms in creating overgrowth and subsequent inflammatory trends such as provoking glycosaminoglycan and remodeling of connective tissue. Since consumption of phenytoin, Niphedipine and cyclosporine does not always result in gingival overgrowth, recent studies have suggested that genetic factors are possibly influent on the pathogens of these lesions in pediatrics and teenagers [62]. One of genetic risk factors is the gingival fibroblasts response to different dosages and cultures in terms of proliferation and the changes in synthesis of proteins such as collagen [54]. Fibroblasts of different strains demonstrate a natural functional heterogeneity in producing collagenase and other tissue inhibitor of matrix metallo proteinases before and after exposure to drugs that can bring about overgrowth. The variety of activities of cytochrome oxidase P450, the agent of metabolism for phenytoin, cyclosporine and Niphedipine in liver, is genetic factors which are related to gingival overgrowth [8]. Pinkham has suggested that the low levels of Cytochromoxidose p450 and its gradual oxidation causes an incomplete and sudden functionality of this enzyme in digestion and detoxification of phenytoin and so the clinical effects of this drug and the probability of toxicity increases as the levels of P450 decrease [63]. Trackman *et al.* have also proposed that the reduced metabolism and liver's dispel of phenytoin could be another cause of gingival overgrowth. This hypothesis, however, is not confirmed yet [27].

Dental plaque is another factor in phenytoin- and cyclosporine-induced gingival overgrowth especially in younger patients. Studies have suggested that the prevalence and intensity of gingival overgrowth is positively correlated with dental plaque [8]. Doufexi *et al.* have reported that incubation of monocytes and macrophages could increase the Platelet-derived growth factor (PDGF) and stimulates gingiva growth [8]. The presence of inflammatory cells and their produced mediators in drug-induced gingival overgrowth condition corroborates the relation between overgrowth and preexisting inflammation. Since the majority of studies are cross-sectional, it is not yet clear whether the improper oral hygiene is the initial reason of gingival overgrowth or not. In a 2-year study on pediatrics, Dalhoof *et al.* [64] showed that proper oral hygiene and educational programs can not solely hinder the phenytoin-induced overgrowth. Nevertheless, oral hygiene could inhibit the subsequent changes in inflammatory condition and so hamper further overgrowth.

Bonding of plasma proteins is another probable cause of difference in overgrowth of gingiva in pediatrics and adults. There is neither linear relationship between the duration nor intensity of treatment and the levels of plasma proteins. Reduction in the levels of these proteins and their bonding to phenytoin causes the drug to disperse in the tissues, especially in the tissues with high affinity to phenytoin, e.g. gingival fibroblast tissues [53].

The discussed reasons for different response of gingival fibroblasts to phenytoin in pediatrics and adults are briefly demonstrated in **Figure 1**.

The present study is the only research that has compared the effects of phenytoin on gingival fibroblasts in children and adults, however, further studies are necessary to confirm the discussed results. Unlike other studies,

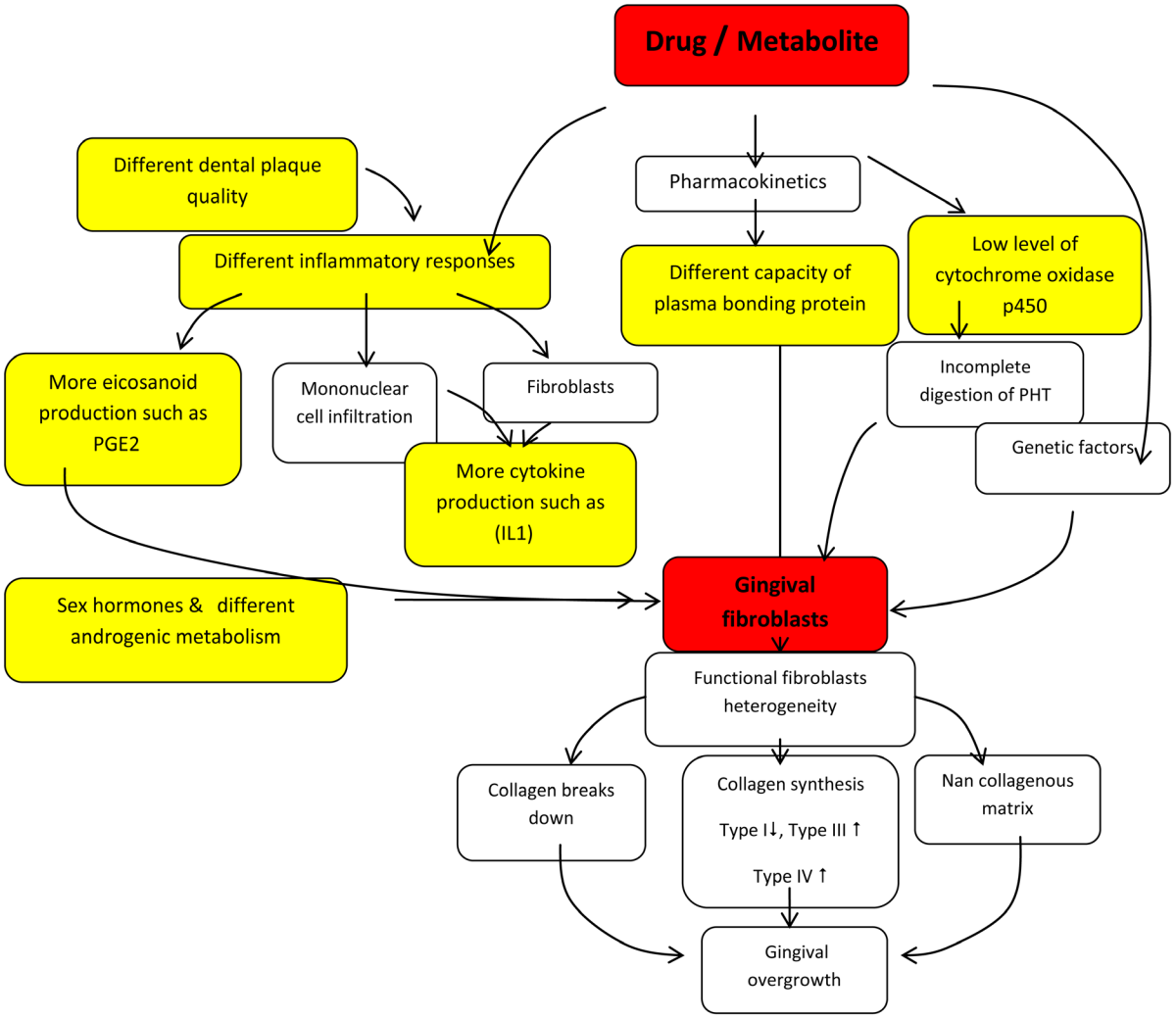


Figure 1. The discussed reasons for different response of gingival fibroblasts to phenytoin in pediatrics and adults.

this study was designed to investigate different aspects of drug influence. This study was conducted without cell lines genetic manipulations or retentive materials, features that are potential advantages of this study.

The different results of variant studies could be attributed to different sources of fibroblasts, the location of biopsies, age of patients or small population of samples. The natural heterogeneity of fibroblasts in response to phenytoin might be another reason for such differences. In order to prevent such issues in this study, those cultures, among cell lines extracted from different patients, were chosen that had shown rather similar responses to phenytoin, and the coefficient of correlation among different wells was calculated at the end [3]. Groups were divided into two responsive and nonresponsive groups on the basis of the perceived growth of gingiva. Genetic differences result in larger dimensions of fibroblasts and greater capacity of secretion of ECM proteins and soluble factors in members of the responder group. Gonzalez *et al.* have not reported any difference among the expression of fibroblast-related markers in healthy, responder and non-responder groups [3] [25]. So, the gingival fibroblasts in 3 groups are capable of responding to LPS intra oral pathogens such as P. gingivalis and F. nucleatum. Fibroblasts can also disturb the toll-like 2, 4 receptors, which play a role in immune processes. As a result, any disturbance in ECM metabolisms during the treatment period can be occurred [27].

5. Conclusion

No significant difference was perceived between proliferations of these two groups. The significant increase in production of IL-1β and PGE_2 in pediatrics as compared to adults might be attributed to genetic differences and

to conditional factors such as gingival plaque and preexisting gingival inflammation.

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