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Effect of Phenytoin and Cyclosporine on IL-17 Production by Gingival Fibroblasts of Adults and Children

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Abstract

Background and aims. Gingival hyperplasia, a relatively common side effect of antiepileptic and anticonvulsant drugs, occurs in 30–50% of patients taking phenytoin and 25–81% of those taking cyclosporine. Gingival hyperplasia due to lack of balance between extracellular synthesis and degradation is associated with increased production of IL-1B, IL-6 and IL-8 by gingival fibroblasts. Tissue level of IL-17 increases in inflammatory conditions. Since the role of IL-17 and patient age in gingival hyperplasia is still unclear, this study aimed to compare the level of IL-17 produced by gingival fibroblasts in children and adults.

Materials and methods. This study was conducted on biopsy specimens obtained from the healthy gingiva of 4 adults, 35–42 years of age, undergoing crown lengthening surgery and 4 children, aged 4–11 years, undergoing impacted tooth surgery. Biopsy specimens were cultured in a mixture of Dulbecco's Modified Eagle's Medium (DMEM), penicillin, 1% streptomycin and 10% fetal bovine serum (FBS) at 37°C and 5% CO_2. The specimens were monitored for contamination and cell proliferation and the medium was refreshed if necessary.

Results. The baseline levels of IL-17 produced by gingival fibroblasts isolated from children and adults were not significantly different from those after the addition of cyclosporine or phenytoin. The two groups of children and adults were not significantly different in terms of the production of IL-17 by gingival fibroblasts. The two groups of children and adults were not significantly different in terms of the production of IL-17 at baseline or after exposure to cyclosporine or phenytoin.

Conclusion. IL-17 inflammatory cytokine does not play a role in gingival hyperplasia in children and adults.

Key words: **Phenytoin, cyclosporine, gingival fibroblast.**

Introduction

Gingival hyperplasia (GH) is a relatively common side effect of antiepileptic and anticonvulsant drugs such as phenytoin and cyclosporine. Phenytoin is an antiepileptic drug and GH is among the side effects of its long-term administration.[1] This medication has long been prescribed for epileptic patients. The first case of GH associated with phenytoin therapy was reported in 1939.[2] GH occurs in 30–50% of patients using phenytoin[3] and involves the buccal gingiva of the maxillary and mandibular anterior teeth.[4] Its underlying mechanism has yet to be fully understood, but its effect on collagen metabolism and formation of fibrous tissue has been confirmed.[5] Phenytoin increases protein synthesis and induces cell proliferation.[6] It regulates macrophage phenotype and expression of cytokines and growth factors[7] by increasing the production of IL-1B, IL-8 and IL-6 by fibroblasts.[8] It also increases NF-κB levels, which mediates the immunological and inflammatory responses. These cytokines activate T cells, induce their proliferation and attract neutrophils to the involved tissue.[9]

Cyclosporine is an immunosuppressant with extensive applications in organ transplantation and autoimmune diseases.[10] GH is among the side effects of cyclosporine administration, especially in the presence of bacterial plaque.[11,12] GH associated with cyclosporine therapy occurs in 25–81% of patients[13] and in up to 97% of children.[14] Age, gender, duration of treatment and the administered dose affect the prevalence and severity of GH.[15–17] Cyclosporine alters the function of fibroblasts[18] and changes the level of cytokines like TGF-B1.[19] Reducing the drug dose, oral hygiene control and surgical treatment may be recommended for patients with GH.[20] However, recurrence is likely to occur and reducing the drug dose is not always feasible. Surgical treatment is usually rendered for esthetic purposes and is not the definite treatment of this condition. Oral hygiene can affect the severity of GH. Increased levels of IL-13, IL-6, IL-1 and TGF-B1[9] have been reported in GH and the role of T-helper 2 as well as IL-4, IL-5 and IL-21 in this respect has been confirmed in an animal model.[9] IL-6 is responsible for activation and proliferation of B and T cells in the fibrous tissue and different organs[21] and IL-8 has a chemotactic effect on PMNs and T cells in human renal interstitial fibrosis.[22] In general, cytokines and their effects on the activity and number of fibroblasts lead to GH in the buccal gingiva of maxillary and mandibular anterior teeth.[4] Drugs like phenytoin and cyclosporine cause an imbalance between the synthesis and degradation of extracellular components causing GH.[11,23] The exact underlying mechanism has yet to be clearly understood but the role of drugs in increasing the level of cytokines is highly probable. IL-17 levels seem to increase in some inflammatory conditions; however, its role in GH is still questionable. The effect of age of patients taking phenytoin and cyclosporine in this respect is not clear either.

This study aimed to compare the IL-17 levels produced by gingival fibroblasts in children and adults.

Materials and Methods

Surgical Process

Biopsy specimens were obtained from the healthy gingiva of 4 adults, 35–42 years of age, undergoing crown lengthening surgery and 4 healthy children, aged 4–11, undergoing impacted tooth surgery. Inclusion criteria consisted of absence of systemic problems and no use of any relevant drugs. The exclusion criteria consisted of systemic conditions, periodontal disease, pregnancy and consumption of anti-inflammatory drugs. Biopsy specimens were taken from the surgically resected tissues during surgery and no excess tissue was resected for the purpose of the study. Informed consent was obtained from donors prior to sampling. The experimental protocol was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences.

Cell Culture

Biopsy specimens were immediately cultured in a mixture of Dulbecco's Modified Eagle's Medium (DMEM; Biochrom AG, Berlin, Germany), penicillin, 1% streptomycin and 10% FBS (FBS; Sigma-Aldrich, St. Louis, MO, USA) at 37°C and 5% CO_2 and continuously monitored for contamination and cell proliferation. The medium was refreshed if necessary. After proliferation of fibroblasts and filling the plate, the cells were treated with trypsin and transferred to tissue culture flasks (Nunc, Copenhagen, Denmark) for passage.

IL-17 Measurement

After 4 passages, the cells were detached from the culture flasks with 0.25% trypsin and transferred to 2-mL culture media. The cells were then counted using Neubauer chamber. Fibroblasts were cultured in a 24-well plate (Nunc, Copenhagen, Denmark) at a density of 60*1000 cells. After 2 days, the wells were divided into 3 groups of control, phenytoin (Sigma-Aldrich, St. Louis, MO, USA) (20 μg/mL) and cyclosporine (Sigma-Aldrich, St. Louis, MO,

USA) (20 µg/mL). The plates were then incubated for 48 hours at 37°C, 5% CO_2 and 95% humidity according to previous studies.

IL-17 levels were assessed using ELISA. IL-17 specific polyclonal antibody was added to the wells (coating). After several steps, OD was measured using ELISA Reader (R and D Systems, Minneapolis, MN, USA).

Kruskal-Wallis test was applied to compare IL-17 levels in children and adults in the control, cyclosporine and phenytoin subgroups. Mann-Whitney test was used to compare IL-17 levels between children and adults.

Results

IL-17 of Children's Fibroblasts

The mean level of IL-17 produced by fibroblasts isolated from the gingival tissue of children was 4.8050 (4.78–4.83). After exposure to cyclosporine under in vitro conditions, this rate reached 5.6400 (5.13–6.15). However, the difference was not statistically significant (P>0.05).

The mean level of IL-17 produced by fibroblasts isolated from the gingival tissue of children after exposure to phenytoin under in vitro conditions was 5.9850 (5.74–6.05), which was not significantly different from the baseline value before exposure to phenytoin (P>0.05)

IL-17 of Adults' Fibroblasts

The mean level of IL-17 produced by fibroblasts isolated from the gingival tissue of adults was 5.1200 (4.35–6.18). After exposure to cyclosporine under in vitro conditions, this rate reached 6.5167 (5.48–7.42). However, the difference was not statistically significant (P>0.05).

The mean level of IL-17 produced by fibroblasts isolated from the gingival tissue of adults after exposure to phenytoin under in vitro conditions was 4.5800 (3.21–5.52), which was not significantly different from the baseline value before exposure to phenytoin (P>0.05).

Comparison of IL-17 between Children and Adults

The mean level of IL-17 produced by fibroblasts isolated from the gingival tissue of children was 4.8050 (4.78–4.83). The mean level of IL-17 produced by fibroblasts isolated from the gingival tissue of adults was 5.1200 (4.35-6.18). The difference was not statistically significant (P>0.05).

After exposure to cyclosporine under in vitro conditions, level of IL-17 produced by fibroblasts of children reached 5.6400 (5.13–6.15). However, the difference was not statistically significant (P>0.05). After exposure to cyclosporine under in vitro conditions, level of IL-17 produced by the fibroblasts of adults reached 6.5167 (5.48–7.42). The difference was not statistically significant either (P>0.05).

The mean level of IL-17 produced by fibroblasts isolated from the gingival tissue of children after exposure to phenytoin under in vitro conditions was 5.9850 (5.74–6.05). The mean level of IL-17 produced by fibroblasts isolated from the gingival tissue of adults after exposure to phenytoin under in vitro conditions was 4.5800 (3.21–5.52). The difference was not statistically significant (P>0.05) (Figure 1).

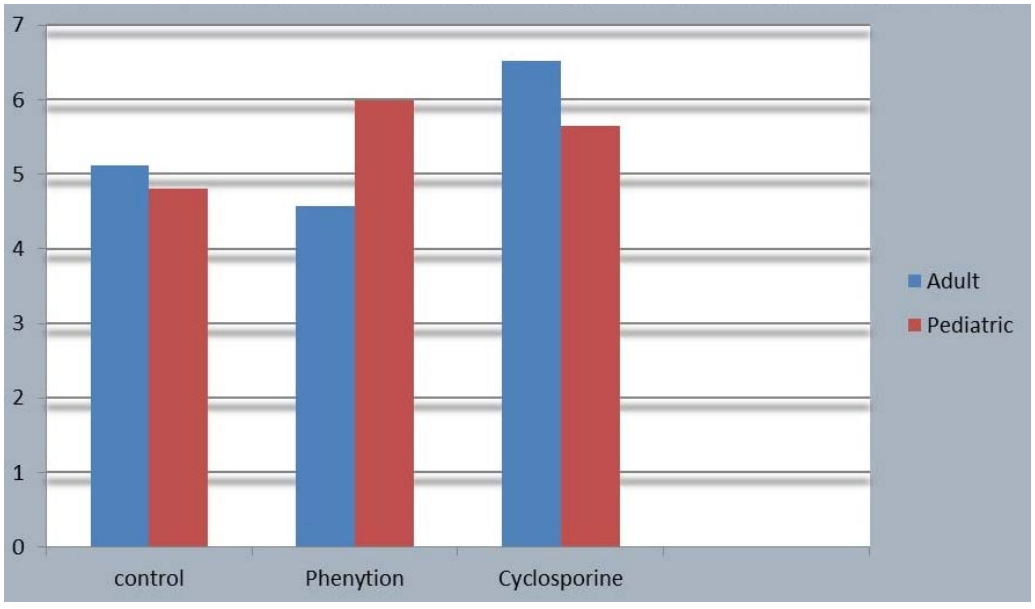

Figure 1. The mean level of IL-17 produced by fibroblasts in 3 groups

Discussion

Incorporation of cyclosporine did not cause a significant change in the production of IL-17 by fibroblasts of children and adults cultured in vitro. This difference after exposure to phenytoin was not significant either.

Chi et al[25] demonstrated a reduction in serum levels of IL-17 in patients with Behçet's disease, receiving cyclosporine,[24] whereas Brandon et al reported an increase in serum levels of IL-17 in mice receiving cyclosporine due to different organ transplants. Chi et al evaluated 6 human subjects whereas the study of Brandon et al was conducted in an animal model. The reason for drug therapy was also different and these factors might explain the different results obtained. Kehlen et al[26] reported an increase in expression, modulation and signaling of IL-17 receptor in fibroblast-like synoviocytes in rheumatoid arthritis (RA) patients, leading to increased proliferation of connective tissue. However, in the present study, no significant association was found between the IL-17 levels and proliferation of gingival fibroblasts.

No significant changes occurred in IL-17 levels in the present study after incorporation of phenytoin. The differences in IL-17 levels between the fibroblasts of children and adults were not significant either.

Gingival connective tissue cells play a principal role in the pathogenesis of GH due to the consumption of certain drugs.[8,27–29] The correlation of inflammatory factors and gingival fibrosis due to the consumption of certain drugs has been documented.[30] In fact, the same factors that increase in the connective tissue due to gingivitis are also present in hyperplastic gingiva due to phenytoin consumption.[30] Biochemical and histological tests indicate alterations in tissue components and cellular activity, attributed to the consumption of drugs that cause GH.[31–35] However, the exact underlying mechanism has yet to be known. Vahabi et al[36] reported differences in mechanisms of action of phenytoin and cyclosporine and demonstrated that they influence different molecules such as lysosomal cysteine proteins; consequently, the inflammatory response to these drugs is different. The difference in this respect between children and adults is usually due to differences in immunological responses and cytokines levels in children. However, it has been well understood that consumption of phenytoin and cyclosporine causes GH due to several known and unknown mechanisms. Vahabi et al[36] showed that these mechanisms are mainly active in adults and do not normally play a role in GH in children.

Lee et al[37] showed that gingival fibroblasts are different from the fibroblasts in other parts of the body (i.e. skin fibroblasts) in terms of behavior and response to growth factors. That explains rapid scar-free healing that occurs in the oral cavity. Gingival and skin fibroblasts normally act similarly in collagen synthesis. However, when exposed to TGF-B1, gingival fibroblasts synthesize much more collagen than skin fibroblasts.

One advantage of this study was evaluation of gingival fibroblasts in children aged 4–11 years since previous studies have been mostly conducted on adults. Based on the literature, the prevalence of phenytoin-induced gingival hyperplasia is 67% in children and 50% in adults,[38] indicating the need for further investigations on children. Moreover, serum levels of enzymes are usually different in children taking phenytoin or cyclosporine from adults, which might be attributed to differences in immunological reactions and cytokine levels between children and adults. Our study evaluated the levels of IL-17, an inflammatory cytokine, using ELISA. After exposure to phenytoin and cyclosporine, small changes occurred in IL-17 levels, which were not significant. However, in systemic sclerosis, overproduction of collagen has been reported due to an increase in IL-17 levels in the skin of patients with early SSC. Increased IL-17 levels have also been demonstrated in RA patients experiencing increased connective tissue proliferation.[39] However, we did not find an increase in IL-17 levels associated with gingival proliferation and collagen synthesis in our study.

We did not notice any changes in fibroblastic response in our study; however, Nazemi Salman et al[40] showed significant effects of TGF-B, IL-1B, IL-8, IL-6 and PGE2 on children and adults after taking phenytoin and cyclosporine. Such difference between their study results and ours might be attributed to our small sample size. Due to ethical issues, finding patients, especially children, was difficult for our study. The in vitro design of the present study is another factor that might have influenced the results. Ethnic and racial differences might also play a role since most studies reporting significant effects of IL-17 on inflammation have been conducted on non-Iranian populations. This study might have yielded different results if it had been conducted on a different population. Differences in the source of isolated fibroblasts, biopsy site, patient age, patient response to medications and diagnosis of drug-induced gingival hyperplasia can all influence the results.

The present study was conducted using ELISA, which is a format of "wet-lab" type analytical assay that uses solid-phase enzyme immunoassay for detection of target materials. It is considered a standard technique for cytokine measurement and is widely used in clinical laboratories and for biomedical research worldwide. ELISA kits are commercially available for commonly measured cytokines from different manufacturers. Its main advantage is that the results are highly quantitative and generally reproducible.[41]

Application of other techniques, such as Mass Spectrometry Assay (MSA) might have yielded different results in relation to the IL-17 levels. MSA is an analytical technique that isolates single- or multi-atom ions and measures the mass-to-charge ratio and frequency of these charged particles in a gaseous phase. MSA works by ionizing chemical compounds to generate charged molecules or molecule fragments and measuring their mass-to-charge ratios using electrical and magnetic fields.[42] Simon et al[43] replaced ELISA with MSA and concluded that the accuracy of MSA was higher than that of ELISA. MACS cytokine secretion assay is another technique suggested for this purpose with high sensitivity for detection of cytokines. In this method, reagents and the fluorescent agent are added to the analyte. Cytokine detection and quantification are carried out using MACS Plex.

This study can be a start point for further investigations on IL-17 and the related differences between children and adults. Further studies are required to better elucidate the mechanisms involved in drug-induced GH in children and adults and possible differences in this regard. The role of other cytokines should also be investigated in GH.

Conclusion

IL-17 inflammatory cytokine does not play a role in gingival hyperplasia in children and adults.

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