Original

Evaluation of Collagen Type-1 Production and Anti-Inflammatory Activities of Human Placental Extracts in Human Gingival Fibroblasts

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Abstract: Placental extracts contain various bioactive agents and are thought to be a prospective medicine for oral diseases. However, detailed information regarding their mechanisms with respect to treatment of periodontal diseases is not available, thereby hindering their reliable application in dentistry. In this study, we demonstrated that human placental extracts increased collagen type-1 production, which is linked to the regenerative capabilities of periodontal tissue, on primary human gingival fibroblasts in vitro. Generally, deterioration of periodontal tissue occurs when various types of cytokines are released by periodontal tissue cells in response to stimulation by plaque. However, we exhibited that human placental extracts hindered the pro-inflammatory cytokines such as interleukin (IL)-6 and IL-8 secretion from primary human gingival fibroblasts in vitro. The results contribute to understanding a therapeutic mechanism underlying the effect of human placenta extracts against periodontal disease by modulating the function of human gingival fibroblasts.

Key words: Collagen, Human gingival fibroblast, Human placental extracts, Periodontal disease

Introduction

Periodontal diseases linked to adult lifestyle-related diseases, are a biological response caused by complicated interactions between the host and mechanical stress or periodontal pathogens residing in the oral cavity1). Generally, deterioration of periodontal tissue occurs when various types of cytokines are released by periodontal tissue cells in response to stimulation by plaque4). In fact, inflamed periodontal tissue is known to exhibit elevated concentrations of cytokines such as interleukin (IL)-6 and IL-85). IL-6 has a variety of functions, including the regulation of immune cell differentiation and multiplication6), stimulation of bone absorption, and exacerbation of periodontal disease5). IL-8 is believed to be involved in the stimulation of neutrophils and exacerbation of periodontal disease6).

Many cells, including macrophages or lymphocytes, are known to be intricately involved in the exacerbation of periodontal disease6). Human gingival fibroblasts (HGFs) are the most abundant cell type in gingival connective tissue and produce collagen while sustaining the structure or homeostasis of the periodontal tissue7-9).

Placental extracts are produced by the purification of placenta through a process involving dialysis, heat treatment, and hydrolysis; they contain various biologically active substances such as amino acids, peptides, glycosaminoglycans, minerals, and growth factors10). Placental extracts are reported to exert effects that have medicinal value, such as the acceleration of anti-oxidative effects11), anti-inflammatory effects11,12), wound healing13), and immune enhancement14). Therefore, placental extracts are processed by a variety of methods and are sold as drugs or quasi-drugs. Even in the field of dentistry, there are many studies demonstrating the benefits of using placenta in periodontal diseases15-18). However, these studies have focused entirely on clinical knowledge and therefore, it is unclear if the placenta has any effect on cellular function with respect to periodontal diseases. It is necessary to accumulate basic research data with cultured cells or with similar in vitro methods because of the complexity of the factors that cause periodontal disease, owing to the network of multiple cells and cytokines19). Recently, we reported that powdered placental extracts, isolated from porcine tissue, could...
increase collagen type-1 production and hinder pro-inflammatory cytokine secretion by HGFs\textsuperscript{20}). To confirm this, we examined the effect of human placenta extracts on the production of type I collagen and inflammatory cytokines by HGFs \textit{in vitro}. In addition, we attempted to clarify part of the mechanism underlying the action of human placenta extracts in periodontal diseases.

Materials and methods

Reagents

The following materials and antibodies were purchased: Melsmon (human placenta extracts) was provided by Melsmon Pharmaceutical Co., Ltd. (Tokyo, Japan). Melsmon (2 ml) contains 100 mg of human placental hydrolysate. Lipopolysaccharide (LPS) from \textit{Porphyromonas gingivalis} (\textit{P. gingivalis}) (InvivoGen, Shatin, Hong Kong); anti-Interleukin (IL)-6, biotinylated anti-IL-6 antibodies (eBioscience, San Diego, USA); anti-IL-8 and biotinylated anti-IL-8 antibodies (R&D Systems, Shanghai, China); biotinylated anti-collagen type I antibody (Rockland, Pennsylvania, USA); 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) (Sigma-Aldrich, Missouri, USA).

Cell culture

HGF cells were obtained according to the literature\textsuperscript{21}). The HGFs were cultured in Dulbecco’s Modified Eagle Medium (DMEM, Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) with 10% fetal bovine serum (FBS), 100 units/ml penicillin G, and 100 µg/ml streptomycin at 37°C in an incubator with 5% CO\textsubscript{2} and 95% humidified air\textsuperscript{22}). The HGFs used in this study were obtained from volunteers after obtaining appropriate informed consent. The Ethics Committee of Osaka Dental University approved the study (protocol 110778).

Statistical analysis

Quantitative data were statistically analyzed using either one-way analysis of variance (ANOVA) followed by Dunnett’s test, Student’s \textit{t}-test or Tukey’s test using the StatMate software (ATMS). Differences were considered to be significant at \(P < 0.05\).

Results

We examined the effect of Melsmon on the viability of HGFs...
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using an MTT assay (Fig. 1). Treatment with Melsmon significantly affect the viability of HGFs at concentrations of up to 1500 µg/ml. The following experiments were conducted using 1500 µg/ml of Melsmon.

We evaluated the effect of Melsmon on the production of type I collagen by HGFs, which is directly linked to the regenerative capabilities of periodontal tissue. In the cell viability evaluation, 1500 µg/mL of Melsmon also enhanced production of type I collagen in HGFs by roughly 2.2 times compared with the control (Fig. 2).

Fig. 3 shows the effect of Melsmon on the secretion of inflammatory cytokines by HGFs. IL-6 and IL-8 production from HGFs was markedly accelerated in the presence of LPS stimulated P. gingivalis, a periodontal pathogenic bacterium that has a strong inflammation-inducing effect. On the other hand, the production of this cytokine in LPS stimulated P. gingivalis was significantly suppressed in the presence of Melsmon.

Discussion

Previous reports have shown that placental extracts contain growth factors such as transforming growth factor-β (TGF-β)\(^{24,25}\). TGF-β is known to improve the production of collagen in HGFs\(^{26}\). In addition, Melsmon contains a variety of vitamins, which improve the production capabilities of collagen in HGFs\(^{27}\). When considering these points, we believe that the growth factors and vitamins in Melsmon can probably act to improve the collagen production by HGFs.

The results of this study show that Melsmon has a suppressive effect on LPS-induced production of cytokines by HGFs (Fig. 3). It has been reported that HGFs express the toll-like receptor (TLR) genes TLR-1, -2, -3, -4, -5, and -9, which are widely known as receptors for pathogens, and that TLR-2, -3, -4, and -5 contribute to the expression of IL-8\(^{28}\). In addition, TLR-2 is known to be involved in IL-6 expression\(^{29}\). Placental cells secrete a variety of antimicrobial peptides and proteins (AMPs) such as bactericidal/permeability-increasing protein (BPI), secretory leukocyte protease inhibitor (SLPI), human β-defensin 2 (hBD2), and cathelicidin (CAP18) as a latent infection preventive barrier\(^{30}\). These proteins and peptides bind with LPS and work to suppress its binding function\(^{31}\). There is a possibility that these proteins and peptides might be present in the Melsmon used in this experiment. In this study, we also believe that AMPs are potentially involved in the Melsmon-mediated suppression of the LPS-induced inflammatory cytokine production\(^{30,31}\).

In this in vitro study, we observed that Melsmon simultaneously enhanced the production of type I collagen and suppressed the production of LPS-induced inflammatory cytokines (IL-6 and IL-8). These results indicate that the pharmacokinetic data from the powdered placenta extracts isolated from porcine tissue is similar to that of human aqueous placenta extracts in Melsmon\(^{20}\). In recent years, reports have questioned the value of animal testing due to the significant differences between animals and humans\(^{32}\). Even periodontal disease-related studies have not yielded models that recapitulate human periodontal disease\(^{33}\). With this background, the approach used here allows the acquisition of data regarding human periodontal diseases using gingival fibroblasts instead of animal testing. However, future studies using a reliable human periodontal disease model will be necessary to...
confirm the in vitro findings. In addition, periodontal disease-related inflammatory cytokines have not been studied comprehensively. In particular, there is a need for the comprehensive analyses of the effects of placental components on HGFs and the optimum concentration of placenta that has an effect on HGFs, in addition to a comparative investigation with various placental drugs. Nevertheless, we believe that the results of this study contribute to clarifying the clinical benefits of placenta.

Conflicts of Interest

The authors declare no COI existed.

References

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