Effect of Endothelin-1 on Cell Viability of Human Periodontal Ligament Fibroblasts

ผลของเอนโดทีลิน-1 ต่อการมีชีวิตของเซลล์สร้างเส้นใยจากเนื้อเยื่อปริทันต์จากมนุษย์

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ABSTRACT

The objective of this study is to clarify the effect of endothelin-1 on viability of human periodontal ligament fibroblasts. Primary human periodontal ligament fibroblasts were seeded in 96-well plate and treated with 0.1, 1, 10, 100 nM ET-1 for 24 hours. MTT assay was performed to determine percentage of cell viability compared with untreated control group. One-way ANOVA followed by Games-howell post-hoc analysis was used to analyze the difference between groups. The result showed that ET-1 in every concentration significantly decreased cell viability in a dose-dependent manner ($P<0.05$). Further investigation is required to determine the non-toxic concentration of ET-1 for human periodontal ligament fibroblasts.

บทคัดย่อ

วัตถุประสงค์ของการศึกษานี้คือเพื่อศึกษาผลของเอนโดทีลิน-1 ต่อการมีชีวิตของเซลล์สร้างเส้นใยจากเนื้อเยื่อปริ-ทันต์จากมนุษย์ เซลล์สร้างเส้นใยจากเนื้อเยื่อปริทันต์จากมนุษย์ถูกเลี้ยงในเพลตเลี้ยงที่มี 96 หลุม และใส่เอนโดทีลิน-1 ที่มีความเข้มข้น 0.1, 1, 10, 100 นาโนโมลาร์ เป็นเวลา 24 ชั่วโมง โดยใช้การทดสอบเอ็มทีทีเพื่อหาเปอร์เซ็นของความมีชีวิตของเซลล์เทียบกับกลุ่มควบคุมที่ไม่ได้รับเอนโดทีลิน-1 และใช้สถิติ one-way ANOVA ร่วมกับการทดสอบภายหลัง Games-Howell เพื่อวิเคราะห์ความแตกต่างระหว่างกลุ่ม ผลการทดสอบพบว่า เอนโดทีลิน-1 ในทุกความเข้มข้น มีผลลดความมีชีวิตของเซลล์ลดลงตามความเข้มข้นที่เพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติ ($P<0.05$) จึงมีความจำเป็นต้องมีการศึกษาเพิ่มเติมเพื่อหาความเข้มข้นของเอนโดทีลิน-1 ที่ไม่เป็นพิษต่อเซลล์สร้างเส้นใยจากเนื้อเยื่อปริทันต์จากมนุษย์

Keywords: MTT assay, Endothelin-1, Human periodontal ligament fibroblasts
คำสำคัญ: การทดสอบเอ็มทีที เอนโดทีลิน-1 เซลล์สร้างเส้นใยจากเนื้อเยื่อปริทันต์จากมนุษย์

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Introduction

In orthodontic treatment, orthodontists apply force through different types of appliance to generate orthodontic tooth movement (OTM). Orthodontic forces induce extracellular matrix remodeling which cause periodontal ligament (PDL) fibroblasts to reorganize and transfer signals to different cell types, including osteoblasts and osteocytes resulting in osteoclast differentiation and bone remodeling (Krishnan, Davidovitch, 2009; Basdra, 1997). It has been studied that several different inflammatory mediators including cytokines and neuropeptides are involved in these processes (Krishnan, Davidovitch, 2009).

Endothelin-1 (ET-1), a potent vasoconstrictor peptide, is involved in OTM as reported by several studies (Sprogar et al., 2010; Sprogar et al., 2008; Sprogar et al., 2007; Drevensek et al., 2006; Sims et al., 2003; Sims, 2001; Yanagisawa et al., 1988). ET-1, the predominant member, is one of a family comprised of three closely related peptides, ET-1, ET-2, and ET-3 (Inoue et al., 1989). The effects of endothelin are expressed through the endothelin receptors, ET-A and ET-B receptors (Kawanabe, Nauli, 2011). It was reported that endothelin is expressed and causes diverse effects among various tissues, both physiological and pathological roles (Barton, Yanagisawa, 2008).

In 2001, Sims demonstrated that ET-1 immunoreactivity was upregulated in PDL in the loaded rat molars (Sims et al., 2003; Sims, 2001). Another study has also revealed that endothelial cells in PDL showed increased ET-1 in the loaded marmoset's molars (Sims et al., 2003). Drevenšek et al. (2006) first described the relationship between ET-1 and OTM by the experiment in rat (Drevensek et al., 2006). They found that the rate of tooth movement increased when treated with dual endothelin antagonist (Drevensek et al., 2006). Therefore, ET-1, through ET-A and ET-B receptors, may be involved in bone remodeling (Drevensek et al., 2006). Another study by Špela et al. (2007) found that the rate of tooth movement was decreased when treated with selective ET-A antagonist (Sprogar et al., 2007). These results implied that ET-1, through ET-A receptors, may be involved in bone remodeling by inducing bone resorption (Sprogar et al., 2007). However, gene expression and protein level of ET-1, ET-A, and ET-B varied throughout 42 days of orthodontic tooth movement in rat experiments (Sprogar et al., 2010; Sprogar et al., 2008). ET-1 and ET-3 gene expression was also observed to be involved in three phases of OTM in rats with a strong increased of ET-1 gene expression in late phase of OTM (Sprogar et al., 2010). These studies suggested that ET-1 may be involved in stimulating osteoclastic differentiation since osteoclast volume was decreased in ET-A antagonist group. Furthermore, it was revealed that ET-1 can stimulate the proliferation of cultured human osteoblastic cells (Kasperk et al., 1997). ET-1 increased osteoblast proliferation via Wnt signaling pathway in rat calvarial cell cultures (Von Schroeder et al., 2003). Apart from the involvement of ET-1 in osteoclast and osteoblast proliferation, it was found that ET-1 stimulated cell differentiation and proliferation in various cell types including PDL fibroblasts (Huetsch et al., 2018; Spinella et al., 2004a; Spinella et al., 2004b; Ricagna et al., 1996; Ruest et al., 2016a). Ruest et al. (2016) reported the significant increase of PDL cell viability by ET-1 at 100 nM (Ruest et al., 2016a).

However, only one study reported the effect of ET-1 on viability or proliferation of PDL cells. This study aims to investigate the effect of various concentration of ET-1 on viability of PDL cells.

Objectives of the study

To study the effect of endothelin-1 on cell viability of human periodontal fibroblasts

Methodology

Cell culture

Primary human periodontal fibroblasts cells (hPDL) were obtained from extracted premolar teeth which was extracted for orthodontic reasons. Those who had systemic diseases, sign of inflammatory periodontal tissue, and previous orthodontic treatment were excluded. The protocol (HREC-DCU 2020-108) was approved from the ethics committee of the Faculty of Dentistry, Chulalongkorn University. The healthy periodontium, non-carious teeth were obtained from four different subjects who aged between 12-24 years old. The hPDL cells were prepared from middle third of the root with a scalpel and placed in tissue culture dishes (60-mm dishes) with the Dulbecco's Modified Eagles Medium (DMEM) (Sigma-Aldrich St. Louis, MO, USA) comprising 10% Fetal bovine serum (FBS, Gibco, USA), 1% L-glutamine (Gibco, USA), and 1% Antibiotic-Antimycotic (Gibco, USA) under the humidified atmosphere with 37°C and 5% carbon dioxide. Confluent cells were trypsinized and subcultured. The third to the fifth passage were used in the experiments.

Cell cytotoxicity test

To investigate the viability of cells after treated with different concentrations of ET-1, MTT cytotoxicity test was performed in triplicate. The hPDL cells were seeded in 96-well plate at a density of 5,000 cells per well and were allowed to attach overnight. The cells were then washed and treated with ET-1 (0.1, 1 10 100 nM) in DMEM with 1% Fetal bovine serum (FBS, Gibco, USA) for 24 hours. After that, the solution was removed and MTT solution was then added in each well and incubated for 3 hours. Optical density was measured at 570 nm and the percent of cell viability comparing to control was calculated.

Statistical analysis

Each experiment was repeated at least 3 times. Using SPSS version 22, Kolmogorov-Smirnov test was performed to determine the normality of the data**.** One-way ANOVA followed by Games-Howell post-hoc analysis was performed to compare mean differences of percent cell viability. The level of statistical significance was set at $P < 0.05$

Result

In this study, it was found that percent of PDL cell viability decreased significantly when cells were treated with 0.1, 1, 10, 100 nM ET-1 (Figure 1, $p<0.05$). Besides, the percent of cell viability was also decreased significantly in a dose-dependent manner. The mean percent of cell viability of 0.1 nM,

1 nM and 10 nM groups are relatively similar, while the group of 100 nM group showed almost 50 percent decrease of cell viability.

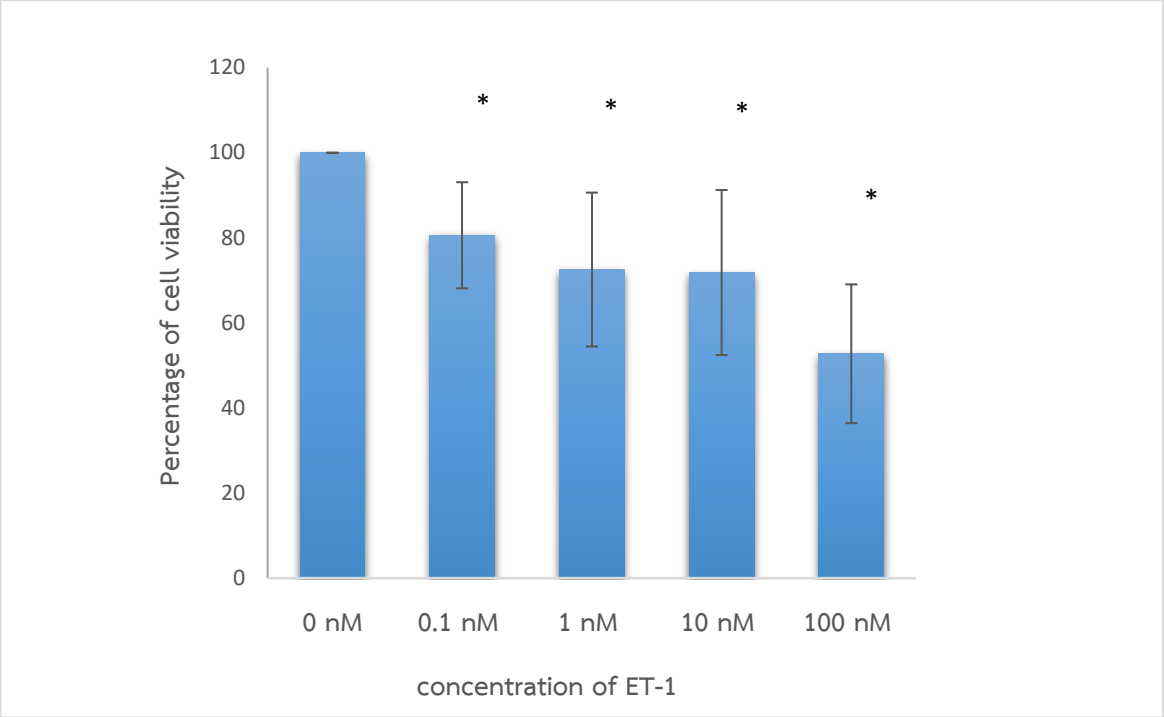

Figure 1 Effect of ET-1 on PDL cell viability. Human periodontal ligament fibroblasts were treated with 0.1, 1, 10, 100 nM ET-1 for 24 hours. MTT cytotoxicity test was used to determine cell viability. Percentage of cell viability of the untreated control group was calculated and presented as mean ± SD. One-way ANOVA followed by Games-Howell post-hoc analysis was performed to determine statistical difference between groups. * $P<0.05$ vs control

Table 1 Mean, standard deviation of percent of PDL cell viability

Concentration of ET-1 (nM)	control	0.1	1	10	100
Mean (Percentage of Cell Viability)	100.00	80.61	72.55	71.86	52.75
SD	0.00	12.47	18.09	19.40	16.32

Discussion and conclusion

The result in the present study demonstrate that ET-1 decreased the cell viability significantly from 0.1 nM to 100 nM, comparing with control. The cell viability was inhibited to almost 50% when treated with 100 nM of ET-1. The concentration of ET-1 used in this study was referred from the previous study of Liang et al. which aimed to investigate the effects of different concentrations of ET-1 on production of inflammatory cytokines from PDL cells (Liang et al., 2016; Liang et al., 2014). In those studies, the lowest concentration was 1 nM, while our study revealed that this concentration significantly decreased the viability of PDL cells at the same level as 10 nM of ET-1. Furthermore, our results also showed that 100 nM of ET-1 is toxic to the PDL cells. However, Liang et al. used the PDL cells obtained from patients with healthy periodontium and those with periodontitis with the age between 26-38 years old while we isolated the PDL cells from younger subjects aged 12-24 years old with healthy periodontium (Liang et al., 2016). In addition, Liang et al. did not report the effect of 1-100 nM of ET-1 on PDL cell viability. Another study from Ruest et al. showed the proliferative effects of 24 hour of ET-1 treatment at 20 and 100 nM in 6^{th}-10^{th} passage periodontal fibroblasts from adults (Ruest et al., 2016b). The MTT assay showed that ET-1 increased the proliferation significantly after 24 hours of 100 nM ET-1 treatment (Ruest et al., 2016b). However, our results contrast with the previous study since the ET-1 decreased cell viability in a dose-dependent manner which might be due to the different age groups of the subjects. Furthermore, the different passage of cell used might also affect the result, since the early passage might be more sensitive to the exogenous ET-1. Another study revealed that ET-1 at 10^{-6}, 10^{-7}, 10^{-8},$10^{-9}$and 10^{-10} M increased proliferation of fetal rat calvarial cells dose-dependently suggesting that different cell types may respond to ET-1 differently. (Von Schroeder et al., 2003). Even though our result is different from other studies, further investigation on the concentration lower than 0.1 nM will be conducted, in order to clarify the concentration which does not decrease the viability of PDL cells for further study on the effect of ET-1 on PDL gene expression.

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