TGF-β1 AS THE TRIGGER OF PULP FIBROBLAST PROLIFERATION

Sri Kunarti
Department of Conservative Dentistry
Airlangga University School of Dentistry

ABSTRACT

Mechanical pulp exposure by a rotary cutting instrument or a hand cutting instrument often occurs in deep caries. The application of a protective dressing can protect the pulp from additional injury by facilitating healing and repair. Pulp capping has been suggested as one treatment of choice after pulp exposure to maintain pulp vitality. TGFβ-1 is a growth factor that has important role in wound healing. The application of exogenous TGFβ-1 as direct pulp capping treatment to induce endogenous TGF-β1 is for reparative dentinogenesis. Samples were taken from orthodontic patients indicated for premolar extraction, between ages 10 – 20 years. The samples were divided into 2 treatment groups: group I was treated by TGFβ-1 and group II as a positive control was treated by Ca(OH)2. A class V cavity preparation was created in the buccal aspect 1 mm above gingival margin until pulp exposure. The sterile absorbable collagen membrane was used as inert carrier of 20ng/ml TGFβ-1 and was soaked with 5 ul. All groups were covered by a teflon pledge to separate pulp capping agent from Glass ionomer cement type II as a restoration. Teeth were extracted in 7, 14 and 21 day after treatment. All samples were examined histopathologically. Data analysis used one way Anova and student t test for parametric data. The exogenous TGFβ-1 increased stellate fibroblast (means TGF-β1 > Ca(OH)2). In conclusion, the exogenous TGF-β1 increased stellate fibroblast development.

Keywords: *stellate fibroblast, TGF-β1, calcium hydroxide, pulp capping*

Correspondence: Sri Kunarti, Department of Conservative Dentistry, Airlangga University School of Dentistry, Jl. Prof Dr Moestopo 47, Surabaya, phone 62-31-5030255, email: attybp@yahoo.com.

INTRODUCTION

Tertiary dentin is secreted by odontoblast as a response against injury that impacts primary and secondary dentin. The process of tertiary dentin secretion can be classified into reactionary or reparative one, depending on the severity of the injury, initial response, and condition during which the new dentin matrix is formed. In moderate injury, odontoblast remains viable and increases the secretion of reactionary type dentin secretion that strengthens the defense of pulp cells. Higher intensity injury may result in the death of local odontoblast (Nanci 2003). The severe the injury, the faster the formation of tertiary dentin, so that the cells are trapped within newly formed matrix and the distortion of tubular pattern ensues. The composition of tertiary dentin has not been disclosed, but so far it has been recognized that collagen production is reduced while the production of non-collagenous matrix protein is increased. This formative process is limited in injury area and designated as tertiary dentinogenesis (Bjorndal & Darvann 1997). The formation of tertiary dentin cannot be separated from the role of pulp fibroblast that plays a role as odontoblast-like cells. Young fibroblast cell is relatively big, having a long and round shape and blunt, sometimes branched, cytoplasmic projection. The nucleus is ovoid, having fine chromatin granule (open face type), basophilic color, and its star-resembling shape produces the name stellate fibroblast. Its cytoplasm extends and intercorrelates, forming a weave. Old fibroblast or fibrosis/fibrocyte has an ovoid dense chromatin type nucleus, as if it was naked, with acidophilic color. This non-active cell is surrounded by fibers which are produced by the cell itself. Fibroblast synthesizes two major macromolecules, the proteoglycan and glycoprotein. The vital characteristic of the surface cell and proteoglycan is its ability to bind growth factor, cytokine and other active biological molecules. The comparative material used in this study was Ca(OH)2 since it remains frequently used as the material for pulp capping.

MATERIALS AND METHODS

Samples involved in this study were the first premolar teeth (P1) planned to be extracted in orthodontic treatment. The condition of the teeth was vital, non-caries and intact, aged 10-20 years, positive cold-test with ethilchloride, percussion and pressure examination revealed no pain, and, clinically, the patient was healthy and the general condition was good. Total sample was 48, divided into TGF-ß1 and Ca(OH)2 groups, each of which was divided into three subgroups. The subgroup

1 was subjected to extraction 7 days post-treatment, subgroup 2 was subjected to extraction 14 days post-treatment, and subgroup 3 was extracted 21 days post-treatment. Initially, anesthesia was applied to buccal fold with 0.6 ml Xylestesin F. Class V preparation was undertaken slantwisely toward the apex using round bur no. 3 with a diameter of 1.5 mm until approaching the pulp, and then using round bur no. 1 with diameter of 0.5 mm to penetrate thin dentin layer until perforating the area of pulp chamber. The cavity was irrigated slowly with 0.5 ml saline solution and dried with sterile cotton pellet.

One mm2 absorbable collagen membrane that had been dripped with TGF-β1 was prepared in a concentration of 20 ng/ml as much as 5 ul. It was carried out in laminar flow hood, put into the eppendorf tube and kept within liquid nitrogen in a temperature higher than -80^{o}C. The membrane was applied on a perforation, covered with teflon pledged, and fixed with restorative material of type II glass ionomer cement and applied with the varnish.

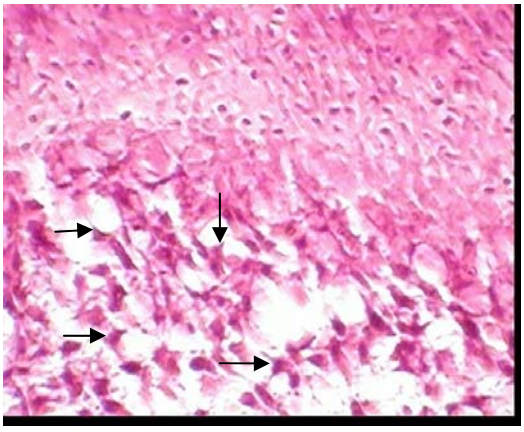

Figure 1. Stellate fibroblast.

Visible light cure Ca(OH)2 was prepared in a tube. The end of the tube has a diameter of 1 mm and the visible light device was also prepared. One mm Ca(OH)2 was put into the base of the cavity and beamed for 40 seconds with the distance with Ca(OH)2 as close as possible. It was subsequently covered with teflon pledged and fixed with restorative material, the type II glass ionomer cement, and applied with the varnish. The preparation to make histopathological preparation was commenced since the extraction of the teeth. Fixation was performed with 10% formalin buffer and 48 hours afterwards decalcification was carried out with solution containing AlCl, formic acid, 37% HCL, and distilled water. The subsequent process was dehydration in order to extract water from the tissue and replace it with hardening media (paraffin). Then, purification was performed using xylene. After paraffin got hardened, the tissue could be excised with microtome in 4 μm thickness and put onto object glass. The resulted preparation was stained with hematoxylin and eosin. Light microscope in a magnification of 400 x was used to count the stellate fibroblast (Figure 1).

RESULTS

Mean and standard deviation of each group in 7, 14, and 21 observation days can be seen in Table 1.

Table 1. Mean and standard deviation of stellate fibroblast in Ca(OH)2 and TGF-β1 groups in 7, 14, and 21 days post-treatment

Materials	7 days		14 days		21 days	
	Mean	SB	Mean	SB	Mean	SB
$Ca(OH)_2$	19.2500	5.2304	10.3750	3.7773	17.7500	3.3274
TGF-β1	21.0000	1.3093	21.0000	2.9761	20.3750	1.1877

The mean of stellate fibroblast in observation day 7, 14, and 21 post-treatment in TGF-β1 group was higher than that in Ca(OH)2 group, while mean reduction from 7, 14, and 21 days was found in both groups. Normal distribution test in Ca(OH)2 and TGF-β1 groups 7, 14, and 21 days post-treatment using Kolmogorov-Smirnov test revealed that all data had normal distribution ($p > 0.05$), fulfilling the requirement of parametric test. Homogeneity test using Levene test in Ca(OH)2 group revealed probability value of more than 0.05, indicating the presence of homogeneity. TGF-ß1 group revealed probability value of < 0.05, not fulfilling the homogeneity assumption, so that it required the application of Dunnet T3 test. The result of Anova between TGF-ß1 groups showing no significant difference ($p > 0.05$), while significant difference was found between Ca(OH)2 groups ($p < 0.05$). The result of Dunnet T3 test between different time of treatment can be seen in Table 2.

P value of Ca(OH)2 group had significant difference in the comparison between 7-14 days and 14-21 days. Whereas, the difference between TGF-β1 groups was not significantly different. The comparison of TGF-β1 and Ca(OH)2 in 7, 14, and 21 days post-treatment showed significant difference in 14 days post-treatment (0.000), while on day 7 ($p = 0.386$) and 21 ($p = 0.066$) post- treatment, the difference was not significant.

Table 2. Significance level of stellate fibroblast in Ca(OH)2 and TGF-β1 group between 7-14 days, 14-21 days, and 7-21 days.

Time groups	p value	
	$Ca(OH)_2$	TGF-β1
7 days - 14 days	0.000*	1.000
7 days - 21 days	0.482	0.689
14 days - 21 days	0.002*	0.924

Note: * = significant difference

DISCUSSION

The histological characteristics of young fibroblast are as follows: The cell is relatively big, having a long and round shape and blunt, sometimes branched, cytoplasmic projection, which is intercorrelating, forming a weave. The nucleus is ovoid, having fine chromatin granule, and its cytoplasm has basophilic color. Its star-resembling shape produces the name stellate fibroblast (Gunawan & Amindariati 2003). Young fibroblast is an indicator of the presence of fibroblast proliferating activity. The administration of TGF-β1 increases young fibroblast proliferation in 7 day observation to 21 day observation. Generally, TGF-β1 stimulates proliferation of mesenchymal cells derivates. According to Pimentel (1994), such stimulation is indirect and involves autocrine pathway. TGF-β1 stimulates DNA synthesis in fibroblast, depending on protein synthesis and secretion that correlate with PDGF. However, acccording to Wahl (1999), growth increase may also occur directly through signaling from mitosis pathway or indirectly as a result of co-factor induction, such as matrix molecules and other growth factors (such as PDGF).

In this study, comparison between Ca(OH)2 and TGF-β1 revealed significant difference on day 14 post-treatment. The mean of stellate fibroblast in TGF-β1 on 7, 14, and 21 days were higher as compared to the mean of Ca(OH)2 on 7, 14, and 21 days. Increasing formation of stellate fibroblast was observable starting from day 7 to day 21 in TGF-β1. This indicated that TGF-β1 provision increased fibroblast proliferation. The binding of growth factor with certain protein receptor had made the plasma membrane to commence the cascade of intracellular molecules and signal transduction that may affect the transcription and control of cell cycle. The first step of growth factors (GF) is to bind with transmembrane receptor at the surface of target cell. Intracellular receptor enhanced molecule production whose task is to become intracellular signaler that transmits stimulus to other molecules. GF activates intracellular phosphorilation cascade that may induce changes in two types of gene, 1) the expression of early-response genes induced in 15 minutes after GF administration and does not require protein synthesis, and 2) delayed response genes induced 1 hour after GF administration and requires protein synthesis. It is possible that delayed response genes are induced by the product of early-response genes. Several GFs are known to ge gene regulatory protein. Both genes are dormant in the phase G0, but their concentration increases if GF is added. If GF addition is ceased (in culture media), the genen concentration is gradually decreasing. Several genes even reaches zero point and several others stay at lower concentration and have a role in cell cycle (Lodish et al. 2000). In several types of cell (fibroblast, smooth muscle, vascular, articular condrocyte), TGF-β1 increases proliferation, retinoblastoma protein phosphorilation, and changes those proteins conformation, which results in the release of their E2F transcription factor as well as other transcription factors. The E2F is brought into the nucleus, resulting in increasing transcription of myc gene. Transcription factor bound with retinoblastoma in early gene also stimulates the transcription of c-jun, jun-B, and c-fos, resulting in increased fibroblast proliferation (Okragly et al. 1994).

CONCLUSION

In conclusion, the exogenous TGF-β1 is found to increase stellate fibroblast development, so that the administration of TGF-β1 enhances fibroblast proliferation.

REFERENCES

Alberts, B, Bray, D, Lewis, J, Raff, M, Roberts, K, Watson, JD 1994, 'Cell signaling' in *Molecular Biology of the Cell*, 3rd edn, Garland Publishing Inc., New York & London, pp. 721-765.

Bjorndal, L & Darvann, L 1999, 'A light microscopic study of odontoblastic and non-odontoblastic cells involved in tertiary dentinogenesis in well-defined cavitated carious lesions', *Caries Res*, vol. 33, pp. 50-60.

Gunawan, A & Amindariati, S 2003, *Histologi I*, 1st edn, Department of Anatomy and Histology, Airlangga University School of Medicine, Surabaya, pp. 4-6.

Lodish, H, Berk, A, Zipursky, SL, Matsudaira, P, Baltimore, D, Darnell, J 2000, 'Regulating of the eukaryotic cell cycle', in *Molecular Cell Biology*, 4th edn, WH Freeman and Company, pp. 496-527.

Nanci, A 2003, 'Repair and regeneration of oral tissue', in *Ten Cate's Oral Histology: Development, Structure and Function*, 6th edn, Mosby, St. Louis, pp. 397-415.

Okragly, A, Balwit, JM, Haak-Frendscho, M 1994, 'Transforming growth factor β-1 (TGF-β-1): A biological paradox', *Promega Notes Magazine*, vol. 47, pp. 10-17.

Pimentel, E 1994, *Handbook of Growth Factor vol. II: Peptide Growth Factor*, CRC Press, Boca Raton, pp. 271-292.

Wahl, SM 1999, 'Transforming Growth Factor-β- (TGF-β-) in the resolution and repair of inflammation', in JI Gallin, R Snyderman (eds), *Inflammation, Basic Principles and Clinical Correlates*, 3rd edn, Lippincott Williams & Wilkins, Philadelphia, pp. 837-842.