ORIGINAL RESEARCH PAPER

Volume-7 | Issue-7 | July-2018 | PRINT ISSN No 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

EFFECT OF ALPHA-TOCOFEROL AND SELENIUM ON PERIODONTAL LIGAMENT FIBROBLASTS

Periodontology

Hazra Madhurima Department of Periodontics, KLE V.K. institute of dental sciences, Belagavi

Walia Pooja* Department of Periodontics, KLE V.K. institute of dental sciences, Belagavi *Corresponding Author

Mulasavalgi Prabhanjan Department of Periodontics, KLE V.K. institute of dental sciences, Belagavi

ABSTRACT

Alpha-tocopherol is the subspecies of vitamin-E antioxidant vital for maintaining cell membrane integrity. Selenium is the cofactor of glutathione peroxidase which detoxifies hydrogen peroxide and other organic peroxides. Alfa-tocopherol and Selenium demonstrate accelerated wound healing. However, little is known about its effect on periodontal tissues. Hence the present study was an attempt to determine the effect of alpha-tocopherol, selenium and alpha tocopherol-selenium combination on periodontal ligament fibroblast cell proliferation and migration. Primary cultures of periodontal ligament fibroblasts cells were treated with the test group compounds alpha tocopherol, selenium and combination of alpha tocopherol and selenium. Migration of periodontal ligament fibroblasts cells was assessed by in vitro scratch test whereas proliferation was determined by the MTT assay. The results of the study indicated that of the three test groups (α-tocopherol, selenium and combination) used for detecting the periodontal ligament fibroblast cell migration and proliferation, combination of α-tocopherol, selenium demonstrated maximum effect.

KEYWORDS

Fibroblast(s), Periodontal Ligament(s), Antioxidant(s)

INTRODUCTION

Periodontitis is a disease of the periodontium characterized by irreversible loss of connective tissue attachment and supporting alveolar bone.[1] These changes often lead to an esthetically and functionally compromised dentition. For many years, periodontists have been interested in regenerating tissues destroyed by periodontitis. Periodontal regeneration can be defined as complete restoration of the lost tissues to their original architecture.[2]

Periodontal surgical approaches offer reliable methods to access root surfaces, reduce periodontal pockets, and improve periodontal architecture.[2] However, these techniques have a limited capability in recuperating tissues destroyed during disease process. The predictable and absolute regeneration of lost periodontium remains an indefinable goal inspite of the advances in surgical procedures and materials.[3]

The classical studies of Nyman, Lindhe, Karring and Gottlow state that only the periodontal ligament cells have the potential for regeneration of the attachment apparatus of the tooth.[4,5]

In the normal physiological process, cells use oxygen to produce energy and as metabolic byproducts reactive oxygen species are produced. These reactive oxygen species are essential for normal function and metabolism of the cell but they are also capable of damaging cell membrane and DNA.[6, 7, 8] There is a continuous balance between ROS and antioxidant defense. Excessive production of ROS has been associated with pathogenesis of many inflammatory diseases, including periodontitis.[6,7,8]

Alfa-tocopherol is one of the subspecies of vitamin E which accelerates gingival wound healing[9,10] and is considered as the most effective lipid soluble antioxidant.[9] Selenium is the essential cofactor of glutathione peroxidase which detoxifies hydrogen peroxide and other organic peroxides.[11] It is effective against oxidative stress, but, when combined with vitamin E, it has a synergistic effect.[12] It was also demonstrated on a rat model that the combination of vitamin E and selenium was protective against ROS-induced collagen breakdown.[11]

Besides the antioxidant properties of alfa-tocoferol and selenium, Vitamin E and the combination of vitamin E and selenium may affect the function of periodontal ligament fibroblast and increase the proliferation and migration, thereby accelerating periodontal wound healing and regeneration.

Therefore, the aim of present study was to evaluate effect of alfa-tocoferol, selenium and their combination of alfa tocopherol – selenium on periodontal ligament fibroblast cell proliferation, migration and differentiation.

MATERIALS AND METHODS

SOURCE OF DATA

All the experimental procedures were approved by the Research and Ethical Committee of KLE University's V K Institute of Dental Sciences, Belagavi and signed informed consent was obtained from all the participants.

The study was conducted in the Department of Periodontics, KLE'S V K Institute of Dental Sciences, Belagavi. The laboratory procedures were undertaken at KLE's Dr. Prabhakar Kore Basic Science Research Center (BSRC), Belagavi.

Primary cultures of periodontal ligament fibroblast cells were prepared from 10 extracted premolar teeth. Tissue samples were taken from systemically healthy male volunteers who were aged 20 to 31 years (mean age: 24 years). The study was carried out on periodontal ligament fibroblast cells from third to fifth passages. A total of 120 wells seeded with periodontal ligament fibroblasts were equally divided to study the cell functions. The experiment included 40 wells each, which were further divided into four groups.

Group 1: PDL fibroblast without any treatment (as control)
Group 2: PDL fibroblasts treated with 60μM α-tocopherol
Group 3: PDL fibroblast treated with 50μM selenium
Group 4: PDL fibroblasts treated with 60μM α-tocopherol and 50μM selenium

METHOD OF COLLECTION OF DATA

I. Periodontal ligament isolation and Cell Culture.

Periodontal ligament fibroblast cells were obtained from the premolars extracted for orthodontic reasons. The extraction was carried out as atraumatically as possible. The transport media used was 5ml of Phosphate buffer saline (PBS) to which 10μl of Penicillin and Streptomycin was added. Extracted teeth were then sent to Dr. Prabhakar Kore Basic Science Research Center for further procedures. Under the vertical laminar air flow, teeth were and transferred to a sterile petri dish. 2ml of Dulbecco's modified Eagles media (DMEM) was added, following which the tissue over the mid root surface of the teeth were scraped with a BP blade no. 11 and macerated into smaller portions.

The obtained tissue was then seeded with a micropipette into a 24 well plate to which Dulbecco's Mod. Eagle's medium (DMEM)

supplemented with Penicillin G (l00U/ml), Streptomycin (100ug/ml), Gentamycin (100ug/ml) and 10% fetal bovine serum (FBS) was added. This plate was then placed in the CO2 incubator at 37°C in a humidified atmosphere containing 5% CO2 and allowed to incubate. The cell count was determined using Neubauer's counting chamber. Dilutions were prepared to get the required cell density. Cells were then seeded in 96 well plates and were allowed to attach to the plate overnight. These periodontal ligament fibroblast cells were then subjected test compounds to perform the migration and proliferation assays.[13]

I.CELL MIGRATION

In vitro scratch test was performed to detect the migration of the periodontal ligament fibroblast.

Principle

This in vitro test is based on the observation that, by creating a new artificial gap, so called "scratch", on a confluent cell monolayer, the cells on the edge of the newly created gap will move toward the opening to close the "scratch" till new cell to cell contacts are formed again.[14]

Procedure

The cells were seeded in 96 wells microtiter plate. Using a sterile micropipette tip, a scratch was made in each well. Only DMEM medium was added in the control wells. Separate wells were treated with test compounds. The scratch was then observed under microscope.The cell culture plate was incubated in 5% CO2, at 37℃. Pictures of the same where taken after 24, 48 and 72 hours. The distance between edges of scratch was measured and the values where calculated.

CELL PROLIFERATION

Proliferation of the periodontal ligament fibroblast cells was determined by MTT assay.

MTT assay is a colorimetric assay which determines cell proliferation. The reduction of yellow 3-(4,5-dimethythiazol- 2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase is evaluated in this assay. MTT that enters the cells is taken up by the mitochondria and is reduced to an insoluble, colored (dark purple) formazan product. The amount of color produced is directly proportional to the number of viable cells.[13]

Procedure

The cells were treated with different reagents, diluted with DMEM media and the control group was added with only DMEM media. The plate was then kept for incubation for 5 days in C02 incubator at 37°C in a humidified atmosphere containing 5%CO2. 20 µl of 5mg/ml MTT reagent was added to the wells after 5 days and the plate was kept for 4hrs incubation in dark place at room temperature. 200 µl of DMSO was added to dissolve the formazan crystals formed. The optical density was measured using spectrophotometer at a wavelength of 570 nm. The proliferation effect of α- tocopherol and selenium was assessed by spectrophotometric determination of color change.

Fig 1. In Vitro Scratch Test (Cell Migration)

CASE STUDY

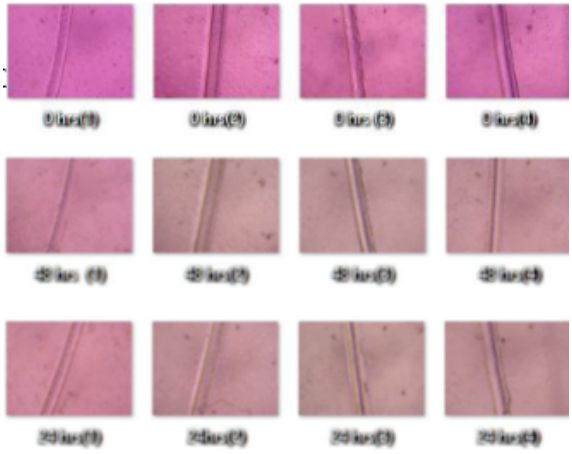
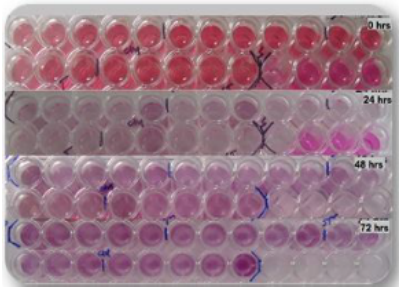


Fig 2. Mtt Assay (Cell Proliferation)



STATISTICALANALYSIS

The mean and the standard deviation of the wound closure (%) in the in vitro scratch test and of the optical densities in the MTT assay of the control and three test groups were calculated. Comparison between the groups was done by One way Analysis Of Variance (ANOVA) to determine if there was any significant difference between the groups.

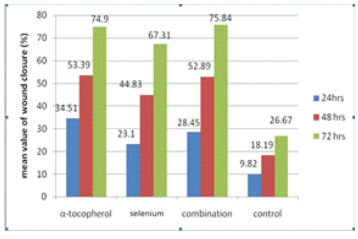
RESULTS

A statistically significant difference was observed in the migration rate (percentage of wound closure) between control and other test groups.

However no statistically significant difference was observed between test groups. Thus, all the three test group significantly increased periodontal ligament fibroblast migration.

A statistically significant difference was seen for mean values of proliferation rate (optic density) with group3 at 48 hours when it was compared with group 1 and thus group3 had showed maximum proliferation in comparison to other groups at 48 hours. However at 24 and 72 hours there was no statistically significant difference of test groups with the control.

Graph 1: Comparison of group 1, group 2, group 3 and group 4 at 24, 48 and 72 hours with respect to migration



Graph 2: Comparison of group1, group2, group3 and group4 at 24, 48 and 72 hours with respect to Optical density of MTT Assay.

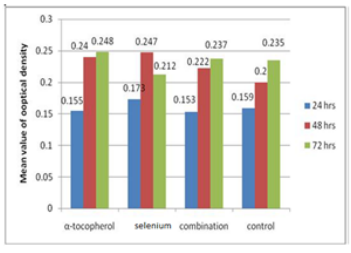


Table 1: Comparison of four groups with respect to wound closure (%) of Scratch test by one way ANOVA at 24, 48 and 72 hours (Migration)

Source of variation	Sum of Squares	Degrees of freedom	Mean Square	F - value	P - value	
24hrs	Between Groups	3321.706	3	1107.235	32.824	<.0001
	Within Groups	1214.356	36	33.732		
	Total	4536.062	39			
48hrs	Between Groups	8229.142	3	2743.047	60.601	<.0001

	Within Groups	1629.509	36	45.264		
	Total	1629.509	39			
72hrs	Between Groups	16313.523	3	5437.841	292.162	<.0001
	Within Groups	670.047	36	18.612		
	Total	16983.570	39			

Table 2: Comparison of four groups with respect to optical density of MTT assay by one way ANOVA at 24, 48 and 72 hours (Proliferation)

Source of variation		Sum of Squares	Degree of freedom	Mean Square	F value	Sig.
24 hrs	Between Groups	3321.71	3.00	1107.24	32.82	<.001
	Within Groups	1214.36	36.00	33.73		
	Total	4536.06	39.00			
48 hrs	Between Groups	8229.14	3.00	2743.05	60.60	<.001
	Within Groups	1629.51	36.00	45.26		
	Total	9858.65	39.00			
72 hrs	Between Groups	16313.52		5437.84	292.16	<.001
	Within Groups	670.05	36.00	18.61		
	Total	16983.57	39.00			

DISCUSSION

The regeneration of the lost periodontal tissue as a consequence of periodontal disease still remains a difficult goal to achieve. It states that for a periodontal therapy to be successful it is necessary to re-establish periodontal attachment apparatus which includes new bone formation, new cementum deposition upon the denuded root surfaces, and insertion of functionally oriented new collagen fiber of the periodontal ligament into the new bone and new cementum[15].

Regeneration of PDL tissues is a complex process. It involves a sequence of properly organized cell to cell interactions between gingival fibroblasts, epithelial cells, periodontal ligament fibroblasts and osteoblasts[16]. The fundamental cellular mechanisms of periodontal regeneration are proliferation and migration of periodontal ligament (PDL) cells and differentiation of cementoblasts and osteoblasts[17].

Alfa-tocopherol is one of the subspecies of vitamin-E, which is vital for maintaining cell membrane integrity[11]. It was previously reported that Alfa-tocopherol has biological effects on human cells besides the antioxidative characteristics. Even though alfa-tocopherol demonstrated accelerated gingival wound healing and has protective effect against alveolar bone loss in vivo, little is known of its effect on periodontal tissues.

Selenium is the essential cofactor of glutathione peroxidase, which detoxifies hydrogen peroxide and other organic peroxides. It is effective against oxidative stress, but, when combined with vitamin E, it has a synergistic effect.[12]

Fibroblasts were evaluated because they are the predominant cells of the periodontal ligament constituting approximately 60% of the PDL cell population. The PDL fibroblast plays a significant role in normal turnover, repair, and regeneration, development, structure and function of the tooth support apparatus[18]

The two important cell functions that were assessed in this study were periodontal ligament fibroblast cell migration and proliferation, which are prerequisite for periodontal regeneration.

Cell migrationis the orchestrated movement of cells in particular directions to specific locations. It is a central process in tissue formation during embryonic development and wound healing[19].

Based on the results of in vitro scratch test, the mean percentage of wound closure of control, (group1), α-tocopherol (group2), selenium (group 3) and combination (group 4) of alfa-tocopherol and selenium, there was a statistically significant difference when the test groups were compared with control (p=0.001*). However there was no statistically difference between the groups when they were compared with each other (Table 2 and Graph 1). Thus the present study showed that there is increase in periodontal ligament fibroblast migration in group4 as compared to the other groups.

The results of the present study are in accordance with the study carried out by Nejat Nizam et al and Feridun Discioglu et al (2013) where alfa tocopherol and alfa-tocopherol and selenium combination was able to accelerate the wound healing[11]. Studies conducted by Shepherd et al (2003), Arnold et al and Barbul et al (2006), Campos et al (2008) stated that vitamin E, magnesium, copper and iron play a significant role in wound healing. [20,21,22]

Cell Proliferation is defined as increase in the number of cells as a result of cell growth and cell division[19].

Yellow MTT (3-(4, 5-Dimethylthiazol-2-yl)-2, 5- diphenyl tetrazolium bromide), is reduced to purple formazan in the mitochondria of living cells. Active mitochondrial reductase enzymes are responsible for the reduction of MTT and thus measures number of viable (living) cells. With an increase in the number of cells there is increase in the amount of purple formazan formed which results in increased measure of absorbance [13].

In the present study the mean optical density was studied in group 1, group 2, group 3 and groups 4. A statistically significant difference was seen with selenium at 48 hours when it was compared with control and thus selenium had showed maximum proliferation in comparison to other groups at 48 hours. However at 24 and 72 hours there was no statistically significant difference of test groups with the control. This shows that proliferation was time dependent with selenium as compared to control and test groups. No difference in the proliferation at 24 and 72 hours was observed (Graph 2). Thus from this study it can be concluded that fibroblast treated with selenium had highest proliferation rates at 48 hours.

Proliferation percentage at 24 hours for group 2 was 100%, group 3 was 106 % and for group 3 it was 106 % over the control group. At 48 hours group2 was 120%, group3 was 125% and for group 4 it was 110 % over the group1 and at 72 hours group 2 104%, group3 was 87.5% and for group4 it was 100 % over the group1. Thus it can be suggested that there was 125 % increase in the proliferation at 48 hours with group3 in relation to control which was highest among all the three test groups.

Hence, it was found that

1. The effect of alfa-tocopherol on periodontal ligament fibroblast at 24 and 48 hours showed maximum migration and at 72 hours maximum proliferation.
2. The effect of selenium on periodontal ligament fibroblast at 48 and 72 hours showed maximum proliferation.
3. The additive effect of alfa-tocopherol and selenium showed maximum migration at 72 hours.

The proliferation produced by combination of alfa-tocopherol and selenium was comparable to that produced by alfa-tocopherol. Hence, it can be concluded that the combination of alfa tocopherol and selenium has maximum effect compared to alfa tocopherol and selenium individually.

BIBLIOGRAPHY

1. Carranza, F., Newman, M., & Takei, H., 2006. *Carranza's Clinical Periodontology*. 10th ed. Philadelphia: St. Louis, Mo. Elsevier Saunders
2. Pandula P., Samaranayake LP., Jin LJ., Zhang C., Wang DK., Varanasi S., Bronckers AL., (2005). Position Paper: Periodontal Regeneration. Journal of Periodontology. 76 (9): 1601-1622.
3. Garrett, S. (1996). Periodontal regeneration around natural teeth. Annals of Periodontology. (1): 621-666.
4. Nyman, S., Gottlow, J., Karring, T., & Lindhe, J. (1982). The regenerative potential of the periodontal ligament. J Clin Periodontol. 9: 257-265.
5. Gottlow, J., Nyman, S., Karring, T., & Lindhe. (1984). New attachment formation as the result of controlled tissue regeneration. J Clin Periodontal. 11: 494-503.
6. Chapple, I.L., Matthews, J.B. (2007). The role of reactive oxygen and antioxidant species in periodontal tissue destruction. Periodontol 2000. 43: 160-232.
7. Halliwell, B., Gutteridge, JMC., & Cross, CE. (1992). Free radicals, antioxidants and human disease: Where are we now? J Lab Clin Med. 119: 598-620.
8. Woods, J.R. (2001). Reactive oxygen species and preterm premature rupture of membranes - A review. Placenta. 22: 38-44.
9. Young, IS., Woodside, JV. (2001). Antioxidants in health and disease. J Clin Pathol. 54: 176-186.
10. Kim, J.E., Shklar, G. (1983). The effect of vitamin E on the healing of gingival 2 in rats. J Periodontol. 54: 305–309.
11. Nizam, N., Discioglu, F. (2014). The Effect of a-Tocopherol and Selenium on Human Gingival Fibroblasts and Periodontal Ligament Fibroblasts In Vitro. J Periodontol. 85:

636-644.
12. Bartfay, W.J., Hou, D., Brittenham, G.M. (1998). The synergistic effects of vitamin E and selenium in iron-overloaded mouse hearts. Can J Cardiol. 14: 937-941.
13. Doyle, A., & Griffiths, J.B. Cell and tissue culture for medical research. 2000 (p.409). Wiley
14. Liang, CC., Park AY., & Guan JL. (2007). In vitro starch assay: a convenient and inexpensive method for analysis of cell migration in vitro. Nature Protocols. 2(2): 329-333.
15. Isaka, J., Ohazama, A., Kobayashi, M., Nagashima, C., Takiguchi, T., Kawasaki, H., & Hasegawa, K. (2001). Participation of periodontal ligament cells with regeneration of alveolar bone. J Periodontol. 72(3): 314-323.
16. El-Sharkawy, H., Kantarci, A., Deady, J., Hasturk, H., Liu, H., Alshahat, M., & Van Dyke TE. (2007). Platelet-rich plasma: growth factors and pro and anti-inflammatory properties. J Periodontol; 78(4): 661-669.
17. Christgau, M., Moder, D., Hiller, KA., Schmitz, G., & Schmalz G. (2006). Growth factors and cytokines in autologous platelet concentrate and their correlation to periodontal regeneration outcomes. J Periodontol. 33(11): 837-845.
18. Blom, S., Holmstrup, P., & Dabelsteen, E. (1992). The effect of insulin-like growth factor-I and human growth hormone on periodontal ligament fibroblast morphology, growth pattern, DNA synthesis, and receptor binding. J Periodontol. 60(6): 293-301.
19. NCI Dictionary of Cancer Terms. http://www.cancer.gov/dictionary
20. Shepherd, A.A. (2003). Nutrition for optimum wound healing. Nurs Stand. 18: 55-58.
21. Arnold, M., & Barbul, A. (2006). Nutrition and wound healing. Plast Reconstr Surg: 117 (7 Suppl): 42S-58S.
22. Campos, A.C., Groth, A.K., & Branco, A.B. (2008). Assessment and nutritional aspects of wound healing. Curr Opin Clin Nutr Metab Care, 11: 281-288.