The Study of Dose- Response Mitogenic Effect of L-dopa on the Human Periodontal Ligament Fibroblasts Cells

M. Zarabian[1], F. Salehipour[1], SN. Ostad[2]

[1] Assistant Professor, Department of Endodontics, Faculty of Dentistry, Tehran University of Medical Sciences, Tehran, Iran
[2] Associate Professor, Department of Toxicology, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

Statement of Problem: Avulsion is one of the most serious emergencies in dental office. Avulsed teeth should be stored in a medium that supports the periodontal ligament cells viability. In some clinical situations, preserving media, contained growth factors and mitogenic products may be used for repair of traumatized (Periodontal Ligament) tissues. It has been previously reported that levodopa (L-dopa) accelerates healing by increasing the growth hormone level.
Purpose: In this study, the local effect of L-dopa, on human periodontal ligament fibroblast (HPLF) cells was evaluated.
Materials and Methods: Samples were taken from impacted or semi-impacted third molar teeth, which didn't show inflammatory reaction. The cells obtained from periodontal ligament of these teeth, were cultured in appropriate medium. The passage number between 3-6 was taken for further experiments. The viability of HPLF cells, which treated, by L-dopa were evaluated by trypan blue dye exclusion and neutral red assay.
Results: Results indicate that low concentration of L-dopa produces significant increase in number of these cells in comparison with control group. These results confirmed previous study about direct action of L-dopa on the viability of HPLF cells.
Conclusion: On the basis of this study and previous reports, presence of L-dopa in preserving media may be useful in increasing the self-life transferring HPLF cells.

Keywords: Fibroblast; Periodontal ligament; L-dopa; Viability; Avulsion

Journal of Dentistry, Tehran University of Medical Sciences, Tehran, Iran (2004; Vol. 1, No. 3)

Tooth avulsion is caused mainly in contact sports and car accidents. It Consist 1-16% of traumatic injuries to permanent teeth and the age of the majority of patients are in the range of 7-10 years old.(1,2) Tooth avulsion is an emergency in dentistry. Immediate replantation is the treatment of choice but is not possible in all cases. When immediate replantation is not possible, the tooth should be stored in a medium that maintain periodontal ligament cell viability until definitive dental treatment can be accomplished.(3)

In avulsion, the tooth socket is filled by a blood clot.(4) After trauma, dental pulp and periodontal ligament undergo ischemia. However, bacteria, chemical irritants and dry condition can aggravate this situation and may destroy pulpal and periodontal cells even in a short extra-oral time.(4)

Theoretically, mitotic promoting agents, such as growth factors, may increase the periodontal ligament cell density.(5) These agents are not

commonly used in dentistry today because of their side effects. In systemic usage, therefore, agents with local action may have positive effects in decreasing of the dose and duration of treatment during replantation.

L-dopa is precursor of catecholamines. There was an increase in plasma level of growth hormone in patients who treated with L-dopa for their Parkinson's disease.[5]

In body, L-dopa changes to dopamine, which could stimulate the anterior part of hypophsis to secret growth hormones.[6] In dogs treated with L-dopa callus formation took place in mandibular defects but this was not found in the control group.[7] Pritchett (1990) pointed to managing fractured long bones, which was not responded to regular therapies with L-dopa usage.[6] It has been reported that L-dopa affect endochondral bone and intramembranous bony repair.[7] Partovi et al. (2001) has been reported the effect of L-dopa on the HPLF cells of two samples.[8]

In this study the effect of L-dopa on the HPLF cells has been evaluated in the more samples. Furthermore the different doses of L-dopa have been examined in order to find the optimum mitogenic dose on HPLF cells.

Materials and Methods

Human periodontal ligament tissue was obtained from the freshly extracted, human unerupted third molars of patients of the Oral and Maxillofacial Department of the Dental School (Tehran University of Medical Sciences) with informed patient consent. Teeth with two thirds of the whole root-length formation, which needed no additional sectioning for extraction, were chosen as samples. Before tooth extraction, the patients were given a mouth rinse of iodine solution (Povidone iodine 10%, Tolidaru Co., Tehran, Iran). The atraumatically extracted teeth were then immersed in Dulbecco's modified essential medium (Gibco, UK) solution that was supplemented with ml of 10% fetal calf serum, 5 ml of penicillin/ streptomycin (1X), and 1200 µl of L-glutamine. The samples were immediately immersed in preserving medium and transferred to cell culture lab in the Faculty of Pharmacy University of Tehran Medical Sciences. (The periodontal ligament tissue from the middle third of the root surface was scraped with a sharp # 15 scalpel). The samples were then washed three times with phosphate buffer saline (PBS) under sterile condition. The extracted tissue was immersed in 25-cm^2 culture dishes (Nunc, Roskilde, Denmark) that contained 4 ml of culture medium. The samples were then incubated at 36.5^{o}C, 5% CO_2 for a mean of 10 days to observe the HPLF cells (passage 0). After confluence (1 week), the cells were passaged by using 0.25% trypsin EDTA (Gibco, UK). Passages numbers 3 to 6 were used in this study. A total of 1.5×10^4 cells were cultured in 24 well dishes (Nunc). After 6 hours, L-dopa with different concentrations (0.25, 0.5, 0.75, 1, 2.5 and 5 µg/µl) (3, 4-dihydroxyphenylalanine, Sigma) was added to each well. Twenty microliters of 10% $NaHCO_3$ was as negative control. The mitogenic effect of L-dopa was evaluated after 100 hrs of cell incubation in 36.5^{o}C and 5% CO_2 by using the trypan blue and neutral red (NR) assays.

Trypan Blue Dye Exclusion Test

20 µl of cell suspension was added to 20 µl of trypan blue (0.4% w/v), and the cells that were not stained by the dye were counted.

The number of cells per milliliter was equal to the average of counted cells multiplied by 2×10^4.

Determination of Cell Viability Using the Neutral Red Uptake

The medium was removed and the wells were washed twice with PBS. One hundred microliter of NR was added to each well, and the wells were incubated at 36.5^{o}C, 5% CO_2 for 90 minutes. The solution was then aspirated and washed with PBS, then 300 µl of neutral red

solubilizer [Absolute ethanol; 0.1 M Citrate Buffer, pH 4.2 (21.01 g citric acid + 200ml of 1 M NaOH per liter [A]; 60 ml of A +40 ml 0.1 M HCl mixed 1:1 v/v] was added to each well, and the well were left at room temperature for 20 minutes. Samples were taken from each well and placed in 96 ELISA well dishes (Nunc, Denmark). The results were obtained by placing 96 well dishes into ELISA plate reader (Stat-Fax 303 plus, Awareness Technology Inc, Palm City, FL) at 570 nm against 690 nm for the blank solution.
The results were analyzed by one-way ANOVA and Tukey HSD post Hoc test.

Results

In different doses of L-dopa used for treating of the HPLF cells (0.25, 0.5, 0.75, 1, 2.5 and 5 µg/ml) the highest dose (2.5 µg/ml) showed remarkable effect on the number of the HPLF cells when it compared with the control. The effect of this dose (2.5 µg/ml) was statistically significant (P<0.03).
The effect of L-dopa on the HPLF cell viability was assessed by the NR assay. The highest dose (2.5µg/ml) had statistically significant positive effect on viability of HPLF cells. The ratio of the effect of L-dopa on the HPLF cells versus the NR assay shows that the mitogenic effect of L-dopa on the HPLF cells is dose dependent. The concentrations of the drug used in this study had no effect on the viability of the HPLF cells (Fig 1). The highest mitogenic dose with the lowest toxicity on HPLF cells was observed at dose of 2.5µg (Fig 2).

Discussion

The primary aim of treating the avulsed teeth is preservation the vitality of periodontal ligament elements (mostly consists of fibroblasts).[9]
Another treatment modality is increasing the number of the viable periodontal ligament cells by using growth factor. These factors mostly are expensive and finding of cheaper agents with similar effects may be very beneficial and helpful. It has been shown that it took 4 days for fibroblasts which derived from the gingival to become confluent and 6 days for fibroblasts derived from the PDL.
However, most of tissues obtained from patient did not grow properly (6 from 44 tissues recovered). Time of keeping in preserving medium, age, cell cycle condition and the number of cells obtained from tissue can be considered the reason of this diversity.

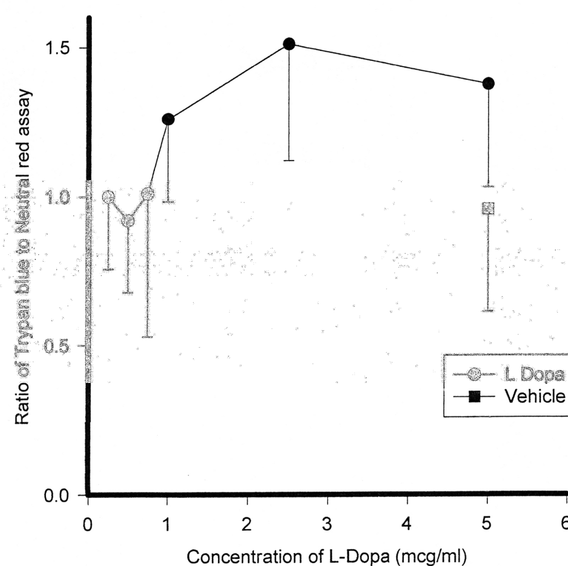

Fig 1- The effect of different concentrations of L-dopa with the number of HPLF cells by using try pan blue methods (Mean±SD, n=6)

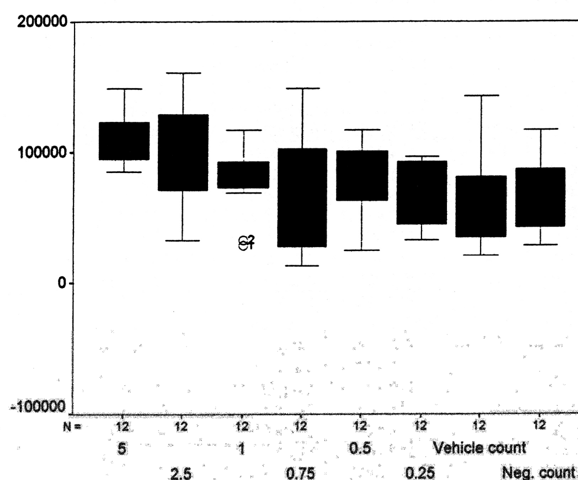

Fig 2- Boxplot of the ratio of direct and indirect measurement of viability (±SD, n=6)

In present study, the HPLF cells which recovered in vitro reached confluence in T-25 flasks after 1 week.
The function of the fibroblasts differs according to age, trauma, and inflammation. Therefore, in this study, young patients with healthy unerupted, third-molar were chosen. Pervious experiment showed that the passage number 3 to 6 produced the best cells for performing the experiment.(10) In this experiment the passage number six was used. Trypan blue exclusion simply reflects the integrity of the cell membrane but gives no indication of the heal thickness of cells that are able to exclude the dye. The neutral red (NR) assay is based on the incorporation of NR into lysosomes and Golgi bodies of the viable cells after incubation with the testing agents. In damaged or dead cells, NR is no longer retained in the cytoplasmic vacuoles, and the plasma membrane does not act as a barrier to retain the NR within cells. Our results confirmed the result of Partovi et al (8) indicating non-toxicity of L-dopa (in the wide range of doses administration) for the HPLF cells. Moreover, Partovi et al(8) showed that in the low dose of L-dopa, the number of cells increases significantly. Despite of this finding, the minimum dose, which shows this effect, was not reported. As it is shown in figure 2 the viability ration results are indicating that L-dopa causes an increase in density of HPLF cells in a dose dependent manner in the range of 1-5 µg/ml.
These results confirmed previous study about direct action of L-dopa on the viability of HPLF cells. On the basis of this study and previous reports, presence of L-dopa in preserving media may be useful to increase the self-life transferring HPLF cells.
Although the most appropriate dose of L-dopa has been determined in this in-vitro study, the in-vivo use of this agent needs to be confirmed by other studies.

References:

1- Cohen S, Burns R. Pathway of the Pulp. 7th ed. St. Louis; Mosby: 1998.

2- Ingle JI, Bakland LK. Endodontics. 4th ed. London: William & Wilkins; 1994.

3- Seltzer S. Endodontology, Biologic Considerations in Endodontic Procedure. 2nd ed. Philadelphia; Lea & Febiger: 1988.

4- Andereasen JO, Andereasen FM, Bakland LK, Flores MT. Traumatic Dental Injuries, a manual. 1st ed. Copenhagen; Munksgaard: 1999.

5- Boyd AE, Lebortiz HE, Pfeiffer JB. Stimulation of human growth hormone secretion by L-dopa. N Engl J Med 1970; 283: 1425-9.

6- Pritchett JW. L-dopa in the treatment of nonunited fractures. Clin Orthop 1990; 255: 293-300.

7- Jones GT, Jian XC, Laskin DM. The effect of L-dopa on the healing of a rat mandibular defect. J Oral. Maxillofac Surg 1996; 54: 470-73.

8- Partovi M, Sadeghein A, Azizi E, Ostad SN. Mitogenic effect of L-dopa on human periodontal ligament fibroblast cells. J Endod 2002; 28(3): 193-96.

9-Trope M, Friedmans A. Periodontal healing of replanted dog teeth stored in viaspan. Endod Dent Treumatol 1992; 8(5): 183-88.

10- Lee A, Baram P. In vitro culture of PDC. J Dent Res 1972; 953-59.