

THE STUDY OF DOSE-RESPONSE MITOGENIC EFFECT OF L-DOPA ON THE HUMAN PERIODONTAL LIGAMENT FIBROBLAST CELLS

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Abstract- Avulsion is one of the most serious emergencies in dental office. In the event of any problem, the tooth should be stored in a medium that supports the periodontal ligament cell viability. In other clinical situations, preserving media, growth factors and mitogenic products may be useful in repairing the traumatized tissues. It has been previously reported that levodopa (L-dopa) accelerates healing by increasing the growth hormone level. In this study, the local effect of L-dopa, as a mitogen, on human periodontal ligament fibroblast (HPLF) cells was evaluated. Samples from impacted or semi-impacted wisdom or canine teeth, which were devoid of inflammation, were taken. The cells obtained from this tissue were cultured in an appropriate medium. The passage numbers between 3-6 were taken for further experiments. The viability of HPLF cells, which were treated by L-dopa, was evaluated by trypan blue dye exclusion and neutral red assay. Results indicated that low concentration of L-dopa produces significant increase of these cells compared to control group. These results confirmed previous studies 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 in increasing the self-life transferring HPLF cells.

Acta Medica Iranica, 42(5): 363-366; 2004

Key words: Fibroblast, periodontal ligament, L-dopa, viability, avulsion

INTRODUCTION

Tooth avulsion occurs primarily in contact sports and car accidents. It consists 1-16% of traumatic injuries to permanent teeth and the majority of patients are in the age range of 7-10 years (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 maintains periodontal ligament cell viability until definitive dental treatment can be accomplished (3).

In avulsion, the tooth socket is filled by a coagulum (4). Biologically, immediately after trauma dental pulp and periodontal ligament are exposed to ischemia. Bacteria, chemical irritants and dry conditions can aggravate the situation and may destroy pulpal and periodontal cells even in a short extra-oral time (3).

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 side effects of systemic use. Therefore, agents with local action may have a

Received: 16 Jun. 2004, Revised: ---, Accepted: 21 Aug. 2004

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beneficial effect to decrease the dose and duration of treatment during replantation.

Levodopa (L-dopa) is a precursor of central nervous system catecholamines. In body, L-dopa changes to dopamine, which could stimulate the anterior part of hypophysis to secrete growth hormone (6). Boyd has reported an increase in plasma level of growth hormone in patients suffering from Parkinson's disease who were treated with L-dopa (5). In a study by Waisman *et al.*, callus formation took place in dogs treated with L-dopa whereas in the control group such callus formation was not observed (7). Pritchett pointed to use of L-dopa for managing fractured long bones with delayed or nonunion healing (6). It has been reported that L-dopa has an effect on endochondral bone repair and intramembranous bony repair (8). Partovi *et al.* reported the effect of L-dopa on the HPLF cells of two samples (9).

In this study mitogenic effect of L-dopa has been evaluated. Furthermore, the different doses of L-dopa have been compared in order to find the optimum mitogenic dose.

MATERIALS AND METHODS

Human periodontal ligament tissue was obtained from the freshly extracted, healthy human unerupted third molars of patients who were referred to the oral and maxillofacial department of the dental school (Tehran University of Medical Sciences) after obtaining an informed 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 solution (Gibco, UK) that was supplemented with 10% fetal calf serum, 5 ml of penicillin/streptomycin (IX), 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, Tehran University of Medical Sciences.

The samples were then washed three times with phosphate buffer saline (PBS) under sterile condition.

To avoid contamination from the gingiva, the periodontal ligament tissue from the middle third of the root surface was scraped with a sharp # 15 scalpel. Then, the extracted tissue was immersed in 25-cm² culture flasks (Nunc, Roskilde, Denmark) that contained 4 ml of culture medium.

The samples were then incubated at 36.5°C, 5% CO₂ 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, Roskilde, Denmark).

After 6 hours, L-dopa with different concentrations (0.25, 0.5, 0.75, 1, 2.5 and 5 µg/ml) (3, 4-dihydroxyphenylalanine, Sigma) were added to each well. Twenty microliters of 10% NaHCO₃ was used as negative control. The mitogenic effect of L-dopa was evaluated after 100 hrs of cell incubation in 36.5°C and 5% CO₂ by using the trypan blue and neutral red (NR) assays.

Twenty 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 .

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°C, 5% CO₂ 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 wells 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 test. *P* value <0.05 was considered significant.

RESULTS

Among different doses of L-dopa administered to the HPLF cells (0.25, 0.5, 0.75, 1, 2.5 and 5 $\mu\text{g/ml}$), the highest dose (2.5 $\mu\text{g/ml}$) showed remarkable effect on the number of the HPLF cells when it is compared to the control. Effect of this dose (2.5 $\mu\text{g/ml}$) 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 (2.5 $\mu\text{g/ml}$) 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 and that 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 in this experiment is observed at dose of 2.5 μg (Fig. 2).

DISCUSSION

The primary aim of treating the avulsed teeth is preservation and vitality of periodontal ligament elements, which consists mostly of fibroblasts (10).

Another treatment modality to consider is increase in the number of the viable periodontal ligament cells by using growth factor. These factors are mostly expensive and finding cheaper agents with similar effects may be beneficial.

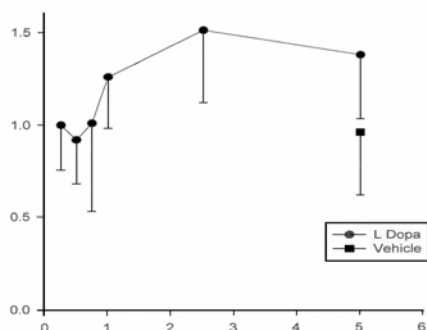


Fig. 1. The effect of different concentrations of L-dopa with the number of HPLF cells by using trypan blue method ($\pm\text{SD}$, $n=6$)

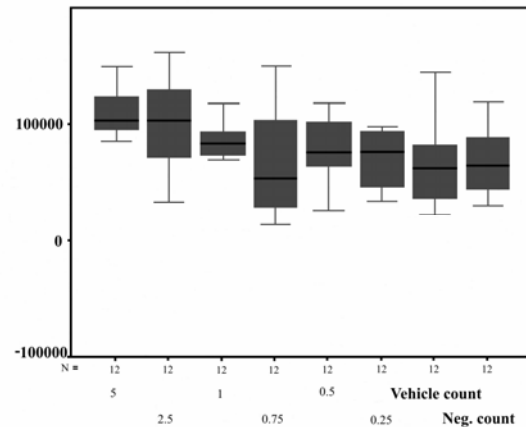


Fig. 2. The ratio of direct and indirect measurement of viability ($\pm\text{SD}$, $n=6$).

It has been shown that it takes 4 days for fibroblasts derived from the gingiva to become confluent and 6 days for fibroblasts derived from the PDL, however, most of tissues obtained from patients do not grow properly. Time of keeping in preserving medium, age, cell cycle condition and the number of cells obtained from tissue may be the reasons of this diversity. In present study, the HPLF cells which recovered in vitro reached confluence in T-25 flasks after 1 week.

The function of the fibroblasts is under the influence of age, trauma and inflammation; in this study, therefore, young patients with healthy unerupted, third molar were chosen. Previous experiments have shown that the passages number 3 to 6 produce the best cells for performing the experiment (11). 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 health 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.* (9) indicating non-toxicity of L-dopa in the wide range of administration for the HPLF cells. Moreover, Partovi *et al.* (9) showed that in the

low dose of L-dopa, the number of cells increased significantly when it was compared to controls. Despite this finding, the minimum dose, which shows this effect, has not been reported. As shown in figure 2, the viability ratio results indicate that L-dopa causes an increase in density of HPLF cells in a dose dependent manner in the range of 1 to 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 in increasing the self-life transferring HPLF cells.

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