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The Effect of Calcium Hydroxide on Fibroblast Cells Viability

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Abstract

Calcium hydroxide [$Ca(OH)_2$] is widely used as medicament in dental pulp and root canal therapy. Previous studies demonstrate the ability of calcium hydroxide to induce necrosis in dental pulp tissue. However, the mechanism of tissue destruction remains unknown. The aim of this study was to investigate fibroblast cell viability in response to calcium hydroxide exposure. In this study, Vero fibroblast cell line was treated with various concentrations of calcium hydroxide for 24 hours. Cell viability was measured by using MTT assay. Our results showed significant decrease in cell viability after exposed with calcium hydroxide at concentration 62.5 and 125 μg/ml. The result indicated that calcium hydroxide induced cell death in Vero cell line in a dose-dependent manner. This study suggests that fibroblast cell death may involved in the mechanism of pulp tissue necrosis caused by calcium hydroxide.

Keywords: Calcium hydroxide, cell viability.

Introduction

Dental pulp is a connective tissue that is uniquely situated within the rigid encasement of mineralizeddentin[1]. Fibroblast is the most distributed cell in the dental pulp tissue and widely distributed in cell-rich zone. Fibroblast main function is to synthesize colagen tipe I and III, which take part in tissue remodelling[1,2].

Endodontic treatment is a procedure to maintain the health of the pulp and the periapical tissue[3]. Calcium hydroxide [$Ca(OH)_2$] has been widely used as medicament in endodontic treatment since Hermann used it for the first time in 1920[4]. This medicament is used in many dental treatments such as pulp capping, pulpotomy,apexification,rootperforation,andinternal or external root resorption[5-11]. Calcium hydroxide has antimicrobial effect[5]that further more can give therapeutic effect and it has the ability to induce reparative dentin and dentinal bridge formation[12]. Previous in vivo study demonstrated the potencial of calcium hydroxide in inducing dental pulp tissue destructions[13]. However the underlying mechanism of tissue destruction is not well understood. As an initial step to clarify the molecular mechanism of $Ca(OH)_2$-induced pulp tissue destruction, this study was subjected to investigate fibroblasts cell viability after $Ca(OH)_2$ exposure in vitro.

Materials and Methods

In this study, Vero fibroblast cells were cultured and maintained in M199 culture medium (Gibco, Paisley, UK) suplemented with 10% fetal bovine serum (Gibco), 2% penicillin and streptomycin (Gibco), and 0.5% fungizone (Gibco). The confluent culture was harvested and plated into 96 wells plate for overnight incubation at 36°C. The first group of cells (2 x 10^4 cells/well) was then treated with various concentrations of calcium hydroxide (62.5µg/ml, 125µg/ml, and 250µg/ml), where as another one was left untreated as a negative control. Each concentration of calcium hydroxide was added to the cells and incubated for 72 hours in 5% CO_2, 37°C. All treatment was performed in 5 replications. Cell viability was then measured by using MTT assay. The following equation was used to find the percentage of Vero fibroblast cells viability[13].

$$\text{Viability (\%)} = \frac{\text{OD in treated group}}{\text{OD in negative control group}} \times 100\% \qquad (1)$$

OD = optical density

To determine whether the treatment of calcium hydroxide had significant effect on the cell viability, the data were analyzed by using one-way analysis of variance (ANOVA). The Post-Hoc least significant difference (LSD) test was done to determine the significance of difference among groups.

Results and Discussion

The result obtained in this study is presented in Figure 1 and Table 1. The results of ANOVA demonstrated a significant decrease in cell viability after exposed with calcium hydroxide ($p<0.05$), indicating that calcium hydroxide might have ability to induce cell death. In addition, Post-Hoc LSD test showed a significant difference ($p<0.05$) between control group with all of the treated groups. The group treated with 62.5µg/ml calcium hydroxide showed a significant difference with the groups treated with 125µg/ml and 250µg/ml. No significant difference was found between the group treated with calcium hydroxide 125 µg/ml and 250 µg/ml.

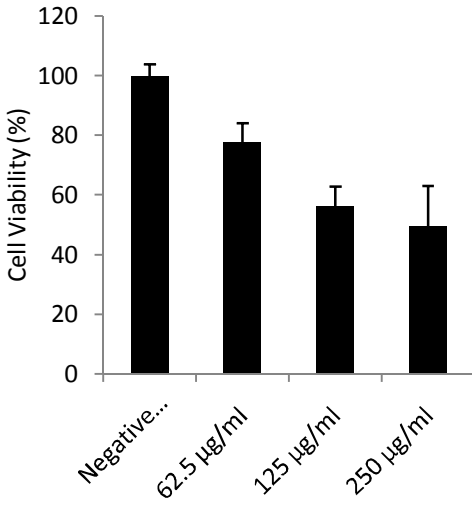

Figure 1. Calcium hydroxide induced cell death in Vero fibroblast cells. The untreated cells showed almost hundred percent living cells. The treatment of various concentrations of $Ca(OH)_2$ caused cell death in dose-dependent manner ($p<0.05$). The data were presented as means ± SD of representative experiments.

Figure 1 and Table 1 demonstrate that the group treated with the largest concentration of calcium hydroxide (250 µg/ml) has the lowest percentage of viable cells. The increasing concentration of the calcium hydroxide added to the cells caused decrease in the percentage of viable Vero cells.

There are many types of preparation of calcium hydroxide used in dentistry. The types are available as points, setting, and non-setting[14]. Pure calcium hydroxide preparation was used in this study. The result of this study showed that $Ca(OH)_2$ might have ability to induce fibroblast cell death. The result revealed that calcium hydroxide at concentration of 62.5 µg/ml or higher might cause cell death in Vero fibroblast cells. This phenomenon may have clinical implications as calcium hydroxide is widely used in dental treatments[4]. The reported clinical evidence provides factual information about the cytotoxic effect of this medicament[13], but little is known about the underlying molecular mechanisms.

Table 1. Means and standard deviations of the percentage of fibroblasts cell viability after calcium hydroxide treatment.

Groups	Cell Viability (%) ± SD
Negative Control	99.842 ± 4.014
$Ca(OH)_2$ 62.5µg/ml	77.59 ± 6.525
$Ca(OH)_2$ 125 µg/ml	56.206 ± 6.595
$Ca(OH)_2$ 250 µg/ml	49.49 ± 13.530

Fibroblast cell death mechanism induced by calcium hydroxide in this study has not been clarified. It was probably caused by the alkaline pH of calcium hydroxide, which is almost 12.5-12.8[15]. Based on the previous in vitro study, pure calcium hydroxide has high initiation pH[14] and that the hydroxyl ions are easily released in pure calcium hydroxide than in other preparations[16]. The pH can influence some of the cellular process such as cell metabolism, the changes of the cell's shape and mobility, the adjustment of transportation process and cell growth, cell proliferation and growth, conductivity and transport through the cell membrane, and isoosmotic cellular volume[17].

The cell death mechanism caused by high alkaline condition is not clearly known, but the previous study demonstrated that mitochondria took an important part in this cell death mechanism. The mitochondrial damage was the initiation of cell death mechanism caused by alkaline condition. This condition induced the increase of mitochondrial trans-membran potential leading to the disfunction of mitochondria. Under normal condition, mitochondria is responsible for cell respiration and therefore maintains the proton gradient. The application of calcium hydroxide may cause an alkaline condition, and it may attract the proton away from mitochondria. The mitochondria adapts by increasing respiration which leads to the increase of radical superoxide. Under these circumstances, the level of mitochondrial trans-membrane potential elevation causes the increase of superoxide diffusion from cytosol to the mitochondrial membrane[18]. Another study suggests that the increasing radical superoxide may cause the de-energization of mitochondria and loss of energy stores, peroxidation, and disruption of lipid membranes, direct DNA damage, and activation of transcriptional factor sactivation of protein-1 (AP-1) and *nuclear factor kappa beta* (NF-κB) both of which promote transcription of cytokines and have been associated with induction of apoptotic cell death[19].

There may be complicated molecular pathways involved in calcium hydroxide-induced cell death. It was reported that calcium hydroxide is able to induce the increase of intracellular calcium ion in $Ca(OH)_2$-pretreated human pulp cells[20]. In fact the increase in intracellular calcium level can generate board spectrum of cellular processes[21].

Conclusion

From our study it isconcluded that in vitro application of calcium hydroxide at certain concentration may cause fibroblast cell death, which may in part involve in pulp tissue destruction.

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