A effect of calcium hydroxide endodontic materials on the differentiation and the activation of osteoclast

Eun-Young Jang, Hoon Kown, Chang-Hee Lee*, Chang-Seop Lee, Sang-Ho Lee

Department of Pediatric Dentistry, M icrobiology*, College of Dentistry, Chosun University

국문초록

수산화칼슘계 근관 충전제가 파골 세포의 분화 및 활성에 미치는 영향

장은영 · 권 훈 · 이장희* · 이창섭 · 이상호

조선대학교 치과대학 소아치과학교실, 미생물학교실

본 실험은 수산화칼슘계 근관 충전제가 파골 세포의 분화 및 활성에 미치는 직접적인 억제 효과의 유무를 고찰하고자 chick embryo tibia의 골수로부터 추출한 파골 세포의 전구세포와 0.1, 0.01, 0.05㎍/ml로 희석된 네가지 실험 물질인 $Ca(OH)_2$ powder, Vitapex®, Metapaste®, pulpdent®를 사용하였다. 파골세포의 분화 및 활성에 미치는 억제 효과를 관찰하고자 분화된 파골 세포의 수와 흡수와 면적이 측정되고 이들 약제의 효과가 세포독성에 의한 결과인지를 알아보고자 U2OS 골아세포에 대한 MTT assay를 시행하여 다음과 같은 결론을 얻었다.

1. 파골세포의 분화에 대한 억제 효과는 다음과 같은 순서로 분화된 파골세포의 수가 통계학적으로 유의한 증가를 보였다.: Metapaste®, $Ca(OH)_2$ powder, Vitapex®. 하지만, 모든 농도에서 분화된 파골세포의 수가 증가한 pulpdent® 군에서는 통계학적으로 유의한 차이를 나타내지는 않았다.
2. 통계학적으로 분화된 파골세포 수의 감소가 유의한 결과를 나타낸 0.1㎍/ml로 희석된 세가지 실험 물질인 $Ca(OH)_2$ powder, Vitapex®, Metapaste® 가운데, Vitapex® 군만이 대조군에 비해 유의한 세포독성을 나타내고 다른 두 집단은 유의한 결과를 보이지 않았다. 또한, 0.2% DMSO군은 통계학적으로 유의한 세포독성을 나타내었다.
3. 0.1㎍/ml로 희석된 세가지 실험 물질인 $Ca(OH)_2$ powder, Vitapex®, Metapaste®과 대조군에서 흡수와의 양상과 면적을 관찰해 보면, $Ca(OH)_2$ powder군을 제외하고 대조군과 실험군사이에 유의한 차이를 나타내었고, 0.2% DMSO군도 통계학적으로 유의한 감소를 보였다.

이상의 결과를 볼때, 수산화칼슘은 파골 세포 분화 및 활성의 직접적인 억제 작용에 기인한 경조직 흡수의 억제에 관여하는 것으로 사료된다.

주요어 : 수산화칼슘, 파골세포, 분화, 활성, 억제작용

Ⅰ. Introduction

Resorption is a condition associated with either a physiologic or a pathologic process resulting in a loss of dentin, cementum, or bone. Invariably, tooth resorption results from injuries to or irrigation of the periodontal ligament and/or tooth pulp. It may arise as a sequela of traumatic luxation injuries, orthodontic tooth movement or chronic infections of the pulp or periondontal structure[1]. It leads to the formation of multinucleated giant

cells referred to as clasts[2,3]. The clastic cell is the key cell type responsible for all hard tissue resorptive processes. It is involved at elaborate interaction between inflammatory cells such as macrophages, monocytes and hard tissue structures[4]. Collectively, these cells orchestrate a complex interplay of molecular biologic events involving cytokines, enzymes, and hormones that influence the progression of resorption. Root resorption is mediated by the odontoclast, a cell identical to the osteoclast in terms of its cytological features and its mineralized tissue resorpive function. Both cell types are identical as pleomorphic multinucleated cells, with many mitochondria and lysosomes, positive for tartrate-resistant acid phosphatase (TRAP) and calcitonin receptors, Because the two cell types appear to differ only in their resorption substrates, one would assume that the differentiation process for the odontoclast would be the same as for the osteoclast and the process of tooth resorption be similar to that of bone resorption[5].

In the many destructive hard tissue diseases, bacterias play an important role. Likewise, bacterias are integral to the process of tooth resorption. The mechanism of bacteria-induced resorption is likely to be induction of osteolytic factors because of the effect of endotoxin, that is, lipopolysaccharides stimulate a number of molecular biologic events, including lysosomal enzyme release, collagenase release from macrophages and osteoblastic secretion of osteolytic factors IL-1, IL-6, M-CSF and PGE_2[6]. Together, these event result in the proliferation of osteoclast and enhanced bone resoption.

In previous study, Hammarstrom et al.[7] had demonstrated that the bacterias in the pulp or dentinal tubules were the final targets for the resorbing cells because the resorption of the dentin did not spread laterally in the experimental cavites but seemed to follow the direction of the dentinal tubules toward the pulp. Therefore, the action mode of therapy for suppression of tooth resorption was thought to relate to action on bacterial infection. In clinical practice, calcium hydroxide is a well established root canal dressing material for treatment of progressive root resoption. Calcium hydroxide was first introduced as a pulp canal capping material and root canal sealer by Hellman at early nineteen century in the endodontic treatment area[8].

Despite extensive research, the mechanism of action of calcium hydroxide is still not fully understood. But several theories[9,10] have been postulated to explain its biological activity. One theory discusses its high alkaline pH, which is important in stimulating matrix formation by the formative cells[11]. Another theory postulates that a high pH neutralizes the acidic products of the resorptive cells, creating an unfavorable environment for them[12]. Furthermore, calcium hydroxide may promote healing because of its antibacterial properties. The high pH of calcium hydroxide, providing an unsuitable environment for the growth and activity of bacteria and resorbing cells appear to be the main reason for the therapeutic effect of calcium hydroxide[13]. More recently, it has also been used in a number of specific endodontic treatment procedure, such as long-term pulpal dressing in teeth with large periapical lesions and temporary root canal filling to arrest root resorption[12,14,15].

However, in spite of the widespread use of calcium hydroxide in endodontic therapy, there seems to be no data concerning the experimental study on effect by interaction between this material and resorbing cell cultured in dircet contact with it.

The purpose of the present study is to investigate the direct inhibitory effect of some available calcium hydroxide materials on differentiation and activation of osteoclast.

Ⅱ. Materials and Methods

A. Experimental materials

Among bone marrow cells collected from tibia that was moved from 14 day-old chick embryo in 99.5℉, 80~82% humidity, progenitor cells of osteoclast were isolated and incubated.

For this study, four commercially available calcium hydroxide endodontic materials, i.e. : $Ca(OH)_2$ powder, Metapaste®(Meta Denta Co., LTD), Vitapex®(Neo Dental Chemical Products Co. Tokyo, Japan), Pulpdent® (PULPDENT Corp. U.S.A.) were used.

B. Experimental methods

1. Isolation of osteoclast progenitor cell

Tibia was dissected aseptically from 14 day-old chick embryo in 99.5℉, 80~82% humidity and placed into 60mm dish containing the HBSS media/antibiotics free of soft tissues. Therefore, cut across their epipyses and moved into 15ml cornical tube containing α-minimum es-

sential medium (α-MEM) and placed in -20℃ for 5min and later, put 60mm dish. In the state of holding of tibia by means of forcep, 1ml syringe needle fulfilled with 1ml medium inserted to bone and pushed the plunger. bone marrow cells flushed were collected in 50ml cornical tube and pipetted by 10ml pipet to increase single cell suspension. Clump was removed by let alone during 5~10min to sediment the cell clump or moving into 15ml cornical tube by pipet. After centrifugation, the cells suspended in αMEM containing 10% fetal bovine serum(FBS). These were plated at 2×10^6 total cells/well in 24-well plate. Cultures were fed at 4~6hr by replacing with fresh medium.

2. Differentiation into osteoclast

Isolated progenitor cells were plated in the medium at 500㎕/well having a similar number of cell. Four experimental groups ($Ca(OH)_2$ powder, Metapast®, Vitapex®, Pulpdent®) were diluted at 0.1, 0.01, 0.05㎍/ml and all experimental materials and their respective controls were plated into three well. Also, Dimethyl sulfoxide, DMSO(SIGMA,CHEMICAL.CO) was diluted at 0.2% concentration and used as a solvent of Vitapex®(Table 1).

Therefore, A total of 48 wells in series of four separative trials were incubated during 36hr at 5% CO_2 Incubator and therefore it was induced differentiation into osteoclast.

3. Measurement of the number of osteoclast

After incubation, each well was washed in a 37℃ prewarm DDW, fixed with fixative consisted of citrate sol 25ml, acetone 65ml, 37% formaldehyde 8ml for 30sec. Cytochemical staining for TRAP was conducted and mixture of it was consisted of the following: fast garnet GBC (wrap in hoil) 0.1ml, DDW 4.5ml, Naphthal AS-BI phosphate sol 0.05ml, acetate sol 0.2ml, tartrate sol 0.1ml(SIGMA DIAGNOSTICS. ST. Louis. Mo). Also, fast garnet GBC which was manufactured by mixing the fast garnet GBC base sol 0.5ml and sodium nitrate sol 0.5ml and was sensitive, thus covered by hoil. After TRAP staining, specimens were incubated for 1 hr at 37 ℃ H_2O bath and washed with distilled water. To examine the osteoclast stained, GEL/MOUNT TM was dropped and put a cover glass. TRAP positive multinucleated cells were counted as osteoclast under the light microscope.

4. Cytotoxicity test

After measuring of number of osteoclast in all experimental materials and their respective controls, cytotoxic effect of calcium hydroxide materials was examined by MTT assay. U2OS osteoblastic cell was plated at 1×10^4 cell/ well in 96-well plate In plate, 5columns were seeded: one was used for the control medium and three to evaluate the cytotoxicity of experimental groups, that is, $Ca(OH)_2$ powder, Metapaste®, Vitapex® at 0.1㎍/ml dilution and 0.2% DMSO was supplemented at remnant one. The 96-well dish was then placed into an incubator with an atmosphere of 5% CO_2 for 24hr at 37℃. The medium was removed and immediately replaced with 20 ㎕/well of a 0.5% solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-dimethyltetrazolium bromide(MTT) prepared in the culture medium. After incubation for 4hr at 37℃, the supernatant was discarded and the intercellar formazan crystals were solubilized with 100㎕/well of 0.04 N HCl/isopropanol. The absorbance of 96-well plate was determined using an automatic microplate spectrophotometer at 540nm.

5. Measurement of area of resorption lacunae

Osteoclast progenitor cells isolated from bone marrow cell of chick embryo tibia were plated at artificial apatite crystal plate (OAAS™, OCT Inc. Dankook University School of Dentistry) in 36 well plate. In plate, 5columns were seeded :one was used for the control medium and three experimental groups, that is, $Ca(OH)_2$ powder, Metapaste®, Vitapex® at 0.1㎍/ml dilution and 0.2% DMSO was supplemented. After incubation for 96hr, 1M NH_4OH was added for removal of osteoclast within re-

Table 1. Materials used in the present study

Materials	$Ca(OH)_2$ Concentration(%)	Manufacturer
Vitapex	30.3	Neo Dental Chemical Products Co. Tokyo, Japan
Metapaste	~30	Meta Denta Co., LTD
$Ca(OH)_2$ powder	95	Meta Denta Co., LTD
Pulpdent	-	PULPDENT Corp. U.S.A

sorption lacunae. The resorbed area was measured using an image analysis system linked to the light microscope.

Ⅲ. Result

Isolated progenitor cells were plated in the medium at 500㎕/well having a similar number of cell. Four experimental groups〔$Ca(OH)_2$ powder, Metapaste®, Vitapex®, Pulpdent®〕were diluted at 0.1, 0.01, 0.05㎍/ml and each was plated into three well. Dimethyl sulfoxide, (DMSO) (SIGMA, CHEMICAL CO.)was diluted at 0.2% concentration and used as a solvent of Vitapex®. All experimental materials and their respective controls were incubated during 36hr at 5% CO_2 Incubator and then measured the number of differentiated osteoclast.

In three experimental groups, that is, $Ca(OH)_2$ powder, Metapaste®, Vitapex® at 0.1㎍/ml dilution that were statistically significant in reduction of the number of differentiated osteoclast and 0.2% DMSO, MTT assay on U2OS osteoblast were conducted to examine cytotoxic effect. Also, pit forming assay was done to prove the effect of calcium hydroxide materials on resorptive activity of osetoclast. Result were as follow.

A. Measurement of the number of osteoclast

The results of the inhibitory effect upon osteoclast differentiation in all experimental groups and their respective controls were shown in Table 2 and Fig. 1. In Vitapex group, it displayed significantly less number at all concentration : These presented a decrease rate at 0.01㎍/ml(20.4%), 0.05㎍/ml(55.8%), 0.1㎍/ml(74.3%). Also, there were statistically significant difference among diluted concentration ($p<0.05$).

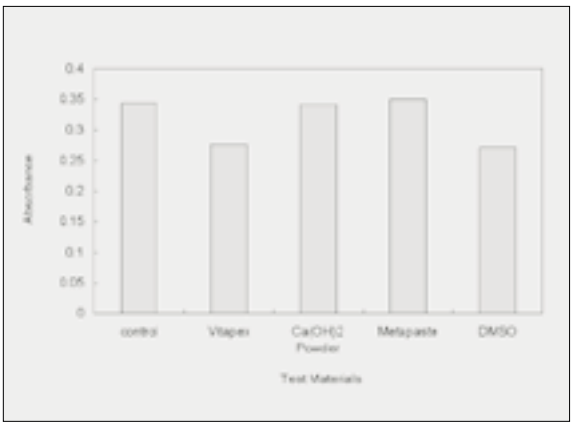

Fig. 2. Absorbance of U2OS osteoblast cell on solution of four experimental groups.

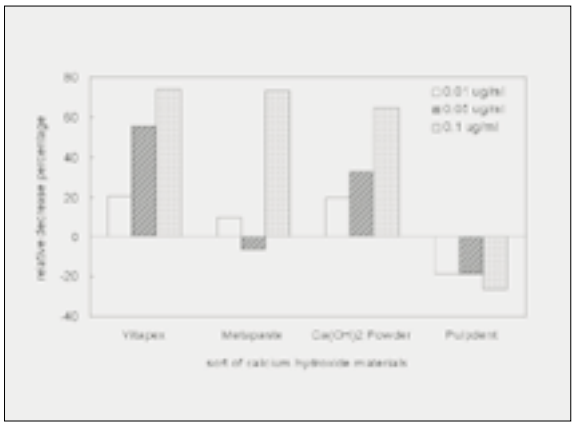

Fig. 1. Relative decrease percentage of the number of osteoclast in the solution of four experimental materials compared to control.

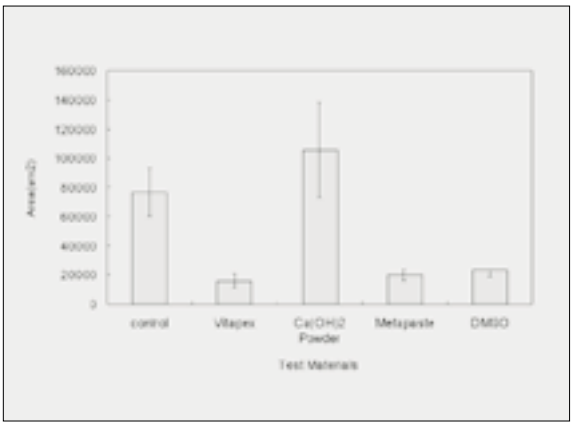

Fig. 3. Area of resorption lacunae on solution of four experimental groups.

Table 2. Number of osteoclast in the solution of four experimental groups

	Vitapex	Metapaste	$Ca(OH)_2$ powder	Pulpdent
control	112.5± 2.12	343 ±96.17	275±35.36	83.67± 5.51
0.01㎍/ml	90 ± 1.41*	309.5±10.6	220±39.60	100 ±12.49
0.05㎍/ml	50 ±11.31*	365 ± 1.41	185± 5.66*	100.3 ± 6.66
0.10㎍/ml	28.5± 7.78*	91 ±36.77*	97±21.21*	106.3 ± 9.45

There were shown a significant difference in Metapaste group at 0.1㎍/ml dilution (73.4%) and $Ca(OH)_2$ powder group at 0.05㎍/ml(32.8%), 0.1㎍/ml (64.7%) dilution and no significant difference was found among different concentrations except 0.1㎍/ml concentration($p<0.05$).

In Pulpdent group, they were exhibited that the number of differentiated osteoclast was increased at all concentration but no significant difference was found(Fig. 4~7).

B. Cytotoxicity test

In three experimental groups, that is, $Ca(OH)_2$ Powder, Metapaste®, Vitapex® at 0.1㎍/ml dilution that were statistically significant in reduction of the differentiated osteoclast, Vitapex® group showed significant difference compared to control and another two groups exhibited no significant difference($p<0.05$).

0.2% DMSO group was shown statistically significant cytotoxicity(Fig. 2).

C. Measurement of area of resorption lacunae

Pattern and measured area of resorption lacunae in the control and the three experimental groups, that is, $Ca(OH)_2$ powder, Metapaste®, Vitapex® at 0.1㎍/ml dilution were presented(Fig. 3).

Except $Ca(OH)_2$ powder group, statistically significant differences were found between experimental groups and control group.: These presented a decreased rate at Vitapex®(79.1%), Metapaste®(73.8%) ($p<0.05$). Also, DMSO group showed statistically significant decrease (68.9%)(Fig. 8~12).

Ⅳ. Discussion

Osteoclasts are highly polarized cells that form ruffled borders and clear zones toward the bone surface and derived from hemopoietic cells of the monocyte-macrophage lineage[16]. These are subject to extensive regulatory mechanism that are facilliated to a large degree by osteoblasts mediating the effects of osteotropic hormones and local mediator on these cells. Osteoclastic formation, regulation, activation are dependent on several local factors including M-CSF, IL-1, IL-6, IL-11, TNF-α and systemic regulatory factors such as PTH, $1,25(OH)_2D_3$, calcitonin[17-20].

The resorptive process itself can be described as being bimodal, involving the degradation of the inorganic crystal structure of hydroxyapatite and the organic structure of collagen. The unique structural arrangement of the osteoclasts to hard tissues allows the cell to establish a microenvironment between the ruffled border and the bone in which resorption takes place. Therefore, osteoclastic bone resorption consists of several complicated processes : osteoclast development, attachment of osteoclast to calcified tissues, development of a ruffled border and clear zone, followed by the secretion of acids and lysosomal enzymes into the space beneath the ruffled border[21].

Considering osteoclast function to be studied biochemically during the past decades, several different therapeutic approaches have been taken to reduction of osteoclastic resorption. A previous report demonstrated that intrapulpal application of an antibiotic/corticosteriod combination effectively eliminates experimentally induced external inflammatory resorpion in monkey teeth through directly inhibited the spreading of odontoclast, suggesting that this medicament acts the detaching resorbing cells from the root surface[22-24].

Another alternatives, bisphosphonates are widely used as anti-bone-resorbing agents in various disease associated with stimulated bone resorption[25]. The mechanism by which bisphosphonates inhibit bone resorption has not been established but proposed that it is achieved by inhibition of differentiation or recruitment of osteoclast[26,27], direct or indirect stimulation of osteoclast apoptosis[28], suppression of the resorption activitiy of mature osteoclast[29], morphologic change of osteoclast.

Pierce et al[30]. demonstrated the potent effects on inflammatory resorption of intra-canal insertion of calcitonin into reimplanted monkey teeth. Residual inflammation found within the periodontal membrane was reduced and this reduction was notably greater than that achieved in earlier studies using alternative therapies such as antibiotics alone or calcium hydroxide. The mode of action of calcitonin in deactivation of resorbing cell is thought to relate both specific and direct inhibition of root-resorbing cell and suppression of Inflammation[31]. According to SUDA et al.[6], calcitonin inhibited osteoclast function by calcitonin-induced morphological changes of the cytoskeleton of osteoclast and by disrupted actin ring and inhibition of pit formation .

Nevertheless, with regard to the management of dental resorption, intracanal application of calcium hydroxide is a popular treatment. Its high pH has been shown to be effective in destroying bacteria in dentinal tubules and thus reducing the inflammatory stimulus of resorption and also ascribed the superfical necrosis of adjacent tissues[1,32]. Hammarstrom et al.[7] found that calcium hydroxide applied intrapulpally caused limited necrosis of both dentin-resorbing cells and cells forming reparative cementum in an experimental groove in the root surface. Thus, a temporary necrotizing effect on reparative cells could encourage increased areas of ankylosis at the expense of the re-establishment of new periodontal attachment[33]. Likelywise, ankylosis was attributed to the potential toxicitiy of the medicament in high concentration on reparative cells[34]. Early investigations showed that calcium hydroxide was highly toxic for HeLa cell or human fibroblast[35-37]. In present study, MTT assay on osteoblast was conducted to examine whether or not effect of calcium hydroxide on inhibition of differentiation of osteoclast is due to cytotoxicity. No statistically significant difference was found between control group and experimental substrate. But in the Vitapex group, it was statistically significant difference that might be resulted from DMSO toxicity.

Zmener and Cabrini[38,39] studied the adverse effects of three calcium hydroxide-based materials on the behavior of a mixed cell population of human blood monocytes and lympocytes and demonstrated that the adverse effects of the test materials would lead to cell detachment from the substratum or to produce alterations in cell morphology. According to their results, it seems that the degrees of inhibition of cell attachment correlated with one of the most undesirable properties of endodontic material, i.e toxicity. In the present study, inhibitory effect of commercially available calcium hyroxide endodontic materials on differentiation of osteoclast increased in the following order : Metapaste , $Ca(OH)_2$ powder, Vitapex . These results may be attributed that difference of concentration of $Ca(OH)_2$ and other components from each material adversly affect differentiation of cells cultured into osteoclast. Although $Ca(OH)_2$ Powder is very higher concentration than that of others, it was shown the lower effect than that of Vitapex because of it's relative insolubility. As another possibility, effect of Dimethyl sulfoxide, DMSO was used as a solvent of vitapex might be considered.

Previously, some researcheres[40-42] have been described the inhibition of cell functions and the suppression of their vital activity when they are cultured in contact with calcium hydroxide or when this material is placed in direct contact with pulpal or periapical tissue.

In present stuty, to examine effect of calcium hydroxide materials on activity of osteoclast, pit forming assay using artificial apatite crystal plate was conducted. Except $Ca(OH)_2$ powder group, calcium hydroxide materials inhibited the formation of resoption lacunae compared to control group. These result may be due to lack of complete solution of $Ca(OH)_2$ powder group. Therefore, there are possible differences in the ability of calcium and hydroxyl ions to egress from $Ca(OH)_2$ powder material, producing different levels of alkalinity in the culture medium. Through this report, it can be proposed that calcium hydroxide is responsible for suppression of hard tissue resorption by a direct inhibition of differentiation and activation of osteoclast. However, on the basis of the molecular mechanism of the regulation of osteoclastic bone resorption, further studies will be established to elucidate the action of calcium hydroxide on formation, regulation, activation of osteoclast.

Ⅴ. Conclusion

Using the osteoclast progenitor cells isolated from bone marrow cell of chick embryo tibia and four experimental materials($Ca(OH)_2$ powder, Metapaste®, Vitapex®, Pulpdent®) diluted at 0.1, 0.01, 0.05㎍/ml, it was evaluated the direct effect of all experimental materials and their respective controls on differentiation and resorptive activity of osetoclast. Also, MTT assay on U2OS osteoblast was conducted to examine cytotoxic effect. Results were as follow.

1. Considering the result of the inhibitory effects upon osteoclast differentiation, there were shown a significantly different increase in the following order: Metapaste®, $Ca(OH)_2$ powder, Vitapex®. But, no significant difference was found in Pulpdent group that the number of differentiated osteoclast was increased at all concentration($p<0.05$).
2. Among three experimental groups, that is, $Ca(OH)_2$ powder, Metapaste®, Vitapex® at 0.1㎍/ml dilution that were statistically significant in reduction of the number of differentiated osteoclast, Vitapex group

showed significant difference compared to control and another two groups exhibited no significant difference. Also, 0.2% DMSO group was shown statistically siginificant cytotoxicity ($p<0.05$).

3. Examining pattern and measured area of resorption lacunae in the control and the three experimental groups, that is, $Ca(OH)_2$ powder, Metapaste®, Vitapex® at 0.1㎍/ml dilution, statistically significant inhibitory effect were found between experimental groups and control group except $Ca(OH)_2$ powder group. Also, DMSO group showed statistically significant decrease($p<0.05$).

Through this report, it can be proposed that calcium hydroxide is responsible for suppression of hard tissue resorption by a direct inhibition of differentiation and activation of osteoclast. However, on the basis of the molecular mechanism of the regulation of osteoclastic bone resorpion, further studies will be established to elucidate the action of calcium hydroxide on formation, regulation, activation of osteoclast.

References

1. Ne RF, Witherspoon DE, Gutmann JL : Tooth resorption. Quintessence international 30:9-25, 1999.
2. Anan H, Akamine A, Maeda K : An enzyme histochemical study of the behavior of rat bone cells during experimental apical periodontitis. J Endod 19:83-86, 1993.
3. Boyde A, Ali NN, Jones SJ : Resorption of dentine by isolated osteoclasts in vitro. Br Dent J 156:216-220, 1984.
4. Akamine A, Anan H, Hamachi T, et al. : A histochemical study of the behavior of macrophage during experimental apical periodontitis. J Endod 20(10):474-478, 1994.
5. Wu SM, Richards DW, Rowe DJ : Production of matrix-degrading enzymes & Inhibition of osteoclast-like cell differentiation by fibroblast-like cells from the periodontal ligament of human primary teeth. J Dent Res 78(2):681-689, 1999.
6. Suda T, Nakamura I, Jimi E : Regulation of osteoclast function. Journal of Bone and Mineral Research 12(6):869-879, 1997.
7. Hammarstrom LE, Blomlof LB, Feiglin B, et al. : Effect of calcium hydroxide treatment on periodontal repair and root resorption. Endod Dent Traumatol 2:184-189, 1986.
8. Rheu E, Jeon SM : PH changes at the surface of root dentin when using root canal sealers containing calcium hydroxide. J of Korean Academy of Conservative Dentistry 23(2):710-717, 1998.
9. Calts S, Serper A, Ozcelik B : PH changes & Ca2+ ion diffusion from calcium hydroxide dressing materials through root dentin. J Endod 25(5):329-331, 1999.
10. Foreman PC, Barnes IE : A review of calcium hydroxide. Int Endod J 23:283-297, 1990.
11. Yousef saad A : Calcium hydroxide in the treatment of external root resorption. JADA 118:579-581, 1989.
12. Stock CJR : Calcium hydroxide ; Root resorption and perio-endo lesions. Br Dent J 158:325-334, 1985.
13. Staehle HJ, Pioch T, Hoppe W : The alkalizing properties of calcium hydroxide compound. Endod Dent Traumatol 5:147-152, 1989,
14. Caliskan MK, Sen BH : Endodontic treatment of teeth with apical periodontitis using calcium hydroxide : A long-term study. Endod Dent Traumatol 12:215-221, 1996.
15. Holland R, Valle GF, Taintor JF, et al. : Influence of bony resorption on endodontic treatment. Oral Surg 2:191-203, 1983.
16. Wedenberg C, Sumita S : Evidence for an inhibitor of osteoclast attachment. Endod Dent Traumatol 6:255-259, 1990.
17. Kim TS, Yu YJ, Gwak WA, et al. : Formation of osteoclasts by cytokine released from periodontal ligament cells in response to interleukin -1β. J of Korean Academy of Pediatric Dentistry 23(1):189-205, 1996.
18. Martin T, Udagawa N : Hormonal regulation of osteclast function. Trends Endocrinol Metab 9:6-12, 1998.
19. Suda T, Takahashi N, Udagawa N, et al. : Modulation of osteclast differentiation and function by the new members of the tumor necrosis factor receptor and ligand families. Endocrine Reviews 20(3):345-357, 1999.
20. Suda T, Jimi E, Nakamura I, et al. : Role of 1α,25-dihyroxyvitamin D3 in osteoclast differentiation and function. Methods Enzymol 282:223-235, 1997.

21. Gillies JA, Carnes DL, Stewart Windeler A : Development of an in vitro culture system for the study of osteoclast activity and function. J Endod 20(7):327-331, 1994.
22. Hammarstrom LE, Blomlof LB, Feiglin B, et al. : Replantation of teeth and antibiotics treatment. Endod Dent Traumatol 2:51-57, 1986.
23. Pierce A, Lindskog S : The effect of an antibiotic/corticosteriod paste on inflammatory root resorption in vivo. Oral Surg Oral Med Oral Pathol 64:216-220, 1987.
24. Pierce A, Heithersay G, Lindskog S : Evidence for direct inhibition of dentinoclasts by a corticosteriod/antibiotics endodontic paste. Endod Dent Traumatol 4:44-45, 1988.
25. Lee IS, Kim HJ, Ryoo HM, et al. : Effect of bisphosphonate on osteoclast differentiation. J of Korean Academy of Pediatric Dentistry 27(2):309-317, 2000.
26. Cocchini MG, Fleisch H : Bisphosphonate in vitro specifically inhibit among the hem atopoietic series, the development of the mouse mononuclear phagocyte lineage. J Bone Miner Res 5:1019-1027, 1990.
27. Hughes DW, MacDonald BR, Russel RGG, et al. : Inhibition of osteoclast-like cell formation by bisphosphonate in long-term cultures of human bone marrow. J Clin Invest 83:1930-1935, 1989.
28. Hughes DE, Wright KR, Sasake A, et al. : Bisphosphonates promote apoptosis in murine osteoclasts in vitro and in vivo. J Bone Miner Res 10:1478-1487, 1995.
29. Sato M, Grasser W : Effect of bisphosphonate on isolated rat osteoclasts as examined by reflected light microscopy. J Bone Miner Res 5:31-40, 1990.
30. Pierce A, Berg GO, Lindskog S : Calcitonin as an alternative therapy in the treatment of root resorption. J Endod 14:459-464, 1988.
31. Wiebkin OW, Cardari SC, Heithersay GS : Therapeutic delivery of calcitonin to inhibit external inflammatory root resorption. Endod Dent Traumatol 12:265-271, 1996.
32. Tronstad L : Root resorption- etiology, terminology and clinical manifestations. Endod Dent Traumatol 4:241-252, 1988.
33. Cotton WR : Bacterial contamination as a factor in healing of pulp exposure. Oral Surg 38:441-450, 1974.
34. Pierce AM : Experimental basis for the management of dental resorption. Endod Dent Traumatol 5:255-265, 1989.
35. Im MK, Lee CS : A study on the cytotoxicity of root canal sealers to several cell lines, J of Korean Academy of Conservative Dentistry 17(2)263-282, 1992.
36. Spangberg L : Biological effects of root canal filling materials toxic effect in vitro of root canal filling materials on HeLa cells and human skin fibroblasts. Odontol Revy 20:1-10, 1969.
37. Torneck CD, Moe H, Howley TP : The effect of calcium hydroxide on porcine pulp fibroblast in vitro. J Endod 9(4):131-136, 1983.
38. Zmener O, Cabrini RL : Effects of three calcium hydroxide-based material on human blood monocytes and lympocytes. Endod Dent Traumatol 3:28-32, 1987.
39. Zmener O, Cabrini RL : Adhesion of human blood monocytes and lympocytes to different endodontic cements. A methodological in vitro study. J Endod 2(4):150-155, 1986.
40. Holland R, Mello WD, Nery MJ, et al. : Reaction of human periapical tissue to pulp extirpation and immediate root canal filling with calcium hydroxide. J Endod 3:63-67, 1977.
41. Schroeder V, Granath L : Early reaction of intact human teeth to calcium hyroxide following experimental pulpotomy and its significance to the development of a hard tissue barrier. Odontol Revy 22:379-396, 1971.
42. Lengheden A, Blomlof L, Lindskog S : Effect of delayed calcium hydroxide treatment on periodontal healing in contaminated replanted teeth. Scand J Dent Res 99:147-153, 1991.

Explanation of figures

Fig. 4. Light micrograph of differentiated osteoclast in respective control and at 0.1㎍/ml dilution of vitapex group(×40)
Fig. 5. Light micrograph of differentiated osteoclast in respective control at 0.1㎍/ml dilution of Metapaste group(×40)
Fig. 6. Light micrograph of differentiated osteoclast in respective control at 0.1㎍/ml dilution of $Ca(OH)_2$ group(×40)
Fig. 7. Light micrograph of differentiatedosteoclast in respective control at 0.1㎍/ml dilution of Pulpdent group(×40)
Fig. 8. Light micrograph of resorption lacunae in Control group(×40)
Fig. 9. Light micrograph of resorption lacunae in vitapex group(×40)
Fig. 10. Light micrograph of resorption lacunae in Metapaste group(×40)
Fig. 11. Light micrograph of resorption lacunae in $Ca(OH)_2$ group(×40)
Fig. 12. Light micrograph of resorption lacunae in DMSO group(×40)

Explanation of figures

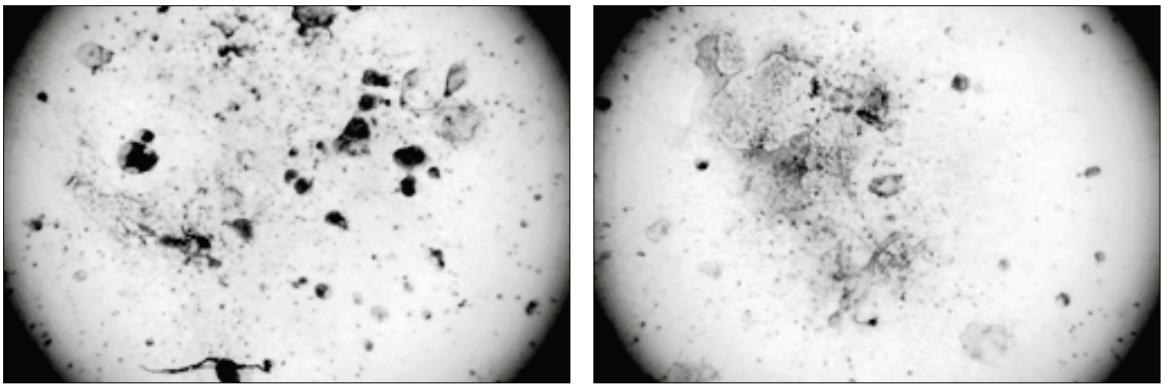

Fig. 4

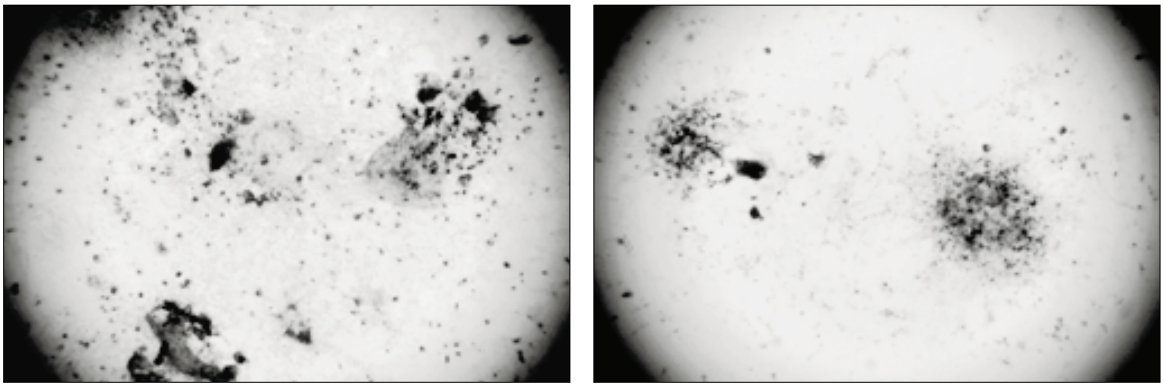

Fig. 5

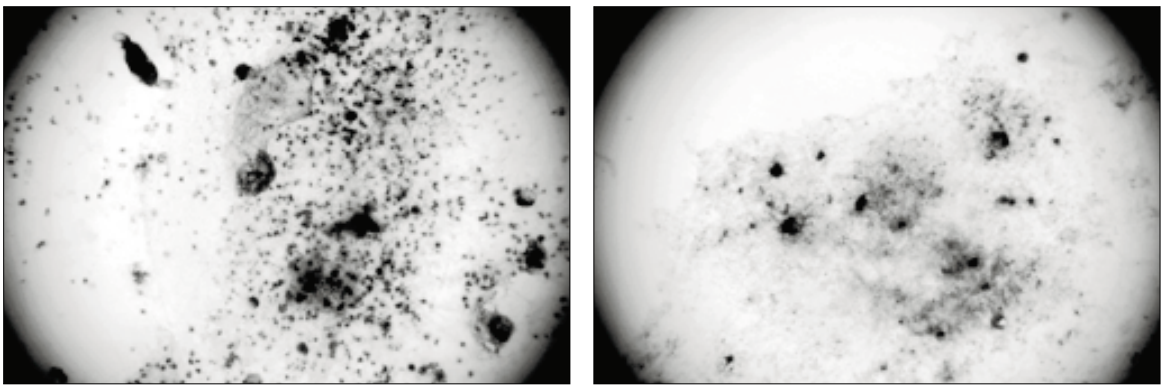

Fig. 6

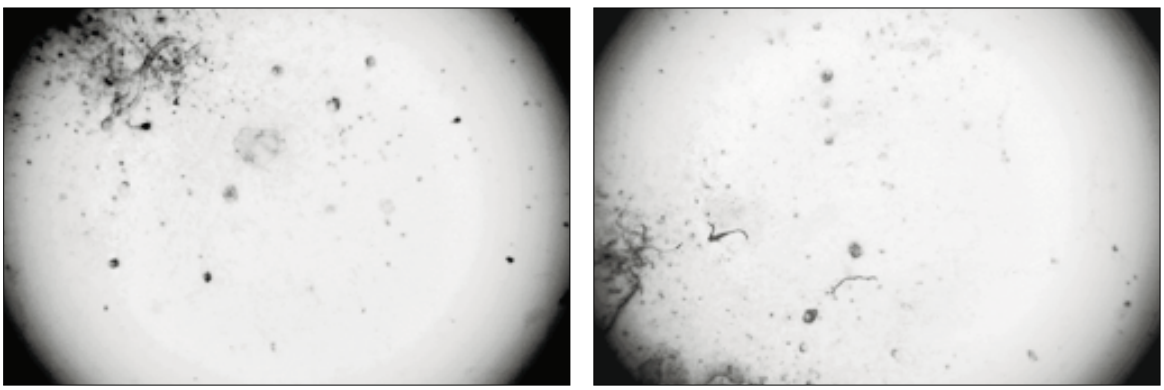

Fig. 7

Explanation of figures

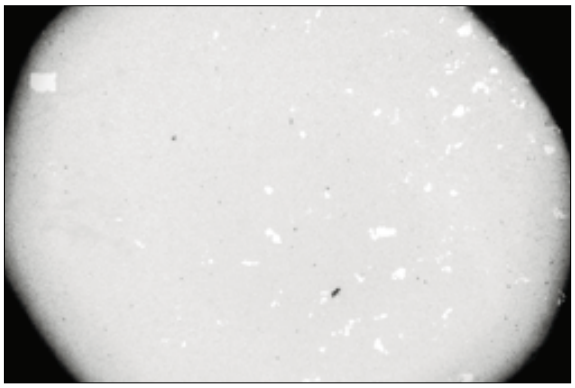

Fig. 8

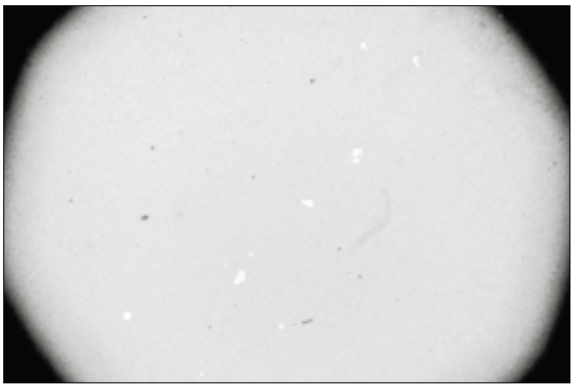

Fig. 9

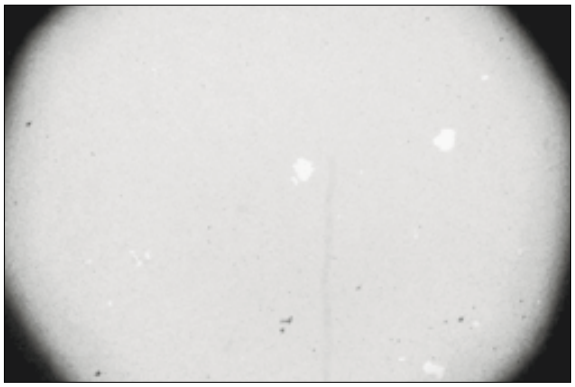

Fig. 10

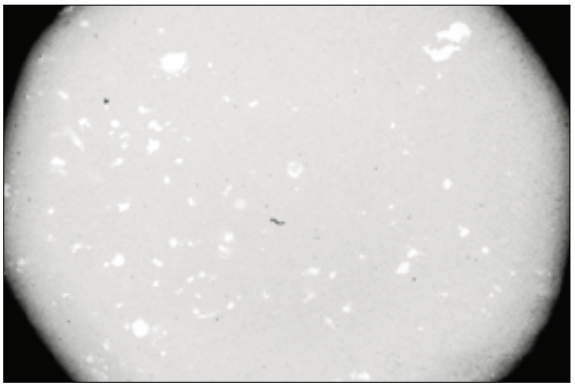

Fig. 11

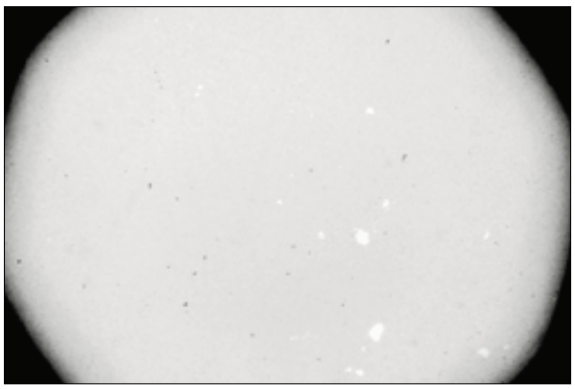

Fig. 12

Abstract

A EFFECT OF CALCIUM HYDROXIDE ENDODONTIC MATERIALS ON THE DIFFERENTIATION AND THE ACTIVATION OF OSTEOCLAST

Eun-Young Jang, Hoon Kwon, Chang-Hee Lee*, Chang-Seop Lee, Sang-Ho Lee

Department of Pediatric Dentistry, Microbiology*, College of Dentistry, Chosun University

The purpose of this study was to investigate the direct inhibitory effect of calcium hydroxide materials on differentiation and activation of osteoclast. we used the osteoclast progenitor cells isolated from bone marrow cell of chick embryo tibia and four experimental materials [$Ca(OH)_2$ powder, Metapaste®, Vitapex®, Pulpdent®] diluted at 0.1, 0.01, 0.05㎍/ml. There were measured both the number of differentiated osteoclast and the area of resorption lacunae. Also, we conducted MTT assay on U2OS osteoblast to examine of cytotoxic effect and obtained following result.

1. Considering the result of the inhibitory effects upon osteoclast differentiation, There were shown a significant difference increased in the following order: Metapaste®, $Ca(OH)_2$ powder, Vitapex®. But no significant difference was found in pulpdent group that the number of differentiated osteoclast was increased at all concentrations($p<0.05$).
2. Among the three experimental groups, that is, $Ca(OH)_2$ powder, Metapaste®, Vitapex® at 0.1㎍/ml dilution that were statistically significant in reduction of the number of differentiated osteoclast. Vitapex group showed significant cytotoxic effect compared to control and another two groups exhibited no significant difference. Also, 0.2% DMSO group was shown statistically siginificant cytotoxicity ($p<0.05$).
3. Examining pattern and measured area of resorption lacunae in the control and the three experimental groups ,that is, $Ca(OH)_2$ powder, Metapaste®, Vitapex® at 0.1㎍/ml dilution, except $Ca(OH)_2$ powder group, statistically significant differences were found between experimental groups and control group. Also, DMSO group showed statistically significant decrease ($p<0.05$).

From these results, we think that calcium hydroxide is responsible for suppression of hard tissue resorption by a direct inhibition of dfferentiation and activation of osteoclast.

Key words : Calcium hydroxide, Osteoclast, Differentiation, Activation, Inhibitory effect.