

Chemical Analysis of the Liberation of Calcium and Hydroxyl Ions from Calcium Hydroxide Pastes in Connective Tissue in the Dog - Part I

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| [Introduction](#) | [Material and Methods](#) | [Results](#) | [Discussion](#) | [Acknowledgments](#) | [References](#) |

The objective of this research is to chemically analyze calcium hydroxide pastes added to three hydrosoluble vehicles having different acid-base characteristics using polyethylene tubes implanted in subcutaneous connective tissue in a dog, evaluating the liberation of calcium and hydroxyl ions over a period of 7, 30, 45 and 60 days. The three vehicles were saline, anesthetic, and polyethylene glycol 400. Chemical analysis of the liberated calcium ions was done by means of conductimetry using EDTA for titration. Liberation of hydroxyl ions was determined by analogy of calcium ions liberated, which are in direct proportion to the molecular weight of calcium hydroxide.

Key Words: calcium hydroxide, intracanal dressing, pulpotomy.

Introduction

The search for effective and practical solutions of disinfection of root canals is not recent. Toward this end various dressings have been proposed. The determining conditions of growth and multiplication of the micro-organisms which influence their enzymatic activities, such as pH, temperature, osmotic pressure, concentration of oxygen and carbon dioxide, substrata, constitute important factors which should be evaluated when an intracanal dressing is selected. The dressing should also favor repair of the affected tissue.

The biological and antibacterial action of calcium hydroxide confer to it a prominent position in this search. It stimulates tissue repair by inducing the formation of mineralized tissue (Holland et al., 1979, 1980). Calcium hydroxide is also effective against micro-organisms present in the root canal (Bystrom et al., 1985; Sjogren et al., 1991; Safavi and Nichols, 1993, 1994; Estrela et al., 1996).

The influence of elevated pH of calcium hydroxide on bacterial and tissue enzymes reinforces the importance of the biological and chemical dynamics since its properties derive from the disassociation of calcium and hydroxyl ions. Estrela et al. (1995), explaining its mechanism of action, report that the effect of its pH alters the conveyance of nutrients and organic components through the cytoplasmic membrane, inhibiting enzymatic activities which are essential to bacterial life, such as metabolism, growth, and cellular division, and liberating a toxic action which is harmful to bacteria. It also activates alkaline phosphatase, a hydrolytic enzyme intimately related to the process of tissue mineralization. For these reasons, the authors believe that calcium hydroxide presents two essential enzymatic properties: inhibition of bacterial enzymes for its antibacterial effect and activation of tissue enzymes, such as alkaline phosphatase, leading to its mineralizing effect.

Various substances and vehicles have been associated with calcium hydroxide as a means of bettering its antibacterial action, its ionic disassociation (which influences dental diffusiveness), and its physical-chemical properties (such as filling of the root canal). The velocity of liberation of calcium and hydroxyl ions is a

result of the hydro-solubility and viscosity of the vehicles as well as the ratio powder/liquid which can alter the biological effects on the bacteria and tissue.

The objective of this research is to chemically analyze the liberation of calcium and hydroxyl ions of calcium hydroxide pastes having vehicles of different acid/base characteristics in connective tissue in a dog.

Material and Methods

Twenty-four polyethylene tubes (Number 10 Sonda Levine) were used. The tubes were 60 mm long and had internal diameters of 3 mm; one of the ends of each tube was sealed by heat and compression.

The tubes were divided into 4 groups of 6 each. Each group of six tubes was further subdivided into three groups of two each. The tubes were then completely filled with 1.2 grams of calcium hydroxide paste (Quimis, USA) with the following vehicles: saline, anesthetic (2% xylocaine with vasoconstrictor) and polyethylene glycol 400. The pastes were prepared to the same viscosity, 3501 centi-Poise at 0.1 rpm, as determined by a Brookfield digital rheometer (cone-plate type); the resulting pastes being compared clinically to the consistency of standard toothpaste.

The four groups of tubes were implanted into the subcutaneous tissue of a two-year-old dog of undetermined breed, two groups on either side of the thorax, separated by 10 cm. The dog was pre-anesthetized intravenously with 0.1 mg/kg acepromazine (0.2% Aceprom) and, after 15 minutes, anesthetized with 3% sodium thiopental (Thionembutal), applied by an oral-tracheal catheter. Anesthesia was maintained with 2% halothane (Fluothane).

After periods of 7, 30, 45, and 60 days the tubes were removed in groups of six and carefully transported for chemical analysis in completely sealed inert flasks containing nitrogen atmospheres.

Chemical analysis of liberated calcium ions

The implanted tubes were removed in groups of six after periods of 7, 30, 45, and 60 days and the samples were transferred from the three pairs according to the vehicles employed in the test. The material from each pair of tubes was discharged with quantitative care and dissolved in a liter of distilled water. A total of 50 ml of the resulting solution, having a concentration of 1.35×10^{-2} mol/l, was taken and mixed with 10 ml of an ammonia tampon-ammonia chloride (pH = 10). The solution was titrated and, after each addition of titrate, the value of solution conductance was recorded with a conductimeter (Digimed CD-21). The measured values of conductance were corrected by a dilution factor because the dilution also reduces the conductance during the titration process. The titration itself was done using a standard disodic solution of EDTA at a concentration of 2.0×10^{-2} mol/l.

At the point of equivalence, the number of mol (n) of EDTA used during the titration corresponds exactly to the number of mol of calcium ions present in the solution. Since this quantity came from 50 ml of solution, one can find the initial concentration of the sample from the equation:

The percentage of calcium ions present was then calculated in the following way:

Since C_{EDTA} equals 2.0×10^{-2} mol/l, one can say that: $\% \text{Ca}^{2+} = 2.96 \times V_{\text{EDTA}}$, where V_{EDTA} is in ml.

In this way, the percent of calcium ions present, and therefore liberated, is calculated by the volume of EDTA used (Basset et al., 1981)

Calculated analysis of liberated hydroxyl ions

From the calculations above of the calcium ions liberated it is also possible to determine the number of hydroxyl ions liberated using the following formulas:

Since the molecular weight of 2 mol hydroxyl ions is 34, and the molecular weight of the 1 mol calcium ion is 40.08, while that of the complete molecule is 74.08, the percent of the two to the total weight can be simply calculated to be 45.89% and 54.11%, respectively. Therefore, in 1 mol of calcium hydroxide there

will be 45.89% hydroxyl ions and 54.11% calcium ions. Once the quantity of liberated calcium ions is determined, it is a simple matter to calculate the quantity of hydroxyl ions since the two are related in direct proportion.

Results

[Tables 1](#) and [2](#) demonstrate the values in percent of calcium and hydroxyl ions liberated by the calcium hydroxide pastes used in all of the experimental periods.

Discussion

Determining the bacterial flora which is predominant in infected root canals is a very important factor in the correct choice of intracanal dressing. This dressing must be effective against the different types of bacteria: aerobic, anaerobic and microaerophilic as well as being active in the process of alkalization of the dentinal tubules, impeding dental resorptions while favoring the process of periapical tissue repair.

Calcium hydroxide influences the enzymatic activity of bacteria which is reflected in growth, cellular division and bacterial metabolism. The enzymatic seat of bacteria is located in the cytoplasmic membrane. Extracellular enzymes act on nutrients, carbohydrates, proteins, and lipids that, through hydrolysis, favor digestion. Intracellular enzymes located in the membrane, on the other hand, relate to the conveyance of substances into and out of the cell, to respiratory activity of the cell, and to cellular wall structure. The pH gradient of the cytoplasmic membrane is altered by the high concentration of hydroxyl ions of calcium hydroxide acting on the proteins of the membrane (proteic denaturation). It is also altered by the state of ionization of organic nutrients, causing chemical conveyance to occur in an unnatural way, provoking toxic effects on the bacteria. Another form of chemical disintegration of the cytoplasmic membrane is through the destruction of unsaturated fatty acids or phospholipids by the high concentration of hydroxyl ions observed through the process of peroxidation of lipids, a saponification reaction (Estrela et al., 1995).

Study of the biological effect of pH on enzymatic activity of bacteria has permitted us to establish the hypothesis of irreversible enzymatic inactivation when the pH of calcium hydroxide remains high for long periods of time which provokes bacterial shock or reversible enzymatic inactivation when there is a return to the ideal pH for the functioning of the enzymes (Estrela et al., 1994). Lehninger (1986) believes that extreme values of pH lead to a loss of biological activities of proteins and that on return to normal values there is a return of native structures in a process called proteic renaturation.

The velocity of ionic disassociation exerts a direct influence on biological activities of bacteria and tissue. For this reason the vehicles used in calcium hydroxide pastes can produce different effects.

In the chemical analysis of the calcium ions liberated from the calcium hydroxide pastes obtained by conductimetric determination of calcium in solution it can be observed that the vehicle which resulted in the greatest liberation over the period of 60 days was the anesthetic, with stabilization from 45 to 60 days. In the paste with saline, the values were intermediate when compared with the other solutions with stabilization from 30 to 60 days. The polyethylene glycol 400 solution showed the lowest initial percent of calcium ion liberation, with the liberation being gradual and uniform as shown in [Table 1](#).

The liberation of hydroxyl ions from these pastes can be calculated from the liberation of calcium ions, using as a parameter the direct relation between the calcium and hydroxyl ions and the molecular weight of calcium hydroxide. In calcium hydroxide the proportion of hydroxyl ions to calcium ions is 45.89 to 54.11. The calculated percent of liberation of hydroxyl ions is thus 84.80% of the measured percent of the liberation of calcium ions ([Table 2](#)).

Estrela et al. (1995) evaluated the apical dental diffusion of hydroxyl ions of calcium hydroxide pastes using these same vehicles in vitro and they observed that the pastes prepared with saline and anesthetic showed an alteration of pH from 6-7 to 7-8 after 30 days, remaining at the latter values for another 30 days. In the group with polyethylene glycol 400, this same alteration occurred only after 45 days and continued unaltered for 15 additional days to the end of the test period. In the interior of the root the pH remained high (>12) over the

test period. The variations in the velocities of ionic disassociations of calcium hydroxide pastes are influenced by their vehicles and by their acid/base characteristics.

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[BACK TO CONTENTS](#)