Effect of Vehicle on Antimicrobial Properties of Calcium Hydroxide Pastes

Carlos ESTRELA[1]
Jesus Djalma PÉCORA[2]
Manoel Damião SOUZA-NETO[3]
Cyntia Rodrigues A. ESTRELA[1]
Lili Luschke BAMMANN[4]

[1]Faculdade de Odontologia, Universidade Federal de Goiás, Goiânia, GO, Brasil
[2]Faculdade de Odontologia de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brasil
[3]Faculdade de Odontologia, Universidade de Ribeirão Preto, Ribeirão Preto, SP, Brasil
[4]Faculdade de Odontologia, Universidade Luterana do Brasil, Porto Alegre, RS, Brasil

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Braz Dent J (1999) 10(2):63-72 ISSN 0103-6440

The current discussion about the importance of intracanal dressings and the effect of vehicles on calcium hydroxide pastes is justified by controversy concerning the achievement of complete disinfection after preparation of infected root canals and the real antimicrobial effect of these vehicles. The aim of this study is to investigate the role of vehicles in the antimicrobial effect of calcium hydroxide pastes. Well-conducted research about the characteristics of calcium hydroxide, such as antimicrobial potential, physico-chemical aspects and histocompatibility, gives credibility to the choice of this medication in several clinical situations. Different vehicles have been added to calcium hydroxide in an attempt to enhance its properties. Scientific reasoning indicates the use of hydrosoluble vehicles (distilled water, saline) associated with calcium hydroxide because of their chemical characteristics of dissociation, diffusibility and filling capability which are decisive for the biological behavior, i.e., antimicrobial qualities and induction of tissue repair.

Key Words: calcium hydroxide, intracanal dressing, vehicle.

Introduction

Knowledge about the mechanisms of microbial aggression after pulpal necrosis and therapeutic resources to neutralize them has motivated the study of substances with antimicrobial and biocompatible characteristics. Because the preparation of an infected root canal does not allow complete microbial elimination, it is necessary to use an intracanal dressing.

Scientific research on intracanal dressings does not mean habits of prescription, beliefs in beneficial effects or even the goal of determining a drug able to heal all illnesses. It only tries to establish appropriate criteria for use, including limits and implications and, at the same time, show the value of research to explain facts that are not yet clear or are mistakenly explained.

Estrela et al. (1995b) reported that the action of calcium hydroxide would explain how its high pH inhibits enzyme activities that are essential to bacterial life, i.e., metabolism, growth and cellular division. The effect of pH on the transport of nutrients and organic components through the cytoplasmic membrane determines its toxic action on bacteria. This also activates the hydrolytic enzyme alkaline phosphatase, which is closely related to the process of tissue mineralization. Thus, this medication presents two fundamental enzyme properties: the inhibition of bacterial enzymes leading to an antimicrobial effect and the activation of tissue

enzymes leading to a mineralizing effect.
Calcium hydroxide is an excellent therapeutic option when the clinical situation requires the use of an intracanal dressing and its biological virtues should be analyzed. The aim of this study is to discuss the role of vehicles in the antimicrobial effect of calcium hydroxide pastes. However, a previous evaluation of chemical characteristics of calcium hydroxide as a way to better understand the influence of vehicles on the pastes is essential.

Chemical characteristics of calcium hydroxide

Calcium hydroxide is a strong base obtained from the calcination of calcium carbonate until its transformation into calcium oxide. Calcium hydroxide is then obtained through the hydration of calcium oxide and the chemical reaction between calcium hydroxide and carbon dioxide forms calcium carbonate. It is a white powder with a high pH (12.5) and is only slightly soluble in water (solubility of 1.2 g/l, at a temperature of 25°C).
Estrela and Pesce (1996) chemically analyzed the liberation of calcium and hydroxyl ions from calcium hydroxide pastes with vehicles of different acid-base and hydrosolubility characteristics by means of conductimeter analysis of their solutions in connective tissue of a dog. The liberation of hydroxyl ions from the pastes can be demonstrated by the liberation of calcium ions 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 values in percent of calcium and hydroxyl ions liberated
by calcium hydroxide pastes over a period of 7, 30, 45 and 60 days are reported in Tables 1 and 2.
The properties of calcium hydroxide resulting from ionic liberation are directly influenced by carbon dioxide which, by forming a weak oxide acid, could cause a partial neutralization of the dressing that is basic. Estrela and Pesce (1997) chemically analyzed the formation of calcium carbonate in connective tissue of dogs and showed that when saline vehicles are used with calcium hydroxide paste the rate of formation of calcium carbonate is practically unaltered after 30 days up to 60 days (Table 3). The values in mass of calcium carbonate are small, with an increase up to 30 days and stabilizing at 30-60 days. Thus, after the initial reaction of calcium hydroxide with tissue, a reduction in the number of intracanal medication changes is indicated, especially after initial inflammatory symptoms.
The presence of calcium carbonate in samples of calcium hydroxide stored for 2 years in containers under varying conditions was determined by means of volumetric analysis of neutralization, using hydrochloric acid, and visualization with methyl orange and phenolphthalein. The level of calcium hydroxide converted into calcium carbonate was not significant to interfere with its properties, ranging from 5 ± 1% to 11 ± 1% (Estrela and Bammann, 1999).
Ionic dissociation and diffusion are essential for activity in the interior of dentinal tubules. The change of dentinal pH caused by hydroxyl ions is slow and depends on several factors that can alter the rate of ionic dissociation and diffusion, such as level of hydrosolubility of the vehicle employed, difference in viscosity, acid-base characteristic, dentinal permeability, and level of existing calcification. The pH on the outer surface of dentin after placement of calcium hydroxide paste ranged from 7 to 8, depending on the root third and remained at 12.6 in the interior of the root canal from 1 to 60 days (Estrela et al., 1995a). However, the pH in mineralized structures is difficult to measure. It is necessary to develop an applicable methodology in order to reproduce, as precisely as possible, the transportation of hydroxyl ions through dentinal tubules and then obtain an exact pH.
High pH values can supply a large amount of hydroxyl ions and influence the viability of microorganisms. The pH of vehicles and calcium hydroxide pastes have presented interesting results, such as the low pH (5.0) of camphorated paramonochlorophenol (CMCP), the intermediate pH (7.8) resulting from the association of calcium hydroxide and CMCP and the high pH (12.6) of calcium hydroxide pastes associated with distilled water, sodium lauryl diethylene ether sulfate, Tween 80, and polyethylene glycol. The molar conductivity of calcium hydroxide pastes did not present significant differences associated with distilled water, sodium lauryl diethylene ether sulfate, or Tween 80 (5057.74, 4976.87 and 4936.45 microSiemens, respectively) (Estrela et al., 1998a, 1999a).
The chemical analysis of essential aspects of the substance being studied is important in order to use it correctly, i.e., influence of vehicle on the rate of ionic dissociation; time necessary for dentinal diffusion to reach the appropriate pH level for microbial control and level of reabsorption; action of carbon dioxide from tissue and atmosphere that favors the transformation of calcium hydroxide into calcium carbonate and interferes in the antimicrobial and mineralizing effects, making dressing changes necessary; antimicrobial effect of vehicle and calcium hydroxide paste.

Biological characteristics: mechanism of mineralizing action

An important property of calcium hydroxide is the ability to activate alkaline phosphatase. The pH necessary for the activation of this enzyme varies from 8.6 to 10.3 according to the type and concentration of substratum, temperature and source of enzymes.
Alkaline phosphatase is a hydrolytic enzyme that acts by means of the liberation of inorganic phosphatase from the esters of phosphate. This enzyme can separate phosphoric esters, freeing phosphate ions which then react with calcium ions from the bloodstream to form a precipitate, calcium phosphate, in the organic matrix. This precipitate is the molecular unit of hydroxyapatite (Seltzer and Bender, 1979), which is believed to be intimately related to the process of mineralization.
Calcium hydroxide in direct contact with connective tissue gives rise to a zone of necrosis, altering the physico-chemical state of intercellular substance which, through rupture of glycoproteins, determines proteic denaturation. The formation of mineralized tissue after contact of calcium hydroxide with connective tissue has been observed from the 7th to the 10th day (Holland, 1971). Studying the repair process of dental pulp after pulpotomy with calcium hydroxide, Holland (1971) reported the existence of massive granulation in the superficial granulosis zone interposed between the zone of necrosis and the deep granulosis zone. These structures are composed of calcium salts and calcium-protein complexes. They are birefringent to polarized light, reacting positively to chloramilic acid and to Van Kossas�s method, providing that part of the calcium ions come from protective material. Below the deep granulation zone are the proliferation cellular zone and the normal pulp. Others hydroxides, such as those of barium and strontium, have shown similar effects, using histochemical methods, to those obtained with calcium hydroxide. Holland et al. (1999a) observed the reaction of rat subcutaneous connective tissue to the implantation of dentin tubes filled with mineral trioxide aggregate (MTA) and calcium hydroxide. The results were similar for both materials. At the tube openings, there were Von Kossa-positive granules that were birefringent to polarized light. Next to these granulations, there was irregular tissue like a bridge that was Von-Kossa-positive. The dentin walls of the tubes exhibited in the tubules a structure highly birefringent to polarized light, usually like a layer and at different depths. It is possible that the mechanism of action of MTA, encouraging hard tissue deposition, is similar to that of calcium hydroxide.
The mechanism of action of calcium hydroxide on tissues, inducing the deposition of mineralized tissue, is an extremely important aspect for the indication of calcium hydroxide, because it demonstrates the excellent biological compatibility of calcium hydroxide.

Biological characteristics: mechanism of antimicrobial action

The essential basis for the choice of an intracanal dressing is the knowledge of its mechanism of action on predominant microbiota in infected root canals. The mechanism of action of calcium hydroxide as an antimicrobial medication may be better understood if knowledge about microbiological and pharmacological properties of antibiotics/chemotherapeutics and their effects on microorganisms and, more specifically, their sites of action is adopted as reference. The antimicrobial substances from antibiotics and/or chemotherapeutics cause two kinds of effects on microorganisms: inhibit growth and reproduction, and induce cellular inactivation. These effects can be observed in cellular wall synthesis, altering the permeability of cytoplasmic membrane, chromosomal replication and intermediate metabolism (Estrela and Bammann, 1999). Calcium hydroxide can hydrolyze bacterial lipopolysaccharides degrading the lipid-A and neutralizing its residual effect after cell lysis (Safavi and Nichols, 1994).
The influence of pH on growth, metabolism and bacterial cell division is important to explain the mechanism of antimicrobial action of calcium hydroxide. Estrela et al. (1994) studied the biological effect of pH on the enzymatic activity of anaerobic bacteria. The authors believe that the hydroxyl ions from calcium hydroxide develop their mechanism of action in the cytoplasmic membrane, because enzymatic sites are located in the cytoplasmic membrane. This membrane is responsible for essential functions such as metabolism, cellular division and growth and it takes part in the final stages of cellular wall formation, biosynthesis of lipids, transport of electrons and oxidative phosphorylation. Extracellular enzymes act on nutrients, carbohydrates, proteins, and lipids that, through hydrolysis, favor digestion. Intracellular enzymes located in the cell favor respiratory activity of the 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). The effect of the high pH of calcium hydroxide alters the integrity of the cytoplasmic membrane by means of chemical injury to organic components and transport of nutrients, or by means of the

destruction of phospholipids or unsaturated fatty acids of the cytoplasmic membrane, observed in the peroxidation process, which is a saponification reaction.

Adjustment of intracellular pH is influenced by different cellular processes such as: a) cellular metabolism, b) alterations in shape, mobility, adjustment of transporters and polymerization of cytoskeleton components, c) activation of cellular proliferation and growth, d) conductivity and transport through the membrane, and e) isosmotic cellular volume. Thus, many cellular functions can be affected by pH, including the enzymes that are essential to cellular metabolism (Putnam, 1995).

The understanding of the mechanism of action of the pH of calcium hydroxide in the control of bacterial enzymatic activity allowed Estrela et al. (1994) to suggest the hypothesis of an irreversible bacterial enzymatic inactivation under extreme conditions of pH for a long period of time and also a temporary bacterial enzymatic inactivation with the restoration of normal activity when the pH returns to the ideal level for enzymatic activity. The irreversible enzymatic inactivation was demonstrated by Estrela et al. (1998b) who determined in vitro the direct antimicrobial effect of calcium hydroxide on different microorganisms (Micrococcus luteus, Staphylococcus aureus, Pseudomonas aeruginosa, Fusobacterium nucleatum, Escherichia coli and Streptococcus sp.) during periods of 0, 1, 2, 6, 12, 24, 48, and 72 hours and 7 days. The changes in the cytoplasmic membrane integrity of the microorganisms that favored their destruction occurred after 72 hours in both pure and mixed cultures. Reversible enzymatic inactivation could be observed in another study carried out by Estrela et al. (1999b) who assessed antimicrobial effect of calcium hydroxide in dentinal tubules infected by different microorganisms, during periods of 0, 48, and 72 hours and 7 days. Calcium hydroxide was ineffective by distance action during a period of 7 days against Streptococcus faecalis, Staphylococcus aureus, Pseudomonas aeruginosa and Bacillus subtillis.

These biological activities of calcium hydroxide in tissues and bacteria can be influenced by vehicles, making necessary greater knowledge about their importance and properties.

Discussion

The processes observed between the infection of the dentin-pulp complex and the development of apical periodontitis and consequential host response encouraged the search for greater knowledge about the structure of endodontic microbiota. Knowledge about the morphology, structure and physiology of microorganisms that are responsible for painful states and destruction of periapical tissues motivated several therapeutic trends. The first step of endodontic treatment is the knowledge of the interrelation between microorganisms and host, together with the chemical and biological dynamics of intracanal dressings.

Calcium hydroxide has been the most used, discussed and studied intracanal medication. Although this does not mean that this medication can cure all illnesses, calcium hydroxide has been the first choice as an intracanal dressing in different clinical situations.

Attempts to relate the release of hydroxyl ions of calcium hydroxide with its influence on enzymatic activities of microorganisms helped Estrela et al. (1995b) to explain the mechanism of action of this substance. Since essential functions like metabolism, growth and cellular division require the participation of the cytoplasmic membrane, which is the seat of important enzymatic systems, the changes in physiological activities of microorganisms can be directly influenced by the release of hydroxyl ions which alter the integrity of the cytoplasmic membrane by means of biochemical injuries to organic components, interfere in the transport of nutrients or destroy phospholipids or unsaturated fatty acids, leading to a saponification reaction. Since the site of action of hydroxyl ions of calcium hydroxide includes the enzymes in the cytoplasmic membrane, depending on its quantity, this medication has a large scope of action and therefore affects a varied and diverse range of microorganisms, irrespective of their metabolic capability. In the microbial world, membranes are similar, irrespective of their morphological, tinctorial and respiratory characteristics, which means that this medication has a similar effect on aerobic, anaerobic, Gram-positive and Gram-negative bacteria. This does not occur with the cellular wall that has distinctive chemical and structural characteristics in accordance with their Gram reactivity. Thus, the choice of hydrosoluble vehicles, such as distilled water and saline solution, that can accelerate ionic dissociation and diffusion and interfere in the bacterial enzymatic and tissue systems is indicated (Estrela and Pesce, 1996). Calcium hydroxide P.A. already has this property and its association with a vehicle might not have a synergic effect or may even show an antagonistic effect (Estrela et al., 2000).

However, in order to enhance the properties of calcium hydroxide, it has been associated with several substances (iodoform, barium sulfate, corticosteroid-antibiotic, antibiotics) and vehicles (distilled water, saline solution, anaesthetic solution, methylcellulose, detergent, glycerin, prophylene glycol, camphorated paramonochlorophenol, chlorhexidine solution).

A controversial factor when choosing a vehicle is the comparison between the antimicrobial effect of calcium hydroxide in association with hydrosoluble vehicles (distilled water or saline solution) and in association with oily vehicles (camphorated paramonochlorophenol). Camphor gives an oily characteristic (with low solubility in water) to CMCP that can hamper the ionic dissociation of calcium hydroxide. CMCP alone has a pH of 5.0 and low superficial tension but in association with calcium hydroxide its pH is increased to 7.8. The low availability of hydroxyl ions and rate of ionic dissociation may not offer antimicrobial effectiveness within dentinal tubules.

Surface tension is a force existing between the surface molecules which causes a drop of liquid to spread or to concentrate when placed on a surface. This phenomenon depends on the values of its cohesive forces (force of attraction resulting from the forces that the liquid molecules exert between themselves) and adhesive forces (forces that the surface molecules exert on contact with those of a liquid). The study of the surface tension of CMCP is performed using a solution rather than a paste. This fact features the surface tension of CMCP and not the surface tension of the calcium hydroxide paste associated with CMCP.

In addition to these characteristics of CMCP, calcium hydroxide has shown expressive antimicrobial superiority during the treatment of infected root canals in comparison with camphorated paramonochlorophenol and with camphorated phenol using bacteriological methods (Byström et al., 1985). CMCP is effective by direct contact and not by volatile action (by distance) within the dentinal tubules (Biral et al., 1982). It also has cytotoxic action. Holland et al. (1999c), assessing the effect of hydrosoluble (saline solution) and non-hydrosoluble (CMCP) intracanal dressings in the healing process of teeth of dogs with periapical lesions, observed that 6 months after root canal filling the best rates of repair were attained using hydrosoluble intracanal dressings with saline solution.

The methodology employed in some experiments must also be analyzed. New research is necessary to compare the efficacy of experimental methods intended to analyze the antimicrobial action of substances with different diffusion and dissociation capabilities. Tests of diffusion in agar culture, which use halos of microbial growth inhibition as reference parameters, often do not offer equivalent conditions to compare substances with different solubility and diffusibility and the correct performance of microbiological technique. Factors such as pre-incubation, dry-up culture medium, and maintenance for periods of time that exceed the ideal time allowed for correct analysis can yield doubtful results. The analysis of medications that have different dissociation and diffusion rates by means of an experimental model using liquid culture medium (broth) is more reliable than diffusion tests using agar as culture medium because some substances have difficulty in dissociating and diffusing in agar (semi-solid medium), not expressing their real antimicrobial effect. The method of evaluation by means of diffusion tests in agar has its value and is largely employed in microbiology. This method is not incorrect, it is just less precise for this kind of comparative analysis and is not the most appropriate for comparing these medications. Also, in concurrent evaluations of different antimicrobial agents, it is not simple to admit that the final result is due to an isolated step or medication. In addition, outcomes from different experiments should be carefully examined and interpreted to avoid misunderstandings. It should not be forgotten that microorganisms acquire resistance. The methodology employed can influence results, and for this reason, the experimental model should be carefully selected.

It is important to remember a study carried out by Holland et al. (1999b) comparing the healing process of tissue in teeth of dogs with periapical lesion using three different calcium hydroxide pastes. Six months after root canal filling, results showed that the addition of CMCP to calcium hydroxide (Calen® + CMCP, SS White, RJ, Brazil) did not improve the results of treatment; the addition of anaesthetic solution to calcium hydroxide did not yield better results than polyethylene glycol. Estrela et al. (2000), analyzing the control of microorganisms by calcium hydroxide pastes, showed that an antimicrobial effect occurred after 48 hours for S. mutans, E. faecalis, S. aureus, P. aeruginosa, B. subtilis, C. albicans and a mixed suspension containing all the microorganisms, irrespective of the vehicle (saline, camphorated paramonochlorophenol, 1% chlorhexidine solution, 3% sodium lauryl sulfate, corticosteroid-antibiotic) associated with calcium hydroxide. The absence of differences in the time required for antimicrobial effect indicates that these vehicles play a supportive role in the process, giving pastes chemical characteristics such as dissociation and diffusion as well as favoring the correct filling of the root canal which are decisive factors for antimicrobial potential and tissue healing. Other vehicles and substances have also been added to calcium hydroxide, such as chlorhexidine, detergent, corticosteroid-antibiotic, iodoform; however, the results have not been encouraging (Estrela and Bammann, 1999).

The biological characteristics of calcium hydroxide, represented by its tissue healing and antimicrobial power, make it the best therapeutic choice as an intracanal dressing. The addition of substances and vehicles with the aim of enhancing its acknowledged biological properties (antimicrobial and mineralizing) have not been effective. If the vehicle exerts an expressive influence on the antimicrobial activity of calcium hydroxide, research would have shown the best choice. However, the outcomes of research and clinical experience have indicated that the vehicle has only a supportive role in this process, giving pastes chemical characteristics (dissociation, diffusibility and filling capability) that are decisive to the antimicrobial potential and tissue healing capability.

During the search to explain the antimicrobial mechanism of calcium hydroxide, it was observed that the enzymes in the cytoplasmic membrane were the targets of pH changes, which can lead to a reversible or irreversible inactivation in aerobic, anaerobic, Gram-positive and Gram-negative microorganisms. Obviously the antimicrobial effect depends on the rate of release of hydroxyl ions, availability, time of contact of direct or indirect action (diffusibility of hydroxyl ions within dentinal tubules) in order to show its real power of microorganism control.

Currently, hydrosoluble vehicles (distilled water and saline solution) have presented the best chemical characteristics in terms of speed of ionic dissociation and diffusion, which helps the already known antimicrobial and tissue healing induction powers of calcium hydroxide.

Thus, in order to choose an intracanal dressing, it is necessary to know the microbiota of the infected root canal, the response of host and the mechanism of action of the target medication. However, to be effective, it is necessary that medication has adequate time to express its antimicrobial effectiveness, and to act by distance (indirect action) and neutralize metabolic products, enzymes, toxins, etc.

Conclusions

1. The assessment of experimental methods used to study the antimicrobial effect of calcium hydroxide should be well conducted.
2. The vehicle added to calcium hydroxide gives the paste chemical characteristics influencing the filling and the capability and rate of ionic dissociation and diffusion.
3. Calcium hydroxide associated with hydrosoluble vehicles has better biological behavior, i.e., antimicrobial qualities and induction of tissue repair.

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Correspondence: Professor Carlos Estrela, Rua B1, Quadra 6, Lote 2, Setor Bueno, 74230-180 Goiânia, GO, Brasil.

Accepted November 23, 1999
Eletronic publication: April, 2000

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