Mechanism of Action of Calcium and Hydroxyl Ions of Calcium Hydroxide on Tissue and Bacteria

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| Introduction | Anti-bacterial | Biological | Conclusions | References |

The biological and bacteriological action of calcium hydroxide confer to it its current success as an intracanal dressing. For this reason the mechanism of action of calcium and hydroxyl ions on tissue and bacteria deserves further study. The objective of the present paper is to analyze and discuss the mechanism of action of calcium and hydroxyl ions on anaerobic bacteria, starting from the isolated study of the influence of pH on these bacteria, as well as the mechanism of action of calcium hydroxide on tissue.

Key words: calcium hydroxide, intracanal dressing, anaerobic bacteria.

Introduction

Recent advances in cellular and molecular biology, biochemistry and microbiology have brought about a better understanding and better definitions of certain mysteries still present in endodontics. Modern thinking has been directed towards the use of an intracanal dressing with a potentially effective action against different types of respiratory bacteria (aerobic, anaerobic and microaerophiles) which act by inhibiting the action of osteoclasts present in the area of dental resorption and which favor the repair process of altered periapical tissue.

The destruction of bacterial life is dependent on the conditions related to their growth and multiplication, among which are physico-chemical factors such as: temperature, pH, osmotic pressure, concentrations of oxygen, carbon dioxide and substrate (Nolte, 1982). It has been noted that the response of the periapical tissues to endotoxins produced by gram-negative bacteria, which are predominant in infected radicular canals, assures an opportunity for the repair of the destroyed tissue architeture. This fact can be observed by means of reinsertion of the periodontal ligament and of the reintegration of the alveolar bone, in conjunction with an osteocemental formation. For this reason, the intracanal dressing must be effective against bacteria which may have escaped and survived after root canal preparation, the control of persistent exudate and the destructive action of the osteoclasts present in external dental resorption.

The success of calcium hydroxide as an intracanal dressing is due to its ionic effect observed by the chemical disassociation into calcium and hydroxyl ions and its action on tissue and bacteria. Its capacity to stimulate tissue repair through the induction of mineralization confirms the biological action of calcium hydroxide (Holland, 1971; Binnie and Mitchell, 1973). The superiority of its antibacterial action when compared to other substances has also been shown (Bystron et al., 1985).

Estrela (1994) advocates that calcium hydroxide has resisted the test of time due to two enzymatic properties: the property of inhibiting bacterial enzymes by means of hydroxyl ions that act on the cytoplasmic membrane of the bacteria (generating the antibacterial effect) and that of activating tissue enzymes, such as alkaline phosphatase, which have an influence on mineralization, leading to the mineralizing effect. The chemical and biological dynamics which occur, respectively, in the ionic disassociation of calcium hydroxide and its effect through tissue and bacterial cellular alterations deserve careful discussion and investigation.

Anti-bacterial action of calcium hydroxide - mechanism of action of hydroxyl ions on anaerobic bacteria

The greatest concern in the selection of any dressing is the knowledge of its mechanism of action on the predominant bacterial flora. Antibiotics provoke two types of effects on bacteria. They either inhibit growth or reproduction or they lead to its death. These actions are exercised essentially by interfering in the synthesis of the cell wall, altering the permeability of the cytoplasmic membrane and interfering in protein synthesis.

From this line of reasoning, one can ask about the site of action of calcium hydroxide. Could its mechanism of action be considered similar to that of Penicillin or Cephalosporine, or identical to Nystatin or Polymyxin? The answer given by the literature is that calcium hydroxide is an exceptional antibacterial agent due to its elevated pH.

However, on adopting as a reference the effects of antibiotics against bacteria, and more specifically the site of action, the phenomenon of the action of calcium hydroxide as an antibacterial should be better elucidated. For this reason it is important to analyze the isolated effect of pH on bacterial growth and metabolism, and cellular division.

The essential enzymatic systems of bacteria have as their locale a cytoplasmic membrane where they involve themselves in the last stages of the formation of the cellular wall, participate in the bio-synthesis of lipids, and are responsible for the conveyance of electrons, as enzymes involved in the process of oxidative phosphorylation. Formed by a double phospholipoproteic layer, it acts as an osmotic barrier to ionized substances and to large molecules, being freely permeable to sodium ions and amino acids (selective permeability). When necessary, they produce proteinase that hydrolyze proteins and amino acids, since bacteria are generally incapable of utilizing macro-molecules.

The enzymes located in the cytoplasmic membrane relate themselves to the conveyance of substances to the interior and to the exterior of the cell, by the structuring of the cellular wall and respiratory activity. Extra-cellular enzymes act on the nutrients, carbohydrates, proteins and lipids which, by means of hydrolysis, favor digestion. To sum up, the enzymatic systems of the cytoplasmic membrane take on primordial functions for the bacteria, such as metabolism and cellular growth and division (Burnet and Schuster, 1982).

On the other hand, the catalytic activity of the enzymes can be regulated by variations of the pH of the medium. Each enzyme possesses an optimum pH at which its velocity of reaction is maximum (Lehninger, 1986). However, there is a difference between the internal pH of the bacteria and that of the medium, possibly being responsible for the influence of pH in bacterial cellular activity. As a matter of fact, the mechanism which maintains the internal neutrality in unknown (Kodukula et al., 1988).

The enzymes present internally and externally in the cytoplasmic membrane influence their complex metabolic reactions, the velocity of the chemical reactions favored by those enzymes influenced by the substrate. It is believed that the control of the flow of nutrients alters chemical conveyance through the membrane which is essential to bacterial life (Nolte, 1982; Orten and Neuhaus, 1982).

The energy necessary for the movement of organic nutrients and components into the cell is obtained through a pH gradient present in the cytoplasmic membrane which can be altered by a change of pH of the medium. The effect of the pH on the chemical movement can be direct or indirect. It is direct when the pH influences the specific activity of the proteins of the membrane, with a combination with the specific chemical group. On the other hand, the indirect effect can lead to alterations in the ionization states of the organic components. The transfer through the membrane is facilitated more to non-ionized components than to ionized ones. Depending on the pH there will be an increase of nutrient availability, and an intense transfer can induce inhibition and toxic effects on the cell. In this way, enzymatic activity of bacteria is inhibited in conditions of elevated pH (high concentration of hydroxyl ions) (Kodukula et al., 1988).

The influence of the pH on the transfer and permeability of the cytoplasmic membrane probably explains the microbiological action of the hydroxyl ions of calcium hydroxide in the control of bacterial enzymatic activity. The conveyance of nutrients and the return of the catabolites through the cytoplasmic membrane must be carried out naturally.

Another explanation about the behavior of hydroxyl ions on the cellular membrane comes from the chemical mechanism which is related to lipidic peroxidation. The loss of integrity of the membrane can be observed through the destruction of unsaturated fatty acids or phospholipids. When the hydroxyl ion removes atoms of hydrogen from the fatty acids, a free lipidic radical is formed which, on reacting with the oxygen molecule, is transformed in a lipidic peroxide radical. The peroxide thus formed can act as a new inductor, drawing another hydrogen atom of a second unsaturated fatty acid, resulting in another lipidic peroxide and another new free lipidic radical, transforming itself into a chain reaction (Rubin and Farber, 1990).

For this reason, the elevated pH of calcium hydroxide, with values reaching 12.6, is due to the great liberation of hydroxyl ions which are capable of altering the integrity of the bacterial cytoplasmic membrane through the toxic effects generated during the transfer of nutrients or through the destruction of the phospholipids of unsaturated fatty acids.

With respect to its antibacterial action, Estrela et al. (1994) raised the hypothesis of the possibility of calcium hydroxide producing reversible and irreversible bacterial enzymatic inactivation. The irreversible inactivation can be observed in extreme conditions of pH over a long period of time during which there is a total loss of biological activity of the cytoplasmic membrane. The reversibility of enzymatic activity is encountered on the return to the ideal pH. Lehninger (1986) relates that extreme values of pH cause the uncoiling of many proteins with loss of their biological activities. For many years the process of denaturation was thought to be irreversible. However, if pH returns to its normal value, there is a return of native structure of the lost biological activity, that is to say, there is renaturation. Kodukula et al. (1988) also consider that a reactivation of catalytic activity is possible when the enzyme resumes operating in an ideal pH.

It has also been observed that pH of the interior of the dental tubules and of the external surface of the cement are not as high as the interior of the canal which is in contact with calcium hydroxide paste. Estrela et al. (1994), using a colorimetric method and universal indicating solution, evaluated in vitro the diffusion of hydroxyl ions of calcium hydroxide through the dentin in an inert atmosphere of nitrogen. They observed small modifications of pH on the external surface of the apical cement as well as in the interior of the radicular canal. In the group in which the vehicles were anesthetic solution and saline solution, the pH of the apical cement at 30 days was around 7 to 8, remaining unchanged at 60 days. Meanwhile, in the group whose vehicle was polyethylene glycol, a pH of 7 to 8 in the apical cement was only attained at 45 days, remaining the same at 60 days. In the interior of the radicular canal all the calcium hydroxide pastes maintained a pH of more than 12 during the 60 days of observation.

This alteration of pH on the surface of the apical cement and in the interior of the radicular canal, when calcium hydroxide is used as an intracanal dressing, is due to a greater or lesser dentinal permeability, to the velocity of diffusion of hydroxyl ions, and to the degree of dentinal calcification present.

Biological action of calcium hydroxide - mechanism of action on tissue

Calcium hydroxide, apart from its bacterial enzymatic inhibition which represents an important antibacterial property, has the capability of activating tissue enzymes which favor tissue restoration through mineralization.

The elevated pH of calcium hydroxide activates alkaline phosphotase (Binnie and Mitchell, 1973; Tronstad et al., 1981), the best pH for the activation of this enzyme varying with the type and concentration of substratum, with the temperature and with the source of enzymes, the limits being from 8.6 to 10.3 (Thompson and Hunt, 1966).

Alkaline phosphatase is a hydrolytic enzyme that acts by means of the liberation of inorganic phosphate from the esters of phosphate. It is believed to be intimately related to the process of mineralization (Granstrom and Linde, 1972). This enzyme can separate the phosphoric esters, freeing phosphate ions which, once free, react with calcium ions from the blood stream to form a precipitate, calcium phosphate, in the organic matrix. This precipate is the molecular unit of hydroxyapatite (Seltzer and Bender, 1979).

Calcium hydroxide in direct contact with conjunctive tissue gives origin to a zone of necrosis, altering the physico-chemical state of intercellular substance which, through rupture of glycoproteins, seems to determine proteic denaturation (Holland, 1971). The formation of mineralized tissue after contact of calcium hydroxide with conjunctive tissue has been observed from about the 7th to the 10th day (Holland, 1971; Binnie and Mitchell, 1973).

In this context, Holland (1971), while studying the repair process of dental pulp after pulpotomy with calcium hydroxide, verified in the superficial granulosis zone interposed between the zone of necrosis and the deep granulosis zone, the existence of massive granulation. He goes on to report that these structures are made up of calcium salts and calcium-protein complexes. They show themselves to be birefringent to polarized light, reacting positively to chloramilic acid and to Von Kossas's method, providing that part of the calcium ions come from the protective material. Below the profound granulation zone are the proliferation cellular zone and the normal pulp. Similar results were obtained by Seux et al. (1991).

Other hydroxides, such as those of barium and strontium, by histochemical methods have shown similar effects to those obtained by calcium hydroxide (Holland et al., 1982), reaffirming the active participation of calcium ions from the protective material in the areas of mineralization. Using a different methodology, electronic sweep microscope and a micro-analyzer of dispersion of X-ray (EDX), Wakabayashi et al. (1993) have obtained results which agree with those of Holland et al. (1982).

However, one observes that the hydroxyl ion and the calcium ion of calcium hydroxide act in a synergic way to mineralization. While the hydroxyl ions activate the alkaline phosphatase favoring mineralization, the calcium ions permit the reduction of permeability of new capillaries in granulated tissue of depulped teeth, diminishing the quantity of intercellular liquid and activating the acceleration of perophosphatase while at high concentration, an important factor during mineralization (Heithersay, 1975), and they act in the complement system activity of the immunological reaction (Tronstad et al., 1981).

The mechanism of calcium hydroxide action can be altered in the presence of carbon dioxide (weak oxidec acid) which, by means of chemical reaction, transforms itself into calcium carbonate. The product thus formed is devoid of biological and bacteriological properties due to pH exhaustion.

Estrela (1994) reports that, through chemical analysis, calcium hydroxide pastes were examined for the calcium carbonate formation in conjunctive tissue of dogs. In the paste containing polyethylene glycol 400 as a vehicle, lower values of calcium carbonate mass were found. In periods of 45 to 60 days there was practically a stabilization. He goes on to point out that after the initial reactions of calcium hydroxide with the tissue there are strong motives to reduce the number of calcium hydroxide paste exchanges during its use as an intracanal dressing, principally when the initial inflamatory alteration is overcome.

Conclusions

It is the ionic disassociation of calcium hydroxide into calcium and hydroxyl ions and their effect on bacteria and tissue which make their use so successful. The mechanism of action of calcium hydroxide is directly influenced by its high pH. This results in enzymatic tissue activation, parting from alkaline phosphatase, and also in the inactivation of enzymes of the cytoplasmic membrane of bacteria. These two enzymatic effects of mineralization, which favors the process of tissue restoration, and bacteriological action, which alters the integrity of the sites essential to bacterial metabolism, growth and cellular division, are what confer to calcium hydroxide an important place among intracanal dressings.

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BACK TO CONTENTS