PROTEIN CONTENT IN IRRIGATING SOLUTIONS IN CONTACT WITH PULP TISSUE

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ABSTRACT

Endodontic irrigating solutions may have different effects, one of which is dissolving pulp tissue. The capacity of different irrigants to dissolve vital and necrotic pulp tissue was evaluated in vitro by means of a quantitative and qualitative study of total soluble pulp protein. Vital pulps and pulps with induced necrosis from young bovine teeth were used. Pulp was cut into smaller pieces, weighed and placed in 1 ml of 1% and 2.5% sodium hypochlorite, 1% and 5% calcium hydroxide, 0.2% chlorhexidine gluconate, 1% tea and distilled water as a control, and kept at 37°. Samples of 20 µl were taken at 30 and 90 minutes and 20 hours. Total protein was dosed using the Lowry method and soluble protein bands were determined by electrophoresis (12% SDS-Page). The results were analyzed using Anova.

Chemical analysis of the electrophoretic runs of bovine pulp protein showed that both concentrations of sodium hypochlorite and calcium hydroxide produce denaturation of proteins. No solvent action was found with chlorhexidine, tea or distilled water.

Key words: *sodium hypochlorite, chlorhexidine gluconate, solvent action, pulp tissue.*

CONTENIDO PROTEICO DE SOLUCIONES DE IRRIGACIÓN EN CONTACTO CON TEJIDO PULPAR BOVINO

RESUMEN

Las soluciones de irrigación endodóntica pueden tener distintas acciones. Una de ellas es la de disolver el tejido pulpar. Se evaluó in vitro la capacidad de diferentes soluciones de irrigación para disolver tejido pulpar vital y necrótico mediante un estudio cuanti y cualitativo de proteínas pulpares totales solubles. Se utilizaron pulpas de dientes de bovinos jóvenes, vitales y con necrosis inducida. Se seccionaron en trozos pequeños, que se pesaron y colocaron en 1 ml de las siguientes soluciones: hipoclorito de sodio al 1% y 2,5%, hidróxido de calcio al 1% y 5%, gluconato de clorhexidina al 0,2%, té al 1% y agua destilada (control). Se colocaron a 37°C y se extrajeron muestras de 20 µl a luego de 30 min, 90 min y 20 hs. Se dosaron proteínas totales utilizando el método de Lowry y por electroforesis (SDS-Page 12%). Se determinaron bandas de proteínas solubles. Los resultados se analizaron por Anova

En el analisis químico, de las corridas electroforéticas de proteínas pulpares bovinas se evidenció que tanto el hipoclorito de sodio en ambas concentraciones como el hidróxido de calcio producen desnaturalización de proteínas. No se demuestra acción solvente con clorhexidina, té y agua destilada.

Key words: *hipoclorito de sodio, hidróxido de calcio, gluconato de clorhexidina, acción solvente, tejido pulpar.*

INTRODUCTION

Clinically, the aim of a proper root canal debridement is to use biomechanical instrumentation to contact, loosen and remove from the pulp walls and lateral canal spaces all suspended debris which is retained due to the complexity and irregularity of the canal system[1].

Various authors have stated that the instruments alone cannot reach all parts of the canals and that careful instrumentation should be complemented by the use of different irrigating solutions[2].

Sodium hypochlorite (NaOCl) is a very effective solvent of pulp tissue[3]. When it comes into contact with organic material, it alters cell biosynthesis and liquefies the tissue[4,5,6].

Moreover, chlorhexidine gluconate is commonly used for irrigation during periodontal treatment, and Delaney suggested its use in endodoncy in 1982[7]. Its antimicrobial effect is achieved by different mechanisms, among which the solvent effect has been studied by Okino et al.[8] and de la Casa et al.[9].

Calcium hydroxide $Ca(OH)_2$ is used in endodoncy for its antimicrobial effect and stimulation of mineralization[10]. The solvent action of $Ca(OH)_2$ paste was described by Hasselgren et al. in 1988[11]. They

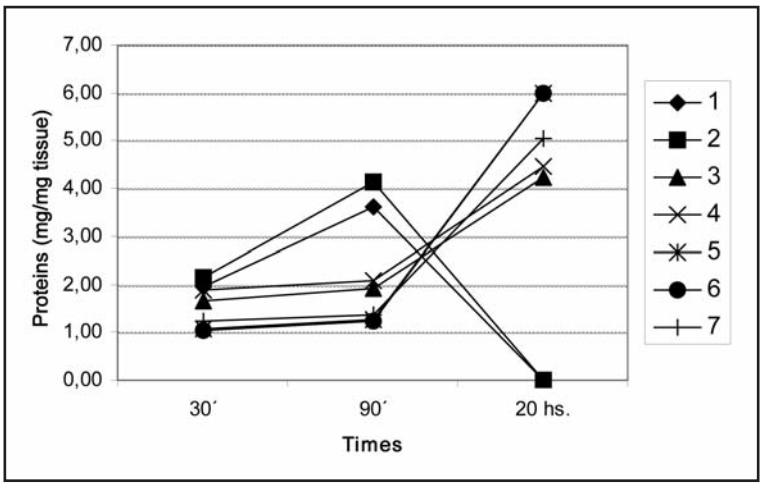

Fig. 1: Total proteins in vital pulps at 30 and 90 min and 20 hs. 1) 1% sodium hypochlorite; 2) 2.5% sodium hypochlorite; 3) 1% calcium hydroxide; 4) 5% calcium hydroxide; 5) 0.2% chlorhexidine gluconate; 6) 1% tea; 7) Distilled water (control).

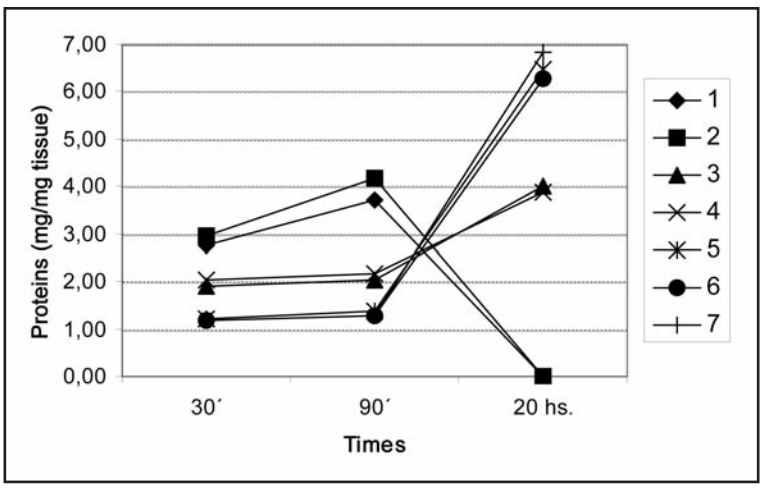

Fig. 2: Total proteins in necrotic pulps at 30 and 90 min and 20 hs. 1) 1% sodium hypochlorite; 2) 2.5% sodium hypochlorite; 3) 1% calcium hydroxide; 4) 5% calcium hydroxide; 5) 0.2% chlorhexidine gluconate; 6) 1% tea; 7) Distilled water (control).

found that treatment with $Ca(OH)_2$ dissolved necrotic muscle tissue of pigs.

Various medicaments have been used for disinfecting root canals. It has also been found that certain components extracted from Japanese green tea or black tea have antimicrobial action[12].

The aim of this study was to measure the solvent capacity of different irrigating solutions on vital and necrotic pulp tissue through a qualitative and quantitative study of pulp protein.

MATERIALS AND METHODS

The following solutions were used:
1% and 2.5% sodium hypochlorite
1% and 5% calcium hydroxide
0.2% chlorhexidine gluconate (ICN Biomedicals Inc. Ohio. USA)
1% tea solution (Green Hills, Argentina)
Distilled water (control).

Tissue preparation

Pulps from teeth of young bovines were used. Vital pulps were extracted from the maxillaries and kept at -14°C until they were processed.

To obtain necrotic pulps, the same extraction process was carried out, after which tissue autolysis was produced by placing them in covered beakers at a temperature of about 25°C for 72 hours. A scalpel was used to cut the pulps thus obtained from both groups into smaller pieces weighing 25 to 35 mg. They were weighed on a watchglass using a precision analytical balance (Acculab, Argentina 221). Pieces of pulp tissue were placed in ten Kahn tubes with 1 ml each of the irrigation solutions, and kept at 37°C in a thermostatized bath (Vicking (mo Masson, Argentina); 1% sodium azide was added to each solution to prevent bacterial growth.

For quantitative analyses, 20 µl samples were taken at 30 and 90 minutes and 20 hours, and total protein was determined using the Lowry method[13]. Qualitative analysis of soluble protein was done by means of 12% SDS-Page, stained with 0.1% Coomassie Brilliant Blue R-250.

The ANOVA Test was applied for statistical analysis.

RESULTS

Figures 1 and 2 show the protein concentration in the liquids in contact with vital and necrotic pulp tissue as a function of time. Both graphs show that the protein content drops completely at 20 hours in sodium hypochlorite solutions. It was found that proteins were present in calcium hydroxide solutions, and in even higher concentrations in

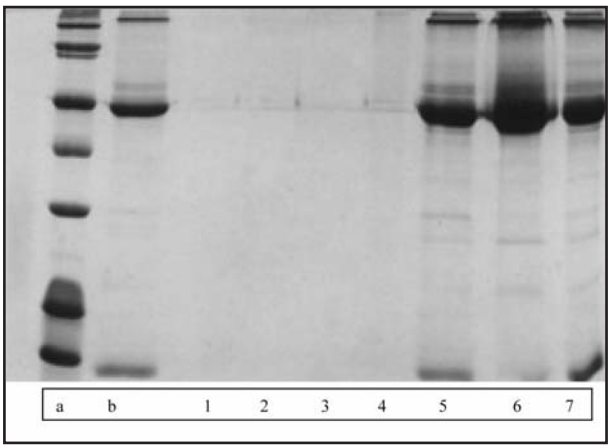

Fig. 3: 12% SDS – PAGE for vital bovine pulps. a) 6 to 200 kDa protein markers; b) Vital bovine pulp extract; 1) 1% sodium hypochlorite; 2) 2.5% sodium hypochlorite; 3) 1% calcium hydroxide; 4) 5% calcium hydroxide; 5) 0.2% chlorhexidine gluconate; 6) 1% tea; 7) distilled water.

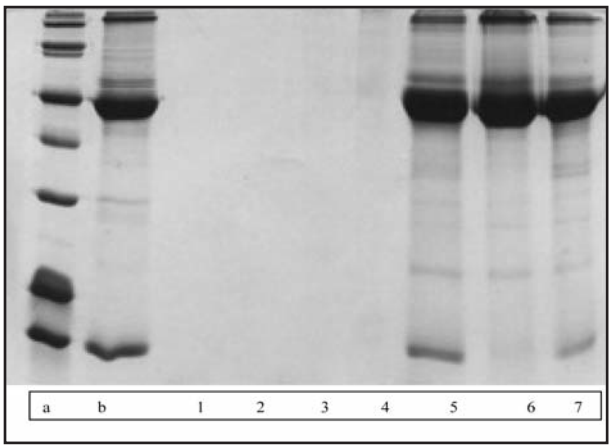

Fig. 4: 12% SDS – PAGE for necrotic bovine pulps. a) 6 to 200 kDa protein markers; b) Necrotic bovine pulp extract; 1) 1% sodium hypochlorite; 2) 2.5% sodium hypochlorite; 3) 1% calcium hydroxide; 4) 5% calcium hydroxide; 5) 0.2% chlorhexidine gluconate; 6) 1% tea; 7) distilled water.

chlorhexidine and tea solutions and distilled water ($p<0.0001$).

Figures 3 and 4 show the electrophoretic runs of the protein in the liquids in contact with the vital and necrotic bovine pulp tissue at 20 hours. Both concentrations of sodium hypochlorite and calcium hydroxide showed no protein, possibly due to the dissolution of the tissue. In contrast, proteins were observed in the chlorhexidine and tea solutions and distilled water, where there was no solvent effect.

DISCUSSION

The concentration of collagen in the pulp of human teeth is 30% to 37% of the protein content[14]. The collagen in the pulp tissue varies considerably among animals of different species, as shown by studies on rats, pigs and bovines[15], and rabbits[16].

Although Orlowski[15] showed that the collagen concentration is lower in bovine pulp than in human pulp, van Amerogen et al.[17], found a similar quantity of type III collagen in human and bovine premolars.

In a previous experiment[18], we determined total protein and hydroxyproline as well as soluble protein using 12% SDS-Page electrophoresis on living human and bovine pulps, and found a higher number of protein bands in humans.

Koskinen et al.[19] obtained the best measurements of solubilization with dry weight and hydroxyproline (an amino acid from collagen) in residual tissue after incubation with different solutions.

The solution most often used in odontological practice is NaOCl, which under experimental conditions was more efficient at 2.5% y 5% than at 0.5%[3]. These findings were supported by histological and gravimetrical methods[20].

Spanó et al.[5], achieved similar results and observed that there is dissolution of pulp tissue and residual chlorine at all the concentrations studied, but that when a higher initial concentration is used, there is greater reduction of surface tension. They tried to provide clinical orientation, suggesting that the concentration to be used could depend on the instrumentation time in canals with necrosis, using lower concentrations for long instrumentations, while in cases where dental rotary instruments are used, more concentrated solutions could be used.

It is worth noting that during the evaluation time in this study, the irrigation solution was in permanent contact with the pulp tissue, without renewal. However, in daily clinical practice the situation is different because irrigation is done by adding new solution each time, with unaltered pH and physical and chemical characteristics.

Moreover, Routh et al.[21] consider that the action of $Ca(OH)_2$ on tissues consists of the denaturation and hydrolyzation of organic matter, which alters the tissue structure. Nevertheless, there are few studies of the solvent capacity of calcium hydroxide solutions[22]. Most studies on the solvent capacity of calcium hydroxide were done with solution and paste[23,24].

For this paper, a chemical study of the irrigating liquid that had been in contact with pulp tissue for different times was done, with total protein determination. Greatest solubilization was observed with both NaOCl concentrations, followed by the calcium hydroxide solutions.

Koskinen et al.[19] were not able to measure hydroxyproline in pulp extracts with the NaOCl solution because it is probably broken down by the NaOCl solution. Conversely, Trepagnier et al.[3] measured considerable quantities of hydroxyproline after treatment with 2.5% and 5% NaOCl.

Yamaguchi et al.[25] conducted research to evaluate the effects of NaOCl on the components of human blood. They showed that the higher the concentration of NaOCl, the lower the molecular weight of the fragments that the protein components in the supernatant break down into. When SDS-Page is used, the protein bands are difficult to see in the NaOCl treatment. This matches our electrophoretic determinations of the liquid in contact with the pulp tissue. Lower visualization of soluble proteins was shown with NaOCl and $Ca(OH)_2$, though not with chlorhexidine gluconate, tea and distilled water.

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