

Analysis of bovine pulp tissue dissolution ability by photodynamic therapy: an in vitro study

Análise da capacidade de dissolução de tecido pulpar bovino através da terapia fotodinâmica: estudo *in vitro*

Abstract

Purpose: To evaluate the bovine pulp tissue dissolution ability of photodynamic therapy.

Methods: Twenty pieces of bovine pulp tissue were weighed and divided randomly into four groups (n=5), according to the cleaning protocol: G1 – distilled water (negative control), G2 – sodium hypochlorite 1% (positive control), G3 – photodynamic therapy, G4 – sodium hypochlorite 1% + photodynamic therapy. The observation of the events of dissolution was performed by two observers blinded in relation to the test using 2x magnification, recording time in minutes until complete tissue dissolution. The total observation time was 2 hours. The dissolution rate was calculated dividing the weight of the fragment pulp (mg) by the time of dissolution (mg/min).

Results: Only group 2 (NaOCl) was able to promote complete dissolution of pulp tissue. In the other groups there was no occurrence of complete dissolution of the samples. The mean dissolution time for samples from group 2 (NaOCl) was 1.26 mg/min.

Conclusion: Only the sodium hypochlorite was able to dissolve the fragments of bovine pulp tissue and photodynamic therapy does not show ability to dissolve tissue.

Key words: Pulp dissolution; sodium hypochlorite; photodynamic therapy

Resumo

Objetivo: Avaliar a capacidade de dissolução de tecido pulpar bovino com o uso da terapia fotodinâmica.

Metodologia: Vinte fragmentos de polpas bovinas foram pesados e distribuídos randomicamente em quatro grupos (n=5) de acordo com os seguintes protocolos de limpeza: G1 – água destilada (controle negativo), G2 – hipoclorito de sódio 1% (controle positivo), G3 – terapia fotodinâmica, G4 – hipoclorito de sódio 1% + terapia fotodinâmica. A observação dos eventos de dissolução foi realizada por dois observadores, cegados quanto aos grupos experimentais, com lupa de 2x de aumento, que registravam o tempo em minutos até a completa dissolução de tecido. O tempo total de observação foi de 2 horas. O cálculo da taxa de dissolução foi feito dividindo o peso do fragmento pelo tempo de dissolução (mg/min).

Resultados: Somente o grupo 2 (NaOCl 1%) foi capaz de promover dissolução completa do tecido pulpar, tendo como média de dissolução 1,26 mg/min.

Conclusão: Apenas o hipoclorito de sódio foi capaz de dissolver os fragmentos de tecido pulpar bovino e a terapia fotodinâmica não mostrou capacidade de dissolução tecidual.

Palavras-chave: Dissolução pulpar; hipoclorito de sódio; terapia fotodinâmica

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Introduction

The persistence of residual pulp tissue, infected dentin or bacteria inside the root canals may be responsible for the failure of endodontic treatment (1). Irrigating agents must present capacity of dissolving pulp remnants (2), since the removal of pulp tissue is inadequate with mechanical preparation alone, due to the complexities of the anatomical root canal system (3). Moreover, it is suggested that postoperative pain is more prevalent in cases of vital pulp than in cases of non-vital pulp (4) and the remaining pulp can cause postoperative pain (5).

Several studies have been conducted in search of an irrigant to provide four major properties: antimicrobial activity, non-toxicity to periapical tissues, solubility in water and ability to dissolve organic matter (6).

Sodium hypochlorite (NaOCl) is considered the main irrigating substance in endodontics because of its broad antimicrobial spectrum, its ability to prevent the formation and dissolving the organic part of the smear layer, and its ability to dissolve organic tissue remnants (2). However, it has been shown a cytotoxic effect on vital tissues, causing severe inflammatory reactions to the periapex, with the concentration of 5.25% producing more toxic and caustic solutions than 0.5 and 1% (7). Moreover, low concentrations of sodium hypochlorite have reduced the ability to dissolve tissue (8), although this can be improved by increasing the temperature (4,5).

Photodynamic therapy (PDT) or photoactivated disinfection uses light of a specific wavelength to activate a non-toxic photoactive dye, known as the photosensitizer in the presence of oxygen (9). The energy transferred from the activated photosensitizer to available oxygen results in the formation of highly reactive oxygen species, which may eliminate microorganisms by damaging their essential cellular molecules, including proteins, nucleic acids and lipid membranes (10). *In vitro* (11,12) and *in vivo* studies (13,14) using photodynamic therapy have shown that this resource has the potential to maximize the disinfection of root canals.

However, there are no studies reporting the capability of photodynamic therapy in the dissolution of organic tissue. The aim of this study was to evaluate the ability of the dissolution of bovine pulp tissue through the action of photodynamic therapy.

Methods

The study was approved by the Ethics Committee of the Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, RS, Brazil.

Tissue preparation

Ten bovine incisors were extracted, immersed in distilled water and stored at a temperature of -20°C until required. The teeth were thawed at room temperature and two longitudinal grooves were prepared in buccal and lingual surfaces using

a diamond disc (KG Sorensen, Barueri, Brazil), running from the crown portion to the apex. The teeth were split in half. The pulp tissue was removed and washed with distilled water. Each pulp sample was divided into two pieces of similar volume, resulting in 20 pieces.

Preparation of solutions

A solution of 1% sodium hypochlorite was prepared at the Endodontic Laboratory, School of Dentistry, Pontifical Catholic University of Rio Grande do Sul, from the solution of 2% sodium hypochlorite (Plus Virex - Johnson Diversey, Sturtevant, USA) diluted in distilled water in the proportion 1:1. A viscous solution of tolonium chloride was provided by the manufacturer of the photodynamic therapy device (PAD Plus, Denfotex Light Systems Ltd., Inverkeithing, Scotland).

Dissolution process

The pulp tissue fragments were weighed on a high precision balance (Sartorius BP61S, Göttingen, Germany), placed in transparent plastic pots and were divided randomly into four groups (n=5) according to initial weight:

- Group 1 – immersion in 1.5 mL of distilled water (negative control)
- Group 2 – immersion in 1.5 mL of 1% sodium hypochlorite (positive control)
- Group 3 – immersion in 1.5 mL of tolonium chloride, introduction of the tip of the PAD Plus (Denfotex Light Systems Ltd., Inverkeithing, Scotland) and activation of low power laser during a period of 120 s on the power of 120 mW
- Group 4 – immersion in 1.5 mL of 1% sodium hypochlorite for a period of 40 min, immersion in 1.5 mL of distilled water, removal of the pulp fragment and immersion again in 1.5 mL of tolonium chloride, introduction of the tip of the PAD Plus (Denfotex Light Systems Ltd., Inverkeithing, Scotland) and activation of low power laser during a period of 120 s on the power of 120 mW.

The observation of the events of dissolution was performed by two observers blinded in relation to the test using 2x magnification, recording in minutes the time until complete tissue dissolution occurred. The total observation time was 2 hours. The time required for dissolution was recorded in minutes (min) and the dissolution rate was calculated by dividing the weight of the fragment pulp (mg) by the time of dissolution (mg/min).

Results were presented in a table with average weights and dissolution times. Since only one experimental group showed total dissolution, no further statistical analysis was needed.

Results

The weight and time of dissolution of each pulp fragment are shown in Table 1. Only group 2 (NaOCl) was able to promote complete dissolution of pulp tissue. In the other groups there was no occurrence of complete dissolution of the samples.

Table 1. Weight and time dissolution of dissolution of each pulp tissue sample.

Group	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5	
	Weight (mg)	Time (min)								
1	99.7	nildiss	138.7	nildiss	71.7	Nildiss	69.7	nildiss	83.0	nildiss
2	67.8	55	140.2	90	83.2	75	102.1	85	79.3	70
3	66.8	nildiss	109.4	nildiss	108.2	Nildiss	72.8	nildiss	102.2	nildiss
4	123.3	nildiss	145.7	nildiss	52.5	Nildiss	49.5	nildiss	52.7	nildiss

The dissolution rate was calculated on the weight of the fragment of pulp tissue (mg) divided by the dissolution time (min), obtaining a value in mg/min. The mean dissolution time for samples from group 2 (NaOCl) was 1.26 mg/min.

Discussion

Previous studies have demonstrated the importance of the ability of an endodontic solvent and emphasized that the elimination of pulp tissue of the root canal was primordial to the success of endodontic treatment (15,16). The bovine pulp tissue was used in our study because it is compared to human pulp tissue despite some minor differences (17). Moreover, previous studies have used the bovine pulp tissue to assess the capability of dissolving various endodontic irrigants (18,19).

As a larger volume of sodium hypochlorite and contact surface leads to a greater ability to dissolve (1,3) it can be speculated that the pots used in this experiment may have reduced the time of dissolution compared with the normal clinical conditions (*in vivo*), as it is most likely to occur according to previously reported *in vitro* investigations (4,18,19).

The tissue dissolution is dependent on three factors: frequency of agitation, amount of organic matter in relation to the amount of irrigating and surface area of contact (1). The present study was standardized by using the same volume of irrigant and dye for the samples of the respective groups, besides the fact that all samples of bovine pulp tissue fragments showed similar mean weights (90 mg).

The results were calculated in dissolution rates and not only time of dissolution, to compensate any variations in the weights of the fragments. The results of this study appear to be in line with previous investigations where only sodium hypochlorite showed ability to dissolve tissue (18,20), although in a previous study the average speed of tissue dissolution to sodium hypochlorite was lower (18). This can be explained by differences in the volume of irrigant in contact with the pulp fragment, specifically the size and weight of the fragments, the concentration of

sodium hypochlorite solutions, and perhaps a difference in temperature of the solutions.

Photodynamic therapy is a new antimicrobial strategy that involves the use of low-intensity laser, which operates through photosensitizing agents (21). The low-intensity laser, beyond been considered harmless to human tissue, has anti-inflammatory and analgesic effects. Its basic mechanism of the operation is based on biostimulation that occurs at the molecular level. The laser penetrates through the tissues and faces a photosensitizer in mitochondria of cells (22).

Disinfection by photo-activation method proves effective against endodontic bacteria, and has proved less toxic and faster than sodium hypochlorite (23). Furthermore, the infiltration of dentin tubules by sensitizers was evaluated microscopically, indicating the effectiveness of this therapy within the tubules (11). There are still records that indicate oral bacteria as susceptible to photodynamic therapy (12).

However, there are no previous studies that elucidate the capacity of tissue dissolution of photodynamic therapy. The results of this study show that photodynamic therapy has not got potential to dissolve the fragments of bovine pulp tissue in the tested groups, both when used alone, with the dye in contact with the pulp fragment and light activation, and when used in combination with sodium hypochlorite, where after immersion in 1% sodium hypochlorite the fragment was placed in contact with the dye and activated through photodynamic therapy.

The idea was that photodynamic therapy could exert an ability to dissolve in a previously unstructured tissue by initial action provided by previous immersion in sodium hypochlorite. However, this could not be observed, since the dissolution process was interrupted from the laser action, without such a development.

Conclusion

In conclusion, only the sodium hypochlorite showed ability to dissolve the fragments of bovine pulp tissue and photodynamic therapy does not aid to provide further dissolution.

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