Received: 12th January 2014 Accepted: 13th April 2014 Conflicts of Interest: None

Source of Support: Nil

Original Research

Tecidual Reaction of Calcium Hydroxide Front Chronic Stress Histological Study in Rats

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How to cite the article:

Semenoff TA, Vieira EM, Borges AH, Bandeca MC, Santos RS, Pedro FL, Silva NF, Semenoff-Segundo A. Tecidual reaction of calcium hydroxide front chronic stress histological study in rats. J Int Oral Health 2014;6(4):4-8.

Abstract:

Background: The Calcium Hydroxide has been widely used as an intracanal dressing and in combination with restorative and endodontic materials and its main goal is the tissue reparation. However, when the patient has chronic stress, the immunological response and tissue repair decreases in both the epithelial and connective tissue. Therefore, the aim was to analyze the effect of chronic stress on the tissue response in rats exposed to calcium hydroxide (CH).

Materials and Methods: A total of 60 wistar rats were anesthetized, and a polyethylene tube containing CH was inserted under the skin. After 24 h, they were divided into two groups: Calcium hydroxide + stress (CHSG) n = 30 and calcium hydroxide (CHG) *n* = 30. They were stressed by physical restraint, for 12 h each day for periods of 7, 15 and 30 days when 10 animals from each group were euthanized. The tissues surrounding the polyethylene tubes were removed, and slides were prepared and stained with hematoxylin and eosin. The analysis was performed with an optical microscope with magnification of 4-400 times by a blinded senior examiner. The sample slides were classified according to the following scores: 0 - absent/1 - present/2 - infiltrate to: Inflammatory infiltrate containing fibrous condensation, lymphocytes, plasmacytes, macrophages, neutrophils, and eosinophils. The data were statistically analyzed using the Student's *t*-test ($P < 0.05$) for paired samples.

Results: The exposure time of 7 days elicited no statistical difference between groups ($P > 0.05$). The 15 days exposure group had higher averages for CHG to eosinophils and inflammatory infiltrate ($P < 0.05$). In 30 days, CHG showed higher averages to inflammatory infiltrate and lower averages to FC ($P < 0.05$).

Conclusions: Some modified patterns of responses in the CHSG were observed at 15 days and 30 days.

Key Words: Calcium hydroxide, laboratory study, rats, stress

Introduction

Calcium hydroxide (CH) is a material of great importance in dentistry. Its uses include protecting the dentin-pulp complex,[1] as an intra-canal medication[2] and endodontic sealer.[3] Its physiochemical properties are widely studied and applied clinically in sanitizing the root canal system[4] and for tissue repair induction.[5] Despite its acknowledged efficiency, there are reports of failure in endodontic therapy.[6] The host response seems to contribute to the progression of the apical periodontium diseases.[7]

The use of CH is associated with repair process of dental pulp and periapical lesions. Initially, the material in contact with tissue will cause a superficial necrosis; adjacent to the release of hydroxyl able to provide a range of immune inflammatory responses will occur.[8,9] In addition, there is the formation of collagen Type I and fibronectin, both may stimulate odontoblasts to form new mineralized tissue.[10] Already in the early stage of the repair tissue mineralization occurs.[9,10]

These tissue responses are much influenced by environmental factors. One of these factors may be the stress that is recognized as an agent that influences the rate of tissue repair and biological systems of mammals.[11]

Local,[12] acquired and environmental factors, as tobacco use,[13] diabetes[14] and vascular problems[15] are connected to the apical periodontal disease. Important to mention that many aspects are still unknown in the pathogenesis of this disease.[16] Among the environmental factors involved in people's lives is stress. In a literature search, the connection of stress and apical periodontitis was little evident. Thus, it is even more difficult to obtain information about the use of CH front of a stressed body. Therefore, the study will seek to understand the effects of stress on wound healing in rats. Stress has significant societal effects.[17] It can stimulate the Hypothalamic-Pituitary-Adrenal triad, exacerbating diseases of emotional, autoimmune, neoplastic and cardio-respiratory origin, in addition to inhibiting tissue repair.[18,19]

Studies in rats produce valuable information about the biological understanding of mammals.[20] These tests help to control behavioral and environmental biases, mainly with the production of information that humans would be impossible to achieve. Thus, given the deleterious effects of chronic stress

on the body, this study sought to evaluate the effect of stress on the biocompatibility of CH paste in rats.

The aim of this study was to analyze the effect of chronic stress on the tissue response in rats exposed to CH.

Materials and Methods

Sixty adult rats-Rattus Novergicus Wistar-were selected, having an average weight of 250 g. They were obtained from the vivarium of UNIC (University of Cuiabá, Cuiabá, MT, Brazil). They were housed in boxes (16 × 40 × 30 polyethylene) in groups of five and were fed a standard diet and water, ad libitum. They were maintained with a light/dark cycle of 12 h at a controlled temperature of 23°C and ± 50% humidity. (Ethics Committee CEP/University of Cuiabá, Brazil, by protocol number 2010-045).

Study design

Initially, the animals were randomly divided into two groups of 30 animals each: Calcium hydroxide group (CHG) and calcium hydroxide + stress group (CHSG). CH within polyethylene tubes was inserted into the subcutaneous tissue on the back of the animals. At intervals of 7, 15 and 30 days, 10 animals were randomly selected from each group for slaughtering. The tissue samples were removed and submitted for histological processing.

Stress model

The CHSG animals were placed in polyvinyl chloride tubing compatible with their size. The tube ends were sealed with wire to allow them to breath and to keep them in a fixed position. The animals were maintained in this confined space for 12 h, in the period between 6 am and 6 pm.

Preparation of calcium hydroxide

For inoculation of the materials to be tested, 60 cylinders 1 cm in length and 1.3 mm internal diameter from polyethylene tubing (19G Venescalp, Feira de Santana, Ba, Brazil) were used. Next, 9 g of CH powder (Iodontosul - Indústria Odontológica do Sul Ltda, Porto Alegre, RS, Brazil) was mixed with 10 ml of distilled water. The solution was injected into the cylinders using disposable syringes (BD-Becton Dickinson Indústrias Cirúrgicas Ltda, Curitiba, PR, Brazil) without needles.

Surgical procedure

All surgical procedures were performed under general anesthesia by intra-muscular injection of 0.1 ml of ketamine (Dopalen, Agribrands. Saúde Animal, Paulínia, SP, Brazil) combined with 0.05 ml of xylazine hydrochloride (Rompun, Bayer, Saúde Animal, São Paulo, SP. Brazil) for each 100 g of body weight. After anesthetizing the animals, we shaved the dorsal right lateral region, followed by disinfection with 2% chlorhexidine (FGM Dental Products, Joinville, SC, Brazil). 1 cm incision was made, and the underlying tissue was released with a blunt instrument. The polyethylene tube containing CH was inserted and sutured with nylon 5.0 (Shalon Fios Cirúrgicos, São Luis de Montes Belos, GO, Brazil).

Histological analysis

After the appropriate days of exposure, the test animals were euthanized. A segment of tissue from the area containing the CH and the higher degree of inflammation was fixed in 10% buffered formalin for 48 h. The tissues were prepared and sectioned into 6 micron thick slices, and were then stained with hematoxylin and eosin. Ten histological slides from each block were optically analyzed at ×4 to ×400 magnification, by a single independent investigator. The analysis looked for the presence of: Inflammatory infiltrate, fibrous condensation (FC), lymphocytes, plasmacytes, macrophages, neutrophils and eosinophils. The slides were then classified according to the following scores: 0 - absent/1 - present/2 - infiltrate.

Analysis of results

The statistical test used in the study was the Student's *t*-test for paired samples with a 5% significance level.

Results

The exposure time of 7 days (Figure 1) elicited no statistical difference among groups for any variable analyzed ($P > 0.05$) (Table 1).

The exposure time of 15 days (Figure 2) presented statistical differences to CHG compared with group CHSG (Table 1) for the variables eosinophils and inflammatory infiltrate ($P < 0.05$). The other variables were not statistically different ($P > 0.05$).

In the exposure time of 30 days (Figure 3), statistically significant differences were found, with higher averages

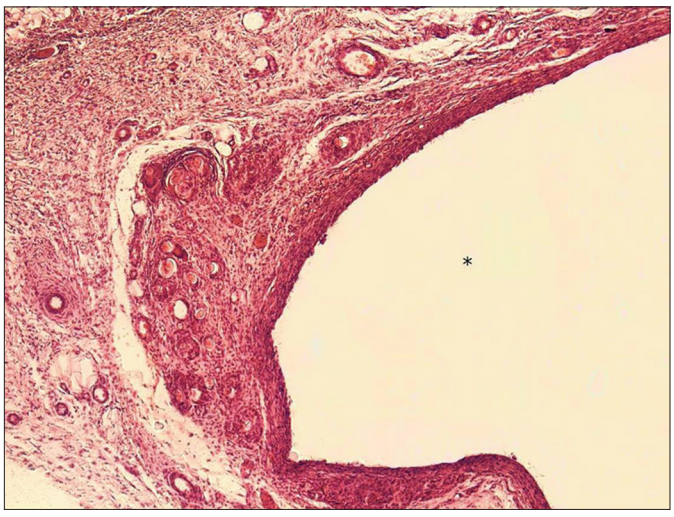

Figure 1: Microscopic aspects observed within 7 days in the calcium hydroxide group. Capsule of variable thickness around the tube area (*) with an intense inflammatory infiltrate (H and E, ×10).

Table 1: Refers to means and standard deviations of the study variables scores.

Day	Groups	II	FC	L	P	M	N	E
7	CH	2.7±0.4	1.1±1.6	2.2±0.4	1.2±0.4	1.8±0.3	1.0±0.0	0.0±0.0
	CHS	2.7±0.4	1.4±1.6	2.4±0.5	1.4±0.5	1.6±0.5	1.0±0.0	0.3±0.4
15	CH	2.0±0.7*	2.7±1.1	1.4±0.7	1.0±0.7	0.8±0.7	0.5±0.5	0.7±0.4*
	CHS	0.7±0.6*	2.3±1.1	1.6±0.7	1.1±0.3	1.1±0.3	0.8±0.4	0.2±0.4*
30	CH	2.5±0.7*	1.2±0.4*	1.6±0.5	1.2±0.4	1.1±0.3	1.3±0.7	0.0±0.0
	CHS	0.8±0.6*	2.3±1.1*	1.8±0.7	1.1±0.4	1.1±0.4	1.0±0.0	0.0±0.0

CH: Calcium hydroxide, CHS: Calcium hydroxide associated with stress, II: Inflammatory infiltrate, FC: Fibrous condensation, L: Lymphocytes, P: Plasmocytes, M: Macrophages, N: Neutrophils, E: Eosinophil. *Indicates statistical differences between groups (P<0.05)

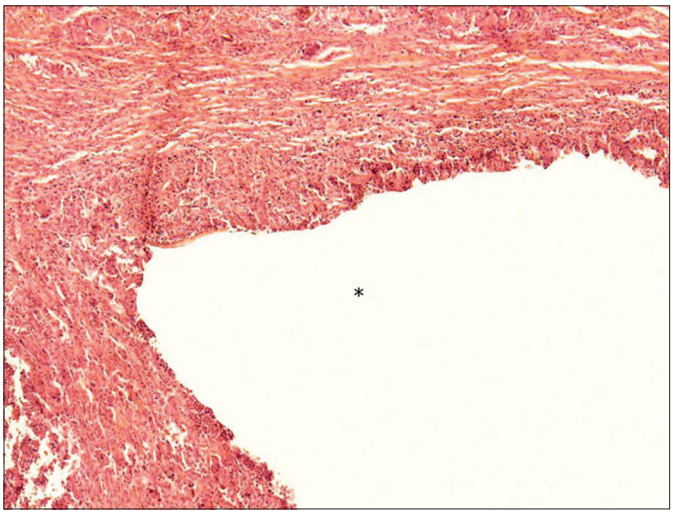

Figure 2: Microscopic aspects observed within 15 days in the calcium hydroxide group. Thick capsule around the area of the tube (*) with moderate inflammatory infiltrate and collagen (H and E, ×10).

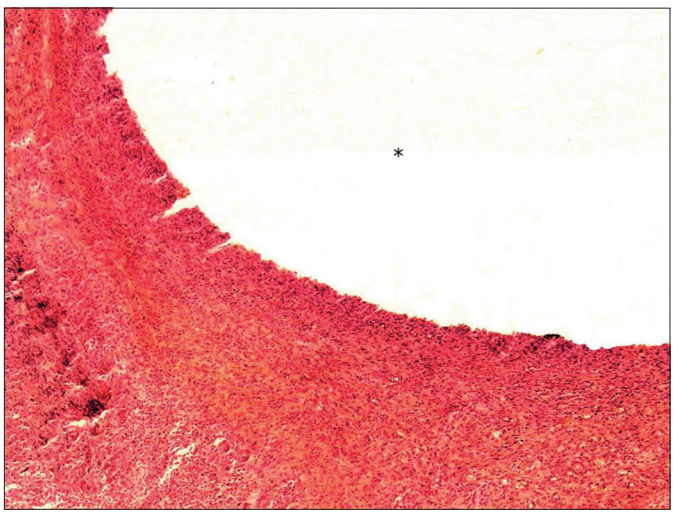

Figure 3: Microscopic aspects observed within 30 days in the calcium hydroxide group. Thick fibrous capsule around the area of the tube (*) with intense fibroblast proliferation and collagen (H and E, ×10).

for CHG for the variables inflammatory infiltrate and FC (P < 0.05). The other variables were not statistically different (P > 0.05) (Table 1).

Discussion

Chronic stress is generally recognized to decrease the immunological response and tissue repair in both the epithelial and connective tissue of humans and animals.[21] Although established as a modulating agent for the deterioration of mammal defenses, making them more susceptible to infectious diseases involving the immune system,[19,22-24] the mechanism of this response is unclear. Observing post-traumatic stress disorders, it seems that some immune cells types are more readily activated.[23] The CH has several actions, among which are the indirect anti-inflammatory effect, biocompatibility, antimicrobial and guidance of mineralized material.[5,25,26] At 15 days of exposure, we observed a decrease in the inflammatory infiltrate in CHSG compared with the CHG groups.

This result corroborates with another study[27] that reported that large amount of cortisol in tissues decreases the immune-inflammatory response and causes a delay in wound repair. One more interesting finding was a decrease of eosinophils. This type of cell is known to be connected to a foreign body reaction. Stress was able to reduce the number of eosinophils to standard scores below normal. At 30 days, no specific reaction to the material was observed.

It is known that CH is able to stimulate immuno-inflammatory cascade response. Given this response there is the production of interleukins (ILs) from inflammatory cells such as macrophages[28] capable to produce mediators such as IL1. It acts directly on corticotropin-releasing hormone; which in turn activates the pituitary-adrenal axis with its powerful anti-inflammatory effect.[29]

Another aspect to be considered was the effect of stress on the growth factors decrease in relation to stimulation of fibronectin and collagen fibers.[10] These two elements are connected to disorganized tissue mineralization by localized inflammation of the pulp or periapical tissue.

At 30 days, with chronic stress, a reduction in inflammatory infiltrate was observed,[30] indicating the effect of CH on tissue organization and maturation. However, the stress decreased the amount of cells, even at a late stage of repair. It seems that the biocompatibility test at 30 days of CHSG showed a improvement in the repair process,[30,31] especially considering that cortisone and adrenaline in greater quantity in bloodstream affect the response.[32]

Stress in 30 days may have caused a stimulus for tissue repair. There is an attempt to modulation of immuno-inflammatory response in the periodontium. Studies have reported an disease progression increase in periodontal tissues with the stimulus of stress.[33] However, the organism show an adaptation to the stress elapsed 30 days.[34] The results of fibrous stimulation condensation and decreased of inflammatory infiltrate seem to

agree with the findings in the literature. However few findings are described in the periodontium until now.

We opted for the HE staining because it is an established technique to cell identification and diagnosis of inflammatory infiltrate.[25] This method of evaluating the effect of physical chronic stress on tissues is widely used in periodontal studies. Several indicators of systemic change include the size of the endocrine organs, body mass, hormonal alterations, and other disease indicators.[32,35,36]

Conclusion

Chronic stress was concluded to have changed some cellular parameters in rats in the presence of CH at 15 days and 30 days.

Clinical significance

It is known that the clinical importance of CH in dentistry. However, little is known about the effect of chronic stress, common in day-to-day life on tissue repair. With the results of this manuscript it's expected that information could be used in clinical trials in order to assist people in improving the quality of life.

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