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ORIGINAL ARTICLE

Evaluation of direct pulp capping with a synthetic chalcone: a preliminary histological study.

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Abstract: The aim of this study is to evaluate the action of the synthetic chalcone 1-phenyl-3-(4-chlorophenyl)-2-propen-1-one to induce pulp healing in rats. Material and Methods: Sixty lower first molars of male Wistar rats were divided into 3 groups (n=20): control (no treatment); calcium hydroxide and chalcone. After relative isolation, the cavities were prepared using a sterile low-speed ¼ round dental bur. After controlling the hemorrhaging, all the pulp exposures were capped with the capping material, by groups. The cavities were sealed with glass ionomer cement and the repair process was assessed at 21 days of procedure. The data were statistically analyzed using the Fisher exact test ($p<0.05$). Results: Moderate inflammation was observed in all the experimental groups and significant ($p<0.05$) reparative dentin (tertiary) formation in the calcium hydroxide and chalcone groups. The chalcone group showed dentinal tubules and a low number of cellular inclusions ($p<0.05$). Conclusion: The chalcone used in this study indicates potential as an inducer of reparative dentine (tertiary) in a rat model.

Keywords: *Chalcone; Dental pulp capping; Wistar rats.*
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INTRODUCTION.

The dental pulp consists of loose connective tissue, and is responsible for dental vitality through its roles such as dentin nutrition, innervation and tooth defense; this occurs by the formation of new dentin against any aggression. The maintenance of its features in case of injury leads to a constant search for drugs that can promote healing and maintenance of its functions[1].

In order to maintain the pulp tissue histophysiology, given that the survival of the tooth depends directly on the maintenance of pulp vitality, the most commonly used procedures are pulp capping and pulpotomy. The success of these treatments depends on an assessment of the degree of pulp impaired by trauma or bacterial infection of the dentin-pulp complex[2].

The procedure known as direct pulp capping is the most indicated in pulps exposed by mechanical agents or traumas. It involves the application of a biomaterial directly over the exposed pulp, in order to stimulate the differentiation of new odontoblasts from stem cells of the pulp. This, in turn, enables a restorative reaction through the formation of tertiary reparative dentin, maintaining pulp vitality and its normal functions[2,3].

The biomaterials used in vital pulp therapy should have the property of stimulating the formation of reparative dentin, maintaining pulp vitality, and providing effective bactericidal and/or bacteriostatic action and pulp sealing[4,5].

Calcium hydroxide (CH) has been widely used for direct pulp capping, and is known to have the potential to induce the repair of mineralized tissue[6]. Although CH has been considered the standard among capping mate-

rials[5]; studies by Delfino *et al.*, Kaiser *et al.*[7,8] describe some factors that may impair the use of CH, such as its accelerated degradation, and tunnel defects in the formation of the dentin bridge making it permeable and less resistant to contamination by bacteria.

Chalcones represent a group of intermediate compounds or final products in the biosynthesis of flavonoids, and have been extensively investigated due to their known high applicability. Pharmacological studies of various synthetic chalcones have shown osteogenic, antioxidante, chemopreventive, anti-inflammatory, analgesic and antibacterial potential[9-11].

A study by Pinero *et al.*[12], that describes the use of chalcones has generated great pharmacological interest due to its biocompatibility, which may suggest new pharmacological perspectives.

Thus, we aimed to evaluate the chalcone 1-pheny l-3- (4-chlorophenyl) -2 - propen -1-one as an agent in direct pulp capping of rat teeth.

MATERIALS AND METHODS.

The study protocol and experimental design were approved by the Ethics Committee of the Universidade do Vale do Itajaí - UNIVALI (Brazil), under no 21. The rats were maintained in propylene cages Beira-Mar Industria e Comércio® (São Paulo–SP-Brazil) with free access to food and water and under dark/light cycle (12/12 hours) at 22°C±2.

Sixty lower first molars of 60 male Wistar rats at 45 days of age were randomly divided into 3 groups (n= 20/ teeth/group): control (no treatment); calcium hydroxide p.a. Quimidrol® (Joinville, SC, Brazil); and chalcone group 1-phenyl-3- (4-chlorophenyl)-2-propen-1-one -0.25mg (Figure 1).

Synthesis of Chalcone

The molecule of chalcone 1-phenyl-3-(4-chlorophenyl)-2-propen-1-one used in this study was synthesized by Claisen-Schmidt condensation from acetophenone. The reactions were monitored by thin layer chromatography using the eluents hexane/ethyl acetate, and the compounds were purified by recrystallization with ethanol.

Figure 1. Chemical structure of a 1-phenyl-3-(4-chlorophenyl)-2-propen-1-one.

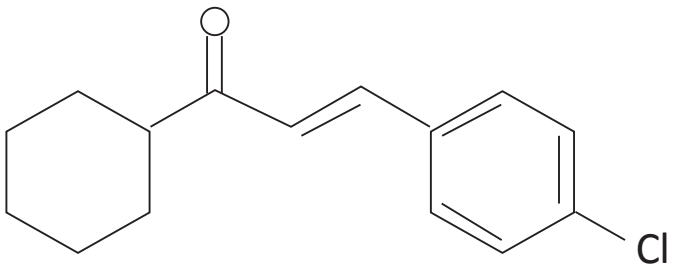
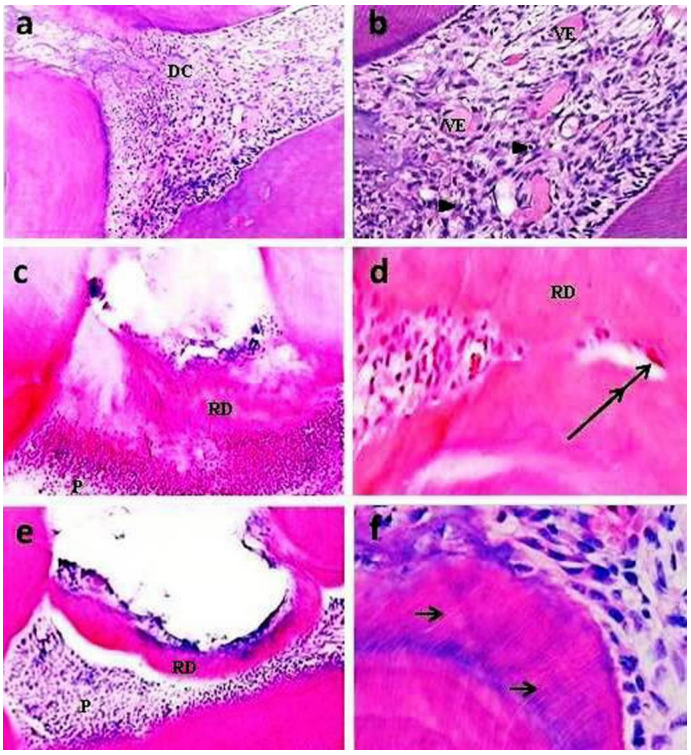


Figure 2. Twenty-one days after the pulp capping procedure.



Hematoxylin- eosin stain. A and B - Control group. A - Original magnification 20x - Dental pulp with diffuse calcifications (DC). B - Original magnification 40x - Inflammatory cells (arrowhead) and vascular engorgement (VE). C and D - Calcium hydroxide group. C - Original magnification 10x - Dental pulp (P) and reparative dentin (RD). D - Original magnification 20x - Cellular inclusions (double arrow) in reparative dentin (RD). E and F - Chalcone Group. E - Original magnification 10x - Dental pulp (P) and reparative dentin (RD) forming a bridge. F - Original magnification 40x - Dentinal tubules (arrows) in the tertiary dentin.

Surgical procedures

All procedures were performed under anesthesia using intraperitoneal injection of ketamine Agener® (Agener União, São Paulo–SP, Brazil) (10mg/kg body weight). Because of the small size of rat molar teeth the use of rubber dam was not possible, using relative isolation and continuous suction. After disinfection of the operational field with iodine and ethanol, the cavities were prepared using a sterile low-speed ¼ round dental bur and thoroughly irrigated with sterile saline solution. Hemorrhaging was stopped by irrigation with chlorhexidine 2% Riorex® (Rioquímica, São José do Rio Preto, SP, Brazil) solution, followed by irrigation with physiological saline solution (Farmax, Divinópolis - MG, Brazil). After controlling the hemorrhaging, all the pulp exposures were capped with the capping material, by groups, and the cavity was filled with glass ionomer cement Vitrebond® (3M ESPE, Sumaré-SP, Brazil), followed by restoration with composite resin Clearfil AP-X® (Kuraray, São Paulo –SP, Brazil).

Histological procedures

After 21 days, the rats were killed by anesthetic overdose and perfused intracardially with paraformaldehyde 4% in phosphate buffer pH 7.4. The mandibles containing the first molars were removed and demineralized in EDTA 7% in phosphate buffer, pH 7.4 for thirty days. After demineralization, dehydration was performed with increasing concentrations of alcohol, clearing with xylene and embedding in paraffin to obtain four semi-serial cuts 7 µm thick, and stained with hematoxyline and eosin.

Qualitative and quantitative analysis

Sections were examined under a light microscope (BX50F4, Olympus®, Japan) at 10x, 20x and 40x magnifications. The data were statistically analyzed using the Fisher exact test with STATA 13.0 (STATA Corp, Texas, USA) to identify differences between the groups, according to the predetermined histological grading criteria of Table 1. The significance level was set at $p<0.05$.

RESULTS.

Qualitative analysis

There was moderate pulp inflammation characterized by leukocyte infiltration and vascular engorgement in all groups of the experiment (Figure 2). In the control group, the tertiary dentin was not observed.

In the group treated with CH, the tertiary reparati-

Table 1. Histological criteria and grading.

	Inflammatory response
Grade	Parameters
0	None inflammatory cells present in the pulpal area
1	Slight inflammatory with polymorphonuclear or mononuclear cells
2	Severe inflammatory cell infiltration involving the coronal pulp
	Tertiary dentin
Grade	Parameters
0	Absence
1	Presence
	Dentinal tubule
Grade	Parameters
0	Absence
1	Presence
	Cellular inclusion
Grade	Parameters
0	Absence
1	Presence

Table 2. Histological evaluation.

Feature	Inflammatory response			Tertiary dentin		Dentinal Tubule		Cellular inclusion	
Scores/ Groups	0	1	2	0	1	0	1	0	1
Control	0	14	6	20	0	20	0	0	20
Calcium Hydroxide	2	12	6	6	14	20	0	0	20
Chalcone	3	12	5	1	19	4	16	17	3
p value	0.596			<0.001		<0.001		<0.001	

ve dentin was irregular, with cellular inclusions, and no dentinal tubules were observed (Figure 2 – C and D).

In the group capped with chalcone, the tertiary dentin exhibited dentinal tubules and little or no cell inclusion (Figure 2–E and F).

Quantitative analysis

The Fisher exact test revealed no significant differences between groups in relation to inflammation of the pulp tissue after 21 days of experiment (Table 2).

Considering the formation of tertiary reparative dentin, the CH and chalcone groups showed significant differences compared to the control group ($p<0.05$). The chalcone group exhibited dentinal tubules on reparative tertiary dentin, showing significant difference ($p<0.05$) compared to the other groups of the experiment (Table 2). Moreover, in the group capped with chalcone, only 3 samples showed cellular inclusions in reparative dentin, a significant result compared to the control and CH groups ($p<0.05$).

DISCUSSION.

Pulp capping is considered successful if there is formation of tertiary reparative dentin. The control of infection and biocompatibility of capping material are important factors in the treatment during direct pulp capping[4,13].

Calcium hydroxide is the material of choice for capping procedures, and has been considered the standard. However, some studies have reported the cytotoxic nature of the calcium hydroxide and its inadequate sealing properties[7,8,14].

The use of substances that induce mineralization as substitutes of calcium hydroxide, such as Portland Cement, Glass Ionomer Cement and Mineral Trioxide Aggregate (MTA), was recently proposed[5, 14-17].

Studies with chalcones have been conducted due to the spectrum of biological activities shown by these substances, such as osteogenic, anti- inflammatory, and antibacterial potential, among others[9-11]. Moreover, many clinical trials have shown that these molecules are biocompatible and non-toxic[9,10,12].

It is worth noting that besides the biocompatibility, materials used for pulp capping should not cause intense inflammation, which impairs the survival and proliferation of the cells.

The results of our study showed moderate infiltration of inflammatory cells in all groups. It is known the processes of tissue repair demands an inflammatory reaction. However, as suggested by Goldberg *et al.*[18], the inflammation should be mild; and in cases of pulp repair, the use of bioactive molecules can promote the differentiation of inflammatory cells into pre-odontoblasts and osteoblasts.

Thus, it is considered that moderate inflammatory reaction in the group treated with chalcone is characteristic of the normal repair process, as found in the group of calcium hydroxide, a substance widely used in pulp capping.

Furthermore, in the control group, no dentin bridge formation was observed, whereas the group capped with calcium hydroxide showed incomplete dentin bridge and many cellular inclusions, this result is in accordance with studies by Murray et al. and Sena *et al.*[19,20]. In our experiment, the group that used chalcone as capping material

shows complete dentin bridge formation and little or no cell inclusion.

It is known that the initial matrix of dentin repair is structurally distinct from that secreted in later periods. In the initial phase, the dentin repair presents an osteoid appearance, containing cellular inclusions, while dentin synthesized later shows a regular form, without cellular inclusions and with the presence of dentinal tubules [21].

In our study, the group treated with chalcone showed dentinal tubules in newly formed tissue repair, similar to the experiments of Demarco *et al.*[22], which used BMPs and also showed homogeneous reparative dentin and dentinal tubules. It is known that BMPs play an important role in cell differentiation, and are of one the most important agents for induction of odontoblast activity[23].

Studies by Kim *et al.*[10] indicate that chalcone may also act on the differentiation of cells that produce mineralized matrix, and interacts with cytoplasmic or membrane receptors such as BMPs, suggesting a likely route of its mechanism of pulp repair.

CONCLUSION.

According to the methodology used in our study, the synthetic chalcone 1- phenyl-3-(4-chlorophenyl)-2-propen-1-one induces pulp repair with tertiary dentin formation and the presence of dentinal tubules in cases of direct pulp capping in a rat model.

Evaluación del recubrimiento pulpar directo con una chalcona sintética: estudio histológico preliminar

Resumen: El objetivo de este estudio es evaluar la acción de la chalcona sintética 1-fenil-3- (4-clorofenil) -2-propen-1-ona para inducir la reparación de la pulpa dentaria en ratas. Materiales y métodos: Sesenta primeros molares inferiores de ratas Wistar machos se dividieron en 3 grupos (n = 20): control (sin tratamiento); hidróxido de calcio y chalcona. Después del aislamiento relativo, las cavidades se prepararon usando una fresa dental redonda de ¼ estéril a baja velocidad. Después de controlar la hemorragia, todas las exposiciones pulpares se taparon con el material de recubrimiento de acuerdo con los grupos del experimiento. Las cavidades fueron selladas con cemento de ionómero de vidrio y el proceso de reparación se evaluó a los 21 días del procedimiento. Los datos fueron analizados estadísticamente mediante la prueba exacta de Fisher (p<0,05). Resultados: Se observó inflamación moderada en todos los grupos experimentales y significativa (p <0,05) formación de dentina reparadora (terciaria) en los grupos de hidróxido de cálcio y chalcona. El grupo de chalcona mostró túbulos dentinarios y un bajo número de inclusiones celulares (p <0,05). Conclusión: La chalcona utilizada en este estudio indica potencial como un inductor de la dentina reparadora (terciaria) en ratas.

Palabras clave: *Chalcona; Recubrimiento pulpar dental; Ratas Wistar.*

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