ORIGINAL RESEARCH Endodontic therapy

Immediate tooth replantation: root canal filling for delayed initiation of endodontic treatment

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Submitted: Aug 16, 2017 Accepted for publication: Nov 29, 2017 Last revision: December 18, 2017 **Abstract:** The aim of this study is to evaluate the action of paramonochlorophenol associated with Furacin followed by calcium hydroxide (CH) dressing in the control of inflammatory root resorption in cases of immediate tooth replantation with delayed endodontic treatment. A total of 28 incisors of 3 male dogs were extracted and replanted after 15 minutes, and randomly divided into 3 groups: Group I (n = 8) - endodontic treatment was performed before the extraction and replantation; Group II (n = 10) - endodontic treatment was performed 30 days after replantation and the root canal was filled with CH dressing; Group III (n = 10) - endodontic treatment was performed 30 days after replantation and root canals received temporary medication of paramonochlorophenol-Furacin followed by CH dressing. The animals were euthanized 90 days after replantation. The histomorphological events analyzed at the epithelial reattachment site were the intensity and extent of acute and chronic inflammatory processes, periodontal ligament (PDL) organization, the intensity and extent of acute and chronic inflammatory processes in the PDL space, root resorption, bone tissue, and ankylosis. Data were submitted to the Wilcoxon Signed Ranks Test for group comparison ($\alpha = 5\%$). In Groups I, II and III the periodontal ligament was regenerated and most of the resorption areas were repaired by newly formed cementum. The depth and extent of root resorption were significantly higher in Group II than in Group III. The use of paramonochlorophenol-furacin followed by CH dressing was more effective in controlling inflammatory root resorption after immediate tooth replantation.

Keywords: Tooth Avulsion; Tooth Replantation; Root Canal Preparation; Calcium Hydroxide.

Introduction

Tooth avulsion is a severe trauma for which the treatment of choice is tooth replantation.^{1,2} According to the International Association for Dental Traumatology protocol, the endodontic treatment of replanted teeth with complete rhizogenesis should be done within 7 to 14 days. However, depending on the clinical situation, a delayed endodontic treatment is recommended; for instance, in cases with established inflammatory resorption.²



External root resorption is frequent if there is periodontal ligament inflammation, especially after replantation. Controlling contamination is essential, since its persistence could maintain the inflammation and provide conditions for sustained resorption.³

The most common infection pathway is through the root canal after pulp necrosis. Therefore, the treatment or prevention of inflammatory resorption requires endodontic treatment.^{1,4,5}

The medication most commonly employed for endodontic treatment after tool replantation is calcium hydroxide (CH),⁶ which has excellent biological properties. However, CH action inside the dentinal tubules and ramifications of the root canal is prolonged and it has little effectiveness against microorganisms, such as *Enterococci faecali*, that resist to high pH values and are the main cause of endodontic failure.^{78,9}

An ideal initial dressing should act quickly and effectively on the septic content of the main root canal and its ramifications, which in combination with biomechanical preparation would significantly reduce the local microbiota. Therefore, the application of CH is the best way to remove residual bacteria and their toxins.¹⁰

The drugs used in the first treatment session should have low toxicity, disperse easily in the dentin and have proven effectiveness, like paramonochlorophenol associated with Furacin. 10,11,12

Paramonochlorophenol is a germicide with a high antibacterial action used as a delay dressing; however, it is also highly cytotoxic. In order to reduce tissue irritation, some substances, such as camphor¹³ and Furacin¹¹, have been used in association with this drug. Although paramonochlorophenol and camphor is the most used drug combination in necropulpectomies, the option for using paramonochlorophenol with Furacin is based on the following characteristics: it has a high antibacterial action and a low cytotoxic effect, is effective against practically all bacteria found in root canals, 11 is water soluble and spreads more easily into the dentin. 14,15,16 These advantages could explain the better results reported by Holland et al.¹⁵, who conducted a study to compare paramonochlorophenol with Furacin and camphorated paramonochlorophenol as intracanal dressing in the treatment of chronic periapical lesions in dog teeth.

Objective

Since there are no studies in the literature that have employed the association of paramonochlorophenol with Furacin followed by CH dressing in tooth replantation, the aim of this study was to evaluate the action of such treatment protocol in the control of inflammatory root resorption in teeth endodontically treated 30 days after immediate tooth replantation.

Material and Methods

The research protocol was approved by the Committee of Animal Care and Research Use (protocol 2010-004113), and all guidelines regarding the care of research animals were strictly observed.

Three young adult male dogs of similar size with an average weight of 30 kg raised in a vivarium registered with the Brazilian Society of Science in Laboratory Animals were used for the study (SBCAL/COBEA). A total of 28 upper central and middle incisors, and lower central, middle and lateral incisors of the animals were extracted, following the protocol below.

The animals were given the pre-anesthetic agent Acepromazine 1% (Vetnil Ind. e Com. de Produtos Veterinários Ltda, Louveira, Brazil) and ketamine 50 mg/mL (Ceva Saúde Animal Ltda, Paulínia, Brazil) via intramuscular injection for sedation. Next, general anesthesia was performed by intravenous administration of Zoletil 100 mg/mL solution (Virbac do Brazil, São Paulo, Brazil). Intravenous access was maintained by means of a scalpel nº 21 (Butterfly, Abbott Ireland, Sligo, Ireland) in order to hydrate the animals with 5% dextrose solution (Laboratório J. P., Indústria Farmacêutica S. A., Ribeirão Preto, Brazil).

The teeth were divided into 3 groups according to the proposed treatment:

Group I (n = 08): endodontic treatment in one session using Sealapex (Sybron Kerr Indústria e Comércio Ltda, Guarulhos, Brazil) as sealer, tooth extraction and replantation. Euthanasia was performed 90 days after replantation.

Group II (n = 10): tooth extraction, replantation and endodontic treatment 30 days after replantation using CH dressing. A paste containing CH, saline and iodoform was prepared and applied into the root canal using a Lentulo spiral (Dentsply-Maillefer

Indústria e Comércio Ltda., Petrópolis, Brazil). Next, the teeth were sealed with gutta-percha and chemically activated glass ionomer cement (Vidrion R, SS White, Rio de Janeiro, Brazil). The dressing was changed after 7 and 60 days, and remained in place until the end of the experiment (90 days).

Group III (n = 10): tooth extraction, replantation and endodontic treatment 30 days after replantation using paramonochlorophenol + Furacin (Biodinâmica Química Farmacêutica Ltda., Abatiá, Brazil) at 5g/28 mL, applied with an absorbent paper cone previously adapted to the root canal; teeth were sealed as described above. After 7 days the crown seal was removed, the canals were irrigated with sodium hypochlorite 2.5%, dried with absorbent paper cones, filled with a paste of calcium hydroxide, saline and iodoform, and then sealed again. This procedure was repeated on day 60, after which the dressing remained in place until the end of the experiment (90 days).

Table 1 illustrates the performed treatment sequence. The extraction procedure was performed through an intrasulcular incision with a #15 scalpel blade, straight lever luxation and seizure with a pediatric forceps (Edlo, Indústria Gaúcha de Instrumentos Cirúrgicos Ltda, Canoas, Brazil).

The teeth were kept dry at room temperature for a period of 15 minutes. The alveoli were irrigated with saline, after which the teeth were replanted and splinted with 0.7 steel wire (Morelli, Sorocaba, Brazil) and composite resin (TPH Spectrum, Dentsply, Petrópolis, RJ, Brazil), which remained in place for 15 days.

All animals received an intramuscular injection of penicillin (Eurofarma Laboratories Ltda., Belo Campo, Brazil) at a dose of 250 mg, an intramuscular injection of Dipyrone Sodium 500 mg/mL (Laboratório Ibasa Ltda, Porto Alegre, Brazil) and anti-inflammatory Keptoprofeno 10% (Laboratórios Vencofarma do Brazil LTDA, Londrina, Brazil) every 12 hours orally for 3 days.

The root canal treatment followed the same protocol for all teeth. Following rubber dam isolation, the root cavity was opened, pulpectomy was performed, the working length was determined, and the root canals were instrumented by a crown-down technique until reaching the apical cemental barrier. Next, the apical cemental barrier was penetrated with a #15 K-file (Maillefer Instruments SA, Ballaigues, Switzerland), and the cemental canal was widened up with a #25 K-file. Throughout the procedure, the root canals were irrigated with sodium hypochlorite 2.5%. The prepared canals were then dried and immediately obturated with gutta-percha cones and Sealapex (Group I), and divided in accordance with the intracanal medication (Group II and III).

The animals (Group I, II, III) were euthanized by anesthetic overdose 90 days after the treatment. Subsequently, the anatomic pieces containing the teeth were removed, fixed in buffered neutral 10% formalin, decalcified in 17% EDTA solution, and embedded in paraffin. The blocks were serially sectioned into 6- μ m thick longitudinal sections that were stained with hematoxylin and eosin, and then examined under light microscopy by a skilled observer blind to the treatment groups.

Table 1. Study treatment sequence.

	Ν	Performed treatment					
Groups		l st session	2 nd session	3 rd session	4 th session	5 th session	6 th session
			15 days	30 days	37 days	60 days	90 days
Group I	8	Endodontic treatment before extraction and replanted	Splint removal				euthanasia
Group II	10	extraction and replanted	Splint removal	CH dressing	CH dressing change	CH dressing change	euthanasia
Group III	10	extraction and replanted	Splint removal	paramonochlorophenol + Furacin dressing	CH dressing	CH dressing change	euthanasia

CH: calcium hydroxide.

The histomorphological parameters used in the present study are based on the criteria described by Panzarini et al.¹⁷ The specimens were examined and scores from 1 to 4 were assigned to each parameter, with 1 indicating the best result and 4 the worst. The histological events analyzed at the epithelial reattachment site were the intensity and extent of acute and chronic inflammatory processes, periodontal ligament (PDL) organization, intensity and extent of acute and chronic inflammatory processes in the PDL space, root resorption (active or inactive, extent, depth and repair), bone tissue, and ankylosis (Table 2, Figure 1).

The data were submitted to the Wilcoxon signed rank test (non-parametric repeated measures analysis) for comparison between groups, at SPSS 20.0, at a significance level of 5%.

Results

Group I – endodontic treatment before tooth replantation

The periodontal ligament was reattached to the cementum and alveolar bone, with fibers arranged perpendicularly to the root surface, preserving structures like the epithelial cell rests of Malassez that confirm regeneration. Newly formed cementum was observer in the root resorption areas (Table 3), preserving the periodontal ligament space (Figure 2A). In the apical region near the delta, a subtle acute and chronic inflammatory infiltrate (Table 4) and active bone resorption (Table 5) were noted (Figure 2B).

Group II - CH dressing

The periodontal ligament was completely preserved. The external root resorption was assigned a score 3 for its extent and depth in 60% of the cases (Table 4), and was mostly repaired by newly formed cementum (Figure 2C). There was a small incidence of ankylosis (Table 5). Acute inflammatory infiltrates near the ramifications were observed (Figure 2D) as well as massive presence of active resorption of bone tissue (Table 5).

Group III - paramonochlorophenol + Furacin followed by CH dressing

The periodontal ligament was reattached to the cementum and alveolar bone with fibers arranged

perpendicularly to the root surface (Table 2). Epithelial cell rests of Malassez were present (Figure 2E). Fifty percent of the root surfaces had no active resorption (Table 3). Most root resorptions were small in extent and depth (Table 3), and repaired by newly formed cementum (Figure 2F). In the alveolar bone tissue, a level 3 active resorption was noted in 100% of the cases (Table 5).

Table 2. Distribution of scores and statistical analysis of the histomorphometric events associated with epithelial reattachment (Wilcoxon signed ranks test at 5% significance level).

(Wilcoxoff signed faths	Epithelial reattachment				
Group/Events/Scores	Endodontic treatment	СН	PMCF + CH		
Site					
1	08/8	10/10	10/10		
2	0/8	0/10	0/10		
3	0/8	0/10	0/10		
4	0/8	0/10	0/10		
Intergroup comparison	Unable to sto	atistics - the	same scores		
Intensity of the acute inflo	ammatory proc	ess			
1	06/8	08/10	02/10		
2	2/8	2/10	08/10		
3	0/8	0/10	0/10		
4	0/8	0/10	0/10		
Intergroup comparison	а	а	а		
Extent of the acute inflam	matory proces	s			
1	03/8	05/10	04/10		
2	05/8	05/10	06/10		
3	0/8	0/10	0/10		
4	0/8	0/10	0/10		
Intergroup comparison	а	а	а		
Intensity of the chronic in	flammatory pro	ocess			
1	06/8	09/10	06/10		
2	02/8	01/10	04/10		
3	0/8	0/10	0/10		
4	0/8	0/10	0/10		
Intergroup comparison	а	а	а		
Extent of the chronic inflammatory process					
1	01/8	01/10	02/10		
2	07/8	09/10	08/10		
3	0/8	0/10	0/10		
4	0/8	0/10	0/10		
Intergroup comparison	а	а	а		

CH: calcium hydroxide; PMCF:

Epithelial insertion				
Score		2	3	
Area of epithelial insertion	Cementoenamel junction.	Ligament below the cementoenamel junction.	Much below the cementoenamel junction (near medium third).	Absence of epithelial insertion.
Score		2	3	
Inflammatory process - Intensity	Absence or occasional presence of inflammatory cells	Small number of inflammatory cells. Up to 10 inflammatory cells.	Moderate number of inflammatory cells. From 11 to 50 inflammatory cells.	Large number of inflammatory cells. More than 50 inflammatory cells.
Score		2	3	4
Inflammatory process - Extension	Absence or occasional presence of inflammatory cells.	Inflammatory process restricted to the lamina propria of the internal aspect of the epithelium.	Inflammatory process extending apically toward the small portion of connective tissue.	Inflammatory process reaching the area of the alveolar bone crest.
*Number of cells per field with 400· magnification.				

Tooth root					
Score			3	4	
Root Resorption	Absence of root resorptions or repaired resorptions.	Areas of inactive resorptions (absence of clastic cells).	Small areas of active resorptions.	Extensive areas of active resorptions.	
Extesion of root resorption	Absence of resorption.	Mean extension of 1–1000 μm.	Mean extension of $1001-5000~\mu m$.	Mean extension larger than 5001 μ m.	
Depth of root resorption	Absence of resorption.	SMean depth of 1–100 μ m.	Mean depth of 101–200 μm.	Mean depth larger than 201 μm.	
Repair of root resorption areas	Absence of resorption or deposition of newly formed cementum all the extension.	Deposition of newly formed cementun in half or more the extension.	Deposition of newly formed cementun in less than half of the extension.	Absence of deposition of newly formed cementun.	

Figure 1. Scores used in the quantification of histological events.

Epithelial insertion				
Score		2	3	4
Organization	Periodontal fibers inserted in bone and cementum throughout the extension of the ligament.	Periodontal fibers inserted in bone and cementum at two-third of the extension of the ligament.	Periodontal fibers inserted in the bone and cementum at one-third of the extension of the ligament.	Absence of periodontal fibers with insertion in bone and cementum.
Score			3	
Intensity of inflammatory process	Absence or occasional presence of inflammatory cells.	Small number of inflammatory cells. Up to 10 inflammatory cells.	Moderate number of inflammatory cells. From 11 to 50 inflammatory cells.	Large number of inflammatory cells. More than 50 inflammatory cells.
Extension of inflammatory process	Absence or occasional presence of inflammatory cells.	Inflammatory process present only in the apical or coronal periodontal ligament, or small lateral area.	Inflammatory process reaching more than half of the lateral periodontal ligament on the tooth root.	Inflammatory process throughout the periodontal ligament.
*Number of cells per field with 400· magnification.				

	Bone tissue				
Score			3 000 000 000		
Bone Resorption	Absence of resorptions areas.	Presence of inactive resorptions areas (absence of clastic cells).	Presence of small areas of active resorption.	Presence of extensive areas of active resorption.	
Score					
Ankylosis	Absence of ankylosis.	Small points of ankylosis.	One-third of the root with of ankylosis.	More than one-third of the root with of ankylosis.	

Figure 1. (Continuation) Scores used in the quantification of histological events.

Table 3. Distribution of scores and statistical analysis of the histomorphometric events associated with root dentin resorption (Wilcoxon signed ranks test at 5% significance level).

	Tooth root				
Group/Events/Scores	Endodontic treatment	СН	PMCF + CH		
Areas of active root resorption					
1	0/8	03/10	05/10		
2	2/8	03/10	03/10		
3	6/8	04/10	02/10		
4	0/8	0/10	0/10		
Intergroup comparison	а	ab	b		
Extent of root resorption					
1	0/8	0/10	01/10		
2	3/8	4/10	09/10		
3	5/8	6/10	0/10		
4	0/8	0/10	0/10		
Intergroup comparison	а	а	b		
Depth of root resorption					
1	0/8	0/10	01/10		
2	04/8	04/10	08/10		
3	04/8	06/10	01/10		
4	0/8	0/10	0/10		
Intergroup comparison	ab	а	b		
Repair of root resorption areas					
1	0/8	03/10	06/10		
2	07/8	07/10	04/10		
3	01/8	0/10	0/10		
4	0/8	0/10	0/10		
Intergroup comparison	а	ab	b		

CH: calcium hydroxide; PMCF:

Discussion

The maintenance of replanted teeth is directly related to the viability of the periodontal ligament cells and contamination control.¹

When the replantation is performed after a short extra-alveolar period, as was the case in this study, a complete regeneration of the periodontal ligament can occur, with all structural components (cementoblasts, fibroblasts, and collagen fibers) reinserted in the bone and cementum, and presence of blood vessels and epithelial cell rests of Malassez.¹

These epithelial cells, along with the cementum, are an important part of the periodontal ligament's defense system against bacteria from the root canal, passing

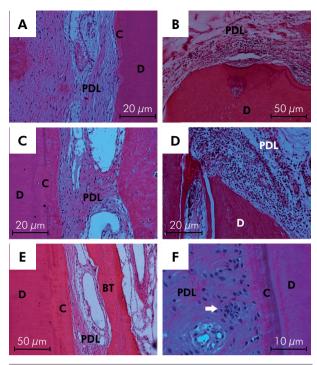


Figure 2. A - Root resorption repaired by newly formed cementum (arrow). B - Presence of acute inflammatory infiltrate in the periodontal ligament near the ramification (arrow). C - Root resorption repaired by newly formed cementum. D - Presence of acute inflammatory infiltrate near a ramification of the main canal (arrow). E - Root resorption repaired by newly formed cementum. F- Presence of epithelial cell rests of Malassez near the root surface (arrow). HE.

through the main canals, lateral ramifications and dentinal tubules. Bacteria promote the proliferation and adhesion of epithelial cells near the contaminated site, with the purpose of forming a barrier against the invaders.^{3,18-21}

Another frequent finding of the treated groups was the repair of root resorption by newly formed cementum, which is also directly related to the presence of epithelial cell rests of Malassez. There is evidence that these cells can activate the secretion of proteins that are normally expressed during tooth development and participate in the formation of acellular cementum.^{22,23}

After tooth avulsion, endodontic treatment is essential to control contamination in the canal and in the periapical region of the tooth. For intracanal dressing, important aspects must be considered that can directly influence the repair of the periodontal ligament, such as toxicity, effectiveness, action spectrum and mechanism of action.

Table 4. Distribution of scores and statistical analysis of the histomorphometric events associated with periodontal ligament (Wilcoxon signed ranks test at 5% significance level).

	Per	Periodontal ligament			
Group/Events/Scores	Endodontic treatment	СН	PMCF+ CH		
Organization					
1	5/8	09/10	09/10		
2	3/8	01/10	01/10		
3	0/8	0/10	0/10		
4	0/8	0/10	0/10		
Intergroup comparison	а	а	а		
Intensity of the acute infl	ammatory prod	cess			
1	6/8	07/10	08/10		
2	1/8	2/10	01/10		
3	1/8	1/10	01/10		
4	0/8	0/10	0/10		
Intergroup comparison	а	а	а		
Extent of the acute inflar	nmatory proces	SS			
1	01/8	05/10	04/10		
2	07/8	05/10	06/10		
3	0/8	0/10	0/10		
4	0/8	0/10	0/10		
Intergroup comparison	а	а	а		
Intensity of the chronic in	nflammatory pr	ocess			
1	07/8	08/10	05/10		
2	01/8	02/10	05/10		
3	0/8	0/10	0/10		
4	0/8	0/10	0/10		
Intergroup comparison	а	а	а		
Extent of the chronic infl		cess			
1	02/8	01/10	04/10		
2	06/8	09/10	06/10		
3	0/8	0/10	0/10		
4	0/8	0/10	0/10		
Intergroup comparison	а	а	а		

CH: calcium hydroxide; PMCF:

Paramonochlorophenol mixed with Furacin promotes a rapid decontamination of the root canal because of its high bactericidal and bacteriostatic proprieties, and low toxicity. This drug acts by contact and spreads easily in the dentinal tubules and ramifications of the main canal, which may explain the lesser extent and depth of root resorptions, as well as the absence of acute inflammatory infiltrate near the ramifications.

Table 5. Distribution of scores and statistical analysis of the histomorphometric events associated with bone tissue (Wilcoxon Signed Ranks Test at 5% significance level).

	Bone tissue			
Group/Events/Scores	Endodontic treatment	СН	PMCF + CH	
Areas of active bone resorp	tion			
1	0/8	0/10	0/10	
2	0/8	0/10	0/10	
3	8/8	10/10	10/10	
4	0/8	0/10	0/10	
Intergroup comparison	Unable to sta	tistics - the	e same scores	
Ankylosis				
1	7/8	9/10	8/10	
2	1/8	1/10	2/10	
3	0/8	0/10	0/10	
4	0/8	0/10	0/10	
Intergroup comparison	а	а	а	

CH: calcium hydroxide; PMCF:

The rapid decontamination favors the repair process of tooth replantation, because inflammatory resorption usually begins within 15 days after replantation, and the main etiological factor is the contamination of the root canal, its ramifications and dentinal tubules.⁴ Furthermore, the deep action of CH requires a long time for the treatment of inflammatory resorption.⁵

In group II, which received only CH as dressing, a presence of acute inflammatory infiltrate was observed near the ramifications, including increased depth and extent of root resorptions compared to group III, probably due to inefficiency or a longer delay in decontamination.

The time until endodontic treatment following replantation is an important factor, ^{24,25} because as time passes, the contamination penetrates deeper into the ramifications and dentinal tubules, promoting inflammatory processes in the periodontal ligament and creating conditions for inflammatory root resorption. ⁵ This fact was demonstrated here by initiating endodontic treatment 30 days post-replantation.

The advantage of using paramonochlorophenolfuracin dressing followed by CH to treat inflammatory resorption is the complementary action of the combination, which contributes to an earlier root canal filling.

Paramonochlorofenol with furacin is a medicament that acts by contact,¹⁴ has a great capacity to diffuse in the dentin and a strong antimicrobial action.¹² Due to these proprieties, which have been confirmed through

studies using dog teeth with periapical lesions,²⁶ the medication is indicated by Holand et al.²⁶ as a delayed dressing for necropulpectomy, remaining in place for a short period of 3 to 7 days. The CH then acts on the residual bacteria and their toxins, which are able to interrupt the resorption process.²⁷

The repair process after immediate tooth replantation is more efficient concerning extent and depth of resorptions, compared to delayed tooth replantation. This indicates the important role of a viable periodontal ligament in the preservation of teeth, even if affected by pulp necrosis.²⁸

Conclusion

The use of paramonochlorophenol-furacin followed by CH dressing was more effective than CH dressing alone regarding the control of inflammatory root resorption after immediate tooth replantation.

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