

Effect of lincomycin treatment of root surface in dental replantation: A study in rats

Efeito da lincomicina como tratamento da superfície radicular no reimplante de dentes: estudo em ratos

Abstract

Purpose: The purpose of studies on antibiotics used topically on the root surface of avulsed teeth is to evaluate their antiresorptive properties which favor the repair of the periodontal ligament and the reestablishment of the dentoalveolar articulation. The aim of the present study was to determine the effect of lincomycin treatment of the root surface on tooth replantation.

Methods: Ten rats were distributed into two groups (I – control; II – experimental). The right maxillary incisor of the animals was extracted and stored in milk for 30 min. The tooth in group I was rinsed with normal saline and replanted; in group II, the incisor was immersed in lincomycin for 5 min before replantation. The animals were euthanized on the 21st day postoperative. The right hemimaxilla was submitted to histological sectioning and stained with H&E for microscopic evaluation.

Results: Group I: the periodontal ligament appeared dispersed, with moderate to intense lymphoplasmacytic infiltrate, and dentoalveolar articulation was not reestablished. Group II: complete repair of the ligament was evident, and there were few inflammatory cells and small areas of inflammatory resorption and ankylosis.

Conclusion: The use of lincomycin allowed the repair of the periodontal ligament and reduced inflammation. There were still areas of inflammatory resorption and ankylosis.

Key words: Tooth replantation; topical antibiotic therapy; lincomycin

Resumo

Objetivo: Estudos sobre antibióticos usados topicamente na superfície radicular de dentes avulsionados buscam avaliar suas propriedades anti-reabsorptivas que favoreçam o reparo do ligamento periodontal e o restabelecimento da articulação dentoalveolar. Neste mesmo propósito, esta pesquisa avaliou o efeito da lincomicina na superfície radicular, antes do reimplante.

Metodologia: Dez ratos foram distribuídos em dois grupos de 05 animais (I – controle e II – experimental). O incisivo superior direito dos animais foi extraído e estocado em leite por 30 min. Em seguida, no grupo I, os dentes foram irrigados com soro fisiológico e reimplantados; no grupo II, os incisivos foram imersos em lincomicina por 5 min, antes do reimplante. Os animais foram eutanasiados no 21º dia pós-operatório e a hemimaxila direita submetida a cortes histológicos, corados em HE, para avaliação microscópica.

Resultados: No grupo I, o ligamento periodontal apresentou-se disperso, com infiltrado linfoplasmocitário moderado a intenso e a articulação dentoalveolar não foi restabelecida; no grupo II, notou-se completo reparo do ligamento, poucas células inflamatórias e pequenas áreas de reabsorção inflamatória e anquilose.

Conclusões: o uso tópico da lincomicina no tratamento da superfície radicular promoveu o reparo do ligamento periodontal, reduziu o processo inflamatório, mas ainda foram observadas áreas de reabsorção inflamatória e anquilose.

Palavras-chave: Reimplante dental; antibioticoterapia tópica; lincomicina

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Introduction

Avulsions occur very often in the anterior teeth of young patients, compromising their aesthetic, mechanical, and biological functions. Replantation is the first choice treatment for avulsions, because it is faced with numerous factors whose interaction will determine the success or not of the treatment, such as intensity of the trauma, vitality of the periodontal ligament, type and time of injury, solutions used for tooth storage in the extra-alveolar period, time elapsed between the avulsion and replantation, maintenance of pulp vitality, preparation of the tooth, and alveolus for replantation, and opportunity for endodontic treatment (1,2). The damage suffered by the periodontal ligament, such as direct mechanical trauma and dehydration, affects the viability of cells of the periodontal ligament, and worsens the prognostics of the replanted tooth (3).

Post-replantation root resorption is frequent and can be classified as: surface resorption, inflammatory resorption, resorption by substitution or dentoalveolar ankylosis, internal resorption, and invasive resorption. The mechanism of action of resorption involves the principal cells of the resorption bone tissue: osteoclasts and osteocytes, besides macrophages and monocytes, where the odontoclasts are responsible for root resorption (3,4). The lack of revascularization and the persistence of inflammatory root resorption have also been attributed to the presence of microorganisms, and therefore, the use of systemic or topical antibiotics is recommended (5).

Lincomycin is a lincosamide which has primary effectiveness against gram-positive pathogens and is often used in the oral cavity (6). It is a broad-spectrum antibiotic synthesized by *Streptomyces lincolnensis*, which shows *in vitro* and *in vivo* activity comparable with erythromycin against staphylococci, streptococci and diplococci. The immediate effect of lincomycin in *S. aureus* is the complete inhibition of protein synthesis. Its action is comparable to that of chloramphenicol and puromycin, where the possible sites of action are the transfer or polymerization of aminoacids (7).

The aim of this study was to evaluate the effect of topical antibiotics on tooth replantation, by conducting a microscopic analysis of the tissue repair process of rat teeth extracted and replanted, after treatment of the root surface with lincomycin hydrochloride.

Methods

Ten rats (Wistar) approximately 120 days old and weighing between 180 and 240 g were used for this study; they were subdivided into two groups of 5 animals each (I-control and II-experimental). After weighing the animals with an analytical balance, the animals were anesthetized with intraperitoneal injections of ketamine hydrochloride (Ketamin[®], Cristália Produtos Químicos Farmacêuticos, Brazil), at a dose of 50 mg/kg body weight, and xylazine hydrochloride (Calmium[®], Agener União, Brazil), at a dose

of 25 mg/kg, to perform the surgical procedure. After peri- and intraoral antiseptics with 0.12% chlorhexidine, syndesmotomy and luxation of the right maxillary incisor were performed with the help of a syndesmotome, followed by extraction, utilizing an osteotome for pediatric use, which was adapted; the procedures were carried out in the most non-traumatic way possible.

In group I (control), after extraction, the teeth were stored for 30 min in a Petri dish containing long-life pasteurized milk, and rinsed with normal saline before replanting. In group II (experimental), after extraction and storage in milk for 30 min, the teeth were immersed in a glass *dappen* dish containing lincomycin hydrochloride (Frademicina[®], injectable solution, Pharmacia S.A. de C.V., Tlalpan, Mexico DF – Mexico), before replanting. The animals received a dose of the analgesic paracetamol (80 mg/kg) intramuscularly just after the replantation of the tooth and during the first 2 days postoperative.

The rats were fed with solid granulated diet (Nuvital[®] Nutrientes S/A, Brazil), administered in a pulverized form in the first 7 days postoperative and water *ad libitum*. On the 21st day of observation, the rats were euthanized by inhalation of isoflurane. Twenty-one days was considered a sufficient period for the wound healing process to occur in rats, and by then, it should be possible to detect the presence or not of root resorption or dentoalveolar ankylosis, by the quantity of neoformed bone tissue occupying the resorption areas (8).

The maxilla of each animal was hemi-sectioned, removed *en bloc*, immersed in 10% formalin, decalcified with 10% nitric acid for 7 days, and then, washed and embedded in paraffin blocks. The blocks were microtomed in the longitudinal direction of the incisor, with sections of 0.6 micron in thickness, which were then stained with hematoxylin and eosin.

The histological slides were analyzed by light microscopy (Olympus BX50 microscope, São Paulo, SP, Brazil), by two independent examiners (one was an experienced specialist in Oral Pathology – considered the gold standard). The calibration was carried out by the *Kappa* inter-examiner agreement test, with a coefficient of 0.85, which is considered almost perfect (9). The images were magnified 400x for analysis of the periodontium (cementum, periodontal ligament, and adjacent alveolar bone), with respect to the tissue repair process.

The descriptive analysis of the histological slides was according to the following criteria:

- periodontal inflammatory process: absent, when there were no inflammatory cells in the field; slight, when few inflammatory cells were present in the field; moderate, when inflammatory cells were present, but not dominating the field; intense, when inflammatory cells dominated the field;
- presence or absence of reinsertion of the periodontal ligament fibers and their disposition;
- presence or absence of resorption by substitution;
- presence or absence of inflammatory resorption;
- presence or absence of dentoalveolar ankylosis.

If inflammatory pulp alterations were observed, these were also described, but this was not the object of the study.

Results

At 21 days postoperative, the histological analysis demonstrated that lincomycin maintained the integrity of the periodontal ligament and promoted its reinsertion in the cementum, which was not seen in the control group. Lincomycin did not prevent inflammatory root resorption and dentoalveolar ankylosis.

In group I, the periodontal ligament appeared disorganized and dispersed, with disarrangement of the fibers and absence of reinsertion in the cementum, except for some isolated points. Lymphoplasmacytic infiltrate, slight to moderate, was observed over all the periodontal ligament, where the most frequent was in focal areas of apical inflammation. The dentin tissue did not show many lacunae of resorption. The presence of some areas with ankylosis was also noted, which were signs of dentin resorption with evidence of cells with characteristics of clastic activity. The pulp appeared inflamed or necrotic in the majority of specimens.

Figure 1 shows specimens of group I, demonstrating a dispersed periodontal ligament, disorganized, without reinsertion in the cementum, and the presence of moderate lymphoplasmacytic inflammatory infiltrate.

In group II, the periodontal ligament appeared healthy, showing functional disposition of the fibers, absence of inflammatory infiltrate and reinsertion in the cementum with an appearance of normality in the majority of specimens (Fig. 2). The dentin tissue showed small and few areas of resorption by substitution in the middle third of the root. Few areas of root resorption and inflammation in the apical portion were noted in this group, as well as pulp with the presence of lymphoplasmacytic or necrotic infiltrate. Dentoalveolar ankylosis was also evident in this group.

Discussion

Even though for an uncertain period, the replanted tooth meets the immediate psychological needs of the patients and their family. When performed immediately after avulsion, the replanted tooth is first aimed at re-establishment of the periodontal ligament. It is important to consider that

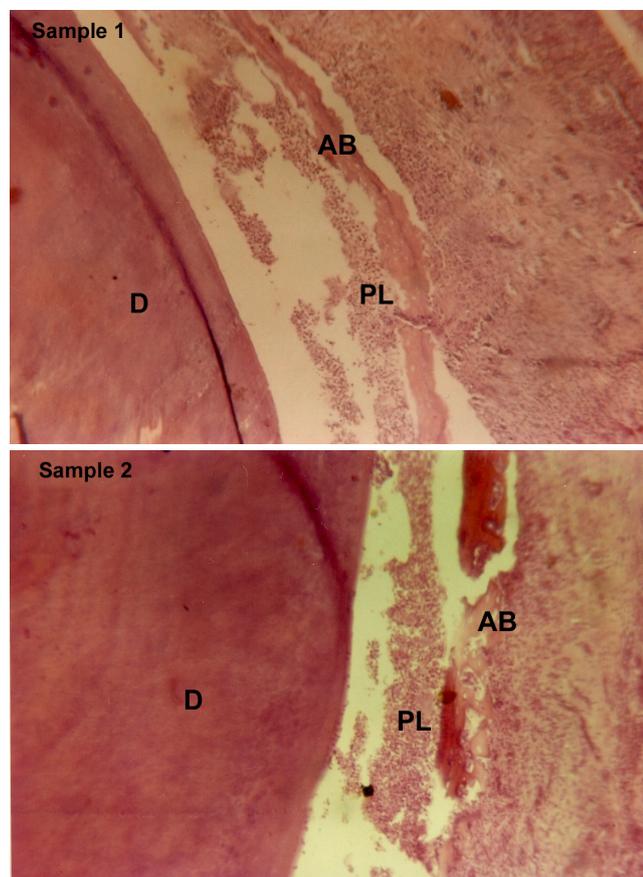


Fig. 1. Control group: periodontal ligament disorganized, dispersed, with disarrangement of fibers and absence of reinsertion in the root surface. D - dentin; PL - periodontal ligament; AO - alveolar bone. (Original magnification, 400X).

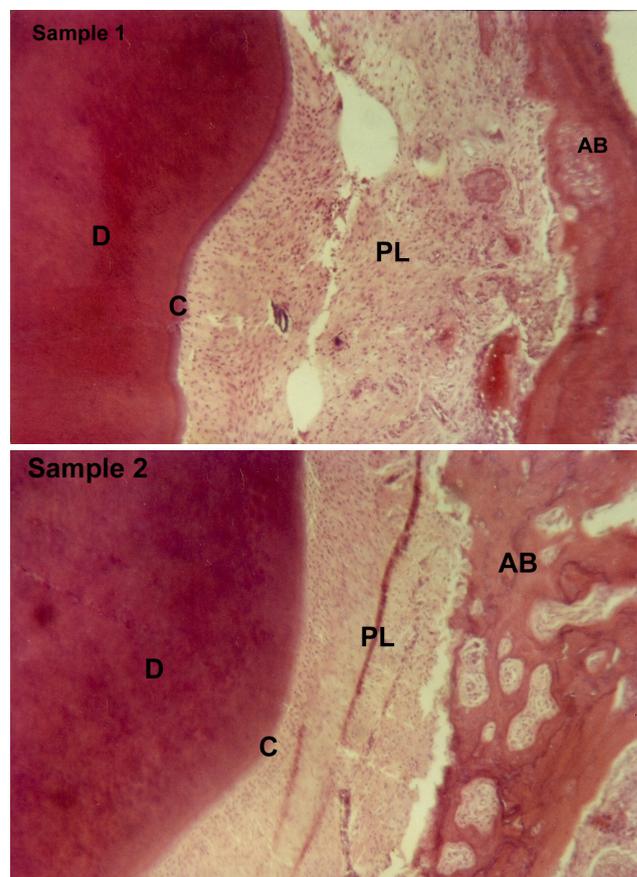


Fig. 2. Experimental group: presence of vital and healthy tissues, showing functional disposition of fibers, absence of inflammatory infiltrate, and reinsertion along the cementum surface in the majority of specimens. D - dentin; C - cementum; PL - periodontal ligament; AO - alveolar bone (Original magnification, 400X).

immediate replantation is not a clinical reality. The delay in replantation and lack of or inadequate storage medium can interfere with the properties of the periodontal ligament. Root resorption is also attributed to preservation or not of the periodontal ligament and of the layer of cementoblasts. Ankylosis occurs due to the removal of the ligament, where the tooth becomes fixed to the bone, whose retention can take years, as progressive resorption can also end in the loss of the tooth (10).

Thus, in the present experiment an extra-alveolar period of 30 min was used to simulate the time that the patient is delayed in seeking a dentist. Compatible physiological solutions, Hanks balanced salt solution, and ViaSpan (11,12) can maintain and/or replace the cellular metabolites of the still viable periodontal ligament. However, due to the limited disclosure and the high cost of these products, the oral surgeon has little access to these substances (11), as does the patient or family. In this period, the teeth were stored in milk to maintain the vitality of the periodontal ligament. Milk has been the storage medium most frequently recommended by studies due to its basic pH and osmolarity, showing good results in laboratory tests and in clinical reports. The type most recommended for use is the long-life milk, because it has a long shelf-life without the need for refrigeration, being able to preserve the periodontal ligament up to 8 h after the trauma. In Brazil, the milk most indicated for this purpose is type A or long-life milk, because it is more reliable, microbiologically (12,13).

Andreasen and Andreasen (1) recommend the use of penicillin as part of the procedures dealing with avulsion, emphasizing that elevated concentrations of penicillin can be achieved in the bone tissue. Systemic antibiotic therapy should also be instituted, since the mouth is an ideal environment for bacterial proliferation. The use of topical antibiotic after replantation has been investigated in many studies (5,14). Studies of antibiotics used topically on the root surface of avulsed teeth aim at evaluating their antiresorptive properties which favor the repair of the periodontal ligament and the reestablishment of dentoalveolar articulation.

Cellular damage due to trauma stimulates an inflammatory response which frequently results in root resorption mediated by osteoclasts. The active resorption process can rapidly degrade the cementum and dentin variable wound healing (3). Ankylosis can also occur substituting the root with bone (10). Various substances have been utilized with the aim of reducing or impeding root resorption and dentoalveolar ankylosis, with promising results, but still not sufficiently satisfactory. Andreasen (2) studied the treatment of avulsed teeth with complete rhizogenesis after an extra-alveolar period in dry medium longer than one hour, where the fibers of the periodontal ligament were removed followed by immersion of the tooth in acidified 2.4% sodium fluorophosphate, pH 5.5, for 20 min. This treatment contributed to the reduction in root resorption in 50% of cases.

Cvek et al. (14) obtained a reduction in the frequency of ankylosis after immersion of teeth in a suspension of doxycycline, a semi-synthetic derivative of tetracycline,

which inhibits the growth of bacteria and is utilized for the treatment of acne. These authors studied the revascularization and periodontal repair in teeth of monkeys, after an extra-alveolar time of 30 and 60 min in dry or moist medium and 5 min of immersion in a suspension of doxycycline diluted in normal saline. Yanpiset and Trope (5), utilizing doxycycline topically on the root surface of teeth of dogs, evaluated pulp revascularization of replanted teeth and noted that the teeth immersed in doxycycline (1 mg/10mL of normal saline) for 5 min showed a significant increase in pulp revascularization (60%).

Minocycline, a tetracycline utilized for the treatment of acne vulgaris, was utilized by Khim and Sae-lim, on the root surface of avulsed teeth of monkeys, before replantation. The group that received topical antibiotic therapy showed better results, demonstrating complete repair in 32.46% of cases, while the group that did not receive minocycline had complete repair in only 16.58% (15).

The choice of lincomycin for topical antimicrobial treatment of the root surface was due to its affinity for bone tissue, predominant action on Gram-positive microorganisms found in the oral microbiota, and water-solubility and stability in an acid environment, allowing it to diffuse through the alveolus without undergoing alterations if there is local infection (6,7). Lincomycin is bacteriostatic and inhibits protein synthesis in sensitive bacteria (17).

Based on our results, it can be seen that lincomycin maintains the integrity of the periodontal ligament and promotes its reinsertion in the cementum, which was not seen in the control group. Lincomycin does not prevent inflammatory root resorption, and by an unknown mechanism can stimulate the appearance of these resorptions, since in group I their occurrence was less frequent. In group I, the periodontal ligament appeared disorganized and dispersed, with disarrangement of the fibers and absence of insertion, except in some isolated points. The pulp appeared inflamed or necrotic in the majority of specimens with slight to moderate lymphoplasmacytic infiltrate throughout the periodontal ligament, being more frequent in focal areas of apical inflammation. Therefore, it appears that with storage in milk without antibiotic treatment, the hard tissues showed satisfactory responses, without the occurrence of dentoalveolar ankylosis and few areas of resorptions, but the behavior of the periodontal ligament when immersed in milk without antibiotic, was not satisfactory, revealing tissue disorganization and chronic inflammatory infiltrate.

The periodontal ligament in group II appeared vital and healthy, showing functional disposition of the fibers, absence of inflammatory infiltrate and reinsertion of fibers in the cementum, with an appearance of normality in the majority of specimens. Few areas of root resorption and inflammation in the apical portion were noted in this group, as well as the pulp with the presence of lymphoplasmacytic or necrotic infiltrate. Dentoalveolar ankylosis was present in the lincomycin group, a finding similar to those of other investigators who utilized topical antibiotic, but different with regard to the results of Cvek et al. (14) who reported a

reduction in the frequency of ankylosis after immersion of the teeth in doxycycline suspension.

The present study showed that the topical use of lincomycin preserves the integrity of the periodontal ligament and promotes its reinsertion in the cementum; however, it does not prevent root resorption or dentoalveolar ankylosis. It is thus evident that lincomycin is not efficacious as an antiresorptive substance. In the case of delayed replantation, based on scientific evidence, sodium fluoride is the substance of first choice in the treatment of the root surface (1). However, despite it being the standard solution, it is not totally effective, because it also does not eliminate the possibility of root resorption. Other substances have been tested for the treatment of the root surface, such as propolis which has antimicrobial activity. In the study by Gulinelli et al. (16), propolis and sodium fluoride were evaluated in the control of external root resorption in the case of delayed replantation. The results demonstrated the presence of inflammatory resorption by substitution and ankylosis in all groups. Lustosa-Pereira et al. (8) used sodium alendronate for treatment of the root surface and the incidence of ankylosis was also not reduced.

It should be pointed out that the results found here on the effect of the topical use of lincomycin in replantation showed positive effects for this drug in reducing the inflammatory process and in the reinsertion of the fibers of the ligament in the cementum. This warrants further studies including other lincosamide derivatives to quantify by means of histomorphometric analyses of the areas of root resorptions and/or dentoalveolar ankylosis and for longer periods of observation; such studies will determine the long-term effects of these drugs on repair after replantation to evaluate the development of resorption lesions.

Conclusions

It can be concluded that lincomycin utilized as treatment of the root surface in immediate replantation helped the repair of the periodontal ligament, favoring the reinsertion of the periodontal ligament fibers in the cementum in many specimens, and reduced the inflammatory process, but there were still areas of inflammatory tooth resorption and dentoalveolar ankylosis.

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