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Antiresorptivni učinak intrakanalne aplikacije alendronata na eksperimentalnom modelu

The Antiresorptive Effect of Intracanal Application of Alendronate in Canine Model

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Sažetak

Koristiti se tvarima koje sprječavaju resorpciju korijena bila bi alternativa slučajevima neuspješnih replantacija zuba. **Svrha:** Željelo se ispitati otopinu alendronata (ALN-a) kao intrakanalnog terapijskog sredstva za kasno reimplantirane zube. **Materijali i postupci:** Upotrijebljeni su zreli korijeni mandibularnih premolara četiriju pasa. Bili su endodontski tretirani, zatim ekstrahirani i sušeni 45 minuta. Nakon toga su podijeljeni u dvije skupine: u prvoj skupini intrakanalno su ispirani otopinom 1mMola ALN-a te topikalnom aplikacijom ALN-a 1mMola; u drugoj skupini učinjena je topikalna aplikacija ALN-a 1mMola. Zubi su replantirani u njihove odgovarajuće alveole. Životinje su žrtvovane četiri mjeseca nakon reimplantacije te su uzorci procesirani za morfometrijsku i mikroskopsku analizu. Preostala korijenska masa mjerena je kako bi se dobio opseg gubitka strukture korijena. **Rezultati:** Statistička analiza bila je obavljena multiplim usporednim testom Scheffe F i pokazala je znatnu razliku između tretiranih skupina ($p < 0,005$). **Zaključak:** Rezultati upućuju na to da alendronat mogu smanjiti resorpciju korijena ako se primjenjuju u obliku intrakanalne i topikalne otopine i to mnogo uspješnije nego kada se rabe samo kao topikalna otopina.

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Ključne riječi

zub, replantacija; zub, korijen; zub, avulzija; alendronat

Uvod

Najčešća terapija kojom se koriste stručnjaci kod pacijenata s avulzijom zuba jest reimplantacija. Kliničke i histološke studije pokazuju da su glavni čimbenici koji djeluju na reimplantirane zube vrijeme provedeno izvan alveole, uvjeti skladištenja i stupanj zrelosti korijena (1,2,3).

Ako se izbijen zub odmah reimplantira ili spremljuje u otopinu koja čuva parodontalne niti na njegovu korijenu, znatno se povećava uspješnost reimplantacije (4-7). Nažalost izbijeni zubi uglavnom dulje ostaju u suhom okolišu ili ekstraalveolarno. Zato se površina korijena mora tretirati te obaviti endodontska terapija (2). Upotrijebljeno je nekoliko tvari za tretman površina korijena reimplanti-

Introduction

The most frequent therapy used on patients with avulsed teeth is the method of replantation. Clinical and histological studies have shown that the main factors that have impact on replanted teeth are: the length of the extra-alveolar period, storage condition and maturation of the root (1,2,3).

When the avulsed tooth is immediately replanted or stored in a solution capable of preserving the periodontal fibers attached to the root, the chances of success in replantation increase considerably (4-7). Nevertheless, in the majority of cases, the avulsed teeth are left exposed to a dry environment or extraalveolar for a long period of time. Thus, the treatment of the root surface and endodontic therapy of the avulsed

ranih zuba kako bi se povećao stupanj njihova zadržavanja, kao što su otopine fluorida, hidrokloridna kiselina, kalcijev hidroksid, antibiotici i slično. (8). Otopina acetazolamida bila je djelotvorna u inhibiciji resorpcije korijena, podržavajući popravak izbijenih i reimplantiranih zuba (9). Bez obzira na vrstu tretmana površine korijena, u podacima iz literature pronašli smo stajalište da se reimplantirani zubi trebaju endodontski tretirati, jer nekrotična pulpa i njezini toksini utječu na periodontalni ligament kroz dentinske tubuse i imaju odlučujuću zadaću u procesu resorpcije (7, 10, 11).

Pokušavajući pronaći mogućnosti za inhibiciju i usporavanje procesa resorpcije unošenjem protuupalne ledermiksne paste u korjenske kanale reimplantiranih zuba kod majmuna, Wong i suradnici dobili su statistički veliku razliku u rezultatima samo u općem cijeljenju (12). A u Tropeovim objavljenim rezultatima, nakon što je aplicirao kalcijev hidroksid u zakašnjelu reimplantaciju zuba kod pasa, dobiven je pozitivan učinak na upalnu resorpciju (13). Korištenjem kalcijeva hidroksida kao intrakanalnog terapijskog uložka može se izbjeći ili ograničiti upalna resorpcija zbog alkalnog pH i baktericidnog učinka te eliminirati infekcija (14, 15). Unatoč takvim terapijama i dalje je mnogo slučajeva resorpcije kostiju te se pogođeni zubi ekstrahiraju u razdoblju od četiri do šest godina (16). Naime, potraga za novim tvarima koje bi mogle spriječiti ili odgoditi resorpciju korijena vrlo je zahtjevna. Nekim se postupcima ona više usporava negoli sprječava. Alendronat (ALN) treća je generacija bisfosfonata s inhibitornom ulogom na osteoklaste koja može usporiti proces resorpcije. Podaci iz literature upućuju na to da je njihov glavni način djelovanja prevencija gubitka koštane strukture i inhibicija funkcije osteoklasta uz narušavanje enzimskih puteva djelovanja koji znatno utječu na aktivnost i preživljavanje osteoklasta (17-23). U dosadašnjim istraživanjima ističe se učinak topikalne aplikacije alendronata u prevenciji resorpcije korijena (24-26). Svrha ovog istraživanja bila je ocijeniti antiresorptivni učinak otopine alendronata (ALN-a) kao intrakanalnog sredstva te topikalnu primjenu ALN-a u usporedbi s topikalnom primjenom 1mMola ALN-a kod kasno replantiranih zuba na modelu traume zuba kod pasa.

tooth must be realized (2). Several substances have been used for the treatment of the root surfaces of the replanted teeth in an attempt to increase their retention rate such as fluoride solutions, hydrochlorid acid, calcium hydroxide, antibiotic, etc (8).

Solution of acetazolamide was efficient in inhibiting root resorption, favoring the repair of avulsed and reimplanted teeth (9). Irrespective of the type of root surface treatment, there is consensus in the literature that replanted teeth should be endodontically treated because the necrotic pulp and its toxins affect the periodontal ligament through the dentinal tubules and play a decisive role to the resorption process (7,10,11).

Searching for the possibility to inhibit and slow down resorption processes through anti-inflammatory component applications of Ledermix paste into the root canals of replanted monkey teeth, Wong et al. showed a statistically significant difference in results only in overall healings (12). On the other hand, the results published by Trope, where the application of calcium hydroxide in delayed replantations of dog's teeth positively affects the inflammatory resorption (13). The use of calcium hydroxide as an intracanal therapeutic agent may avoid or limit inflammatory resorption, due to its alkaline pH and bactericidal effect, and therefore may eliminate the infection present (14,15). Even with such treatment possibilities, there are still many cases of root resorption, and affected teeth are extracted over a period of 4–6 years (16). Hence, the search for new substances that may inhibit or delay the effects of root resorption is demanding. Some treatment modalities aimed at slowing rather than inhibiting root resorption are used.

Alendronate (ALN) is a third-generation bisphosphonate, which demonstrates the osteoclast inhibitory activity that can slow down the resorptive process. To date research indicates that the main mode of action of bisphosphonates in the prevention of bone structure loss is inhibition of osteoclast function through the break-down of enzyme passages, which are very important for osteoclast activity and survival (17-23).

Previously published studies have demonstrated efficacy of topical application of alendronate in preventing root resorption (24-26).

The purpose of this study was to evaluate antiresorptive efficacy of a solution of Alendronate (ALN), as an intracanal therapeutic agent with topical application of ALN in comparison with topical application of ALN 1mMol for late reimplanted teeth on a dog dental trauma model.

Ispitanici i postupci

Korišteni su psi mješanci srednje težine 13,5 kg. Svi su bili skeletalno zreli. Životinje su držane u skladu s međunarodnim standardima za dobrobit životinja koja je prihvatilo Povjerenstvo za istraživanje i bioetiku Stomatološkog fakulteta Sveučilišta u Sarajevu (Izdavanje # 09/133-3/04). Svi eksperimentalni postupci obavljani su pod općom anestezijom. To je postignuto preanestetičkom sedacijom Acepromazinom maleatom (Vetranquil, Sanofi, Francuska) u dozi od 0,05 mg/kg IM, te indukcijom anestezije s Propofolom (Propofol Abbott, Pakistan) u dozi od 4mg/kg IV. Nakon toga je slijedila intubacija i održavanje uz pomoć 2,5-postotnog Halothana (Fluothane, Zeneca, Velika Britanija) i protokom kisika od 2 litre u minuti. Odmah nakon intubacije životinje su dodatno dobile jednokratnu dozu Tramadol-hidrochlorida (Lumidol, Belupo, Hrvatska) u količini od 7 mg/kg SC. U ovom istraživanju rabljeno je 35 mandibularnih premolarnih korijena četiriju pasa, a svaki je služio i sam sebi kao kontrola. Koristili smo se dvokorijenskim mandibularnim premolarima (P2, P3 i P4). P1 je bio isključen zbog veličine korijena i nemogućnosti imobilizacije. Zbog oštećenja korijena ili okolne kosti bilo je isključeno 13 korijena. Dvokorijenski premolari separirani su hemisekcijom, zatim trepanirani, obrađeni K-tipom čeličnih instrumenata do apikalne delte, te irigirani sterilnom fiziološkom otopinom. U prvoj su skupini (17 korjenova) sterilni papirnati šiljci bili natopljeni u 1 mMola ALN-a i postavljeni u korijenski kanal. Zatim je okluzalni pristupni kavititet zatvoren privremenim ispunom (Herculite XRV™). U drugoj skupini (18 korijena) kanali su bili osušeni papirnatim šiljcima i i intrakanalno napunjeni kondenziranom gutaperkom (VDW, GmbH, Njemačka) i Rothovim cementom 801 (Roth International LTD, SAD).

Zubi su bili atraumatski ekstrahirani koliko je to bilo moguće i sušeni 45 minuta na zraku. Nakon toga su korjenovi obiju skupina bili pet minuta u otopini 1mMola ALN-a te replantirani.

Eksperimentalne životinje s intrakanalno postavljenim ALN-om, ponovno su sedirane (kako je već opisano) nakon sedam dana te je tada papirnati šiljak s ALN-om zamijenjen lateralno kondenziranom gutaperkom (VDW, GmbH, Njemačka) i Rothovim cementom 801 (Roth International LTD, SAD).

Otopina Alendronata (Fosamax® 70mg alendronat sodium oral solution, Merck &Co Inc.Whitehouse station NJ, 08889, SAD) bila je pripremljena otapanjem u destiliranoj vodi. Zbog stabilnosti reimplantiranih zuba odlučeno je da nije potrebno

Material and Methods

We used four mongrel dogs with mean weight 13,5 kg. All dogs were skeletally mature. The animals were handled according to international standards of animal welfare that are accepted by The Research and Bioethics Committee of the Dentistry School, of the University of Sarajevo (Issue number 09/133-3/04). All the experimental procedure was performed with the animals under general anesthesia. This was accomplished by preanesthetic sedation with acepromazin maleate (Vetranquil, Sanofi, France) at the dose of 0.05 mg/kg IM, and anesthetic induction with Propofol (Propofol Abbott, Pakistan) at the dose of 4mg/kg IV, followed by intubations and maintenance with 2,5% halothane (Fluothane, Zeneca, UK) with the oxygen flow of 2 liters/minute. Additionally, immediately after intubations, the animals were given a one-time dose of tramadol hydrochloride (Lumidol, Belupo, Croatia) at the amount of 7mg/kg SC. 35 mandibular premolar mature roots of four dogs were used in the study, each dog served as its own control. We used two rooted mandibular premolar teeth (P2, P3, and P4). P1 premolar was excluded because of the size of the root and impossibility of immobilization. 13 roots were excluded due to root or bone damage. The two-rooted premolars were hemisected and accessed, instrumented with stainless steel K-type files to the apical delta, and irrigated with sterile saline.

In the Group I (17 roots), sterile paper points were soaked in 1 mMol ALN and placed in the root canal. Occlusal accesses in this group were filled with temporary filling (Herculite XRV™)

In the Group II (18 roots), canals were dried with paper points, and obturated with laterally condensed guttapercha (VDW, GmbH, Germany) and Roth's 801 sealers (Roth International LTD, USA)

Teeth were extracted as atraumatically as possible, and bench dried for 45 minutes. Furthermore, group I and II roots were soaked in: 1mMol of ALN for 5 min and replanted.

The experimental animals with intracanal ALN placement were again sedated (as previously described) after seven days and intracanal paper points of ALN was changed with laterally condensed gutta-percha points (VDW, GmbH, Germany) and Roth's 801 sealers.

Alendronat solutions were prepared by dissolving Alendronat (Fosamax® 70mg alendronat sodium oral solution, Merck &Co Inc.Whitehouse station NJ, 08889, USA) in distilled water. Due to the

splintiranje. Zbog divergencije korjenova mandibularnih premolara kod pasa, za fiksaciju su korišteni samo šavovi (Mersilk 3-0, 26mm 1/2c, okruglo tijelo, Ethicon Inc.SAD). Psi su dva dana dobivali mekanu hranu. Dobro su podnosili proceduru, pa se njihove prehrambene navike i ponašanje nisu promijenili u odnosu prema onome prije zahvata. Nakon četiri mjeseca životinje su uspravane smrtnom dozom od 6 posto otopine natrijeva pentobarbitala IV, te su koštani blokovi pripremljeni za analizu. Blokovi uzoraka sa zubima i okolnom kosti disecirani su dijamantnim diskovima te fiksirani u 10-postotnom neutralnom formalinu. Nakon toga su sedam dana bili uronjeni u 5-postotnu dušičnu kiselinu (HNO_3) te zatim 24 sata u 5-postotni natrijev sulfat (Na_2SO_4). Koštani blokovi postavljeni su u uređaj za tkivno procesiranje (Microm STP 120, MICROM International GmbH, Njemačka) zbog procesa dehidracije u različitim koncentracijama alkohola - od 70 posto (mijenjanjem otopine svakih 60 minuta po tri puta - I, II, III), 96 posto i 100 posto (promjenom otopine svakih 60 minuta dva puta - I, II), zatim u organsko otapalo Xylol (promjenom otopine svakih 60 minuta dva puta - I, II) kako bi se konačno dobila impregnacija tkiva u tekućem parafinu na temperaturi od 56°C . Nakon završenog postupka uzorak je bio uložen u parafinske blokove veličine $2 \times 2 \times 2$ cm te izrezan na rotacijskom mikrotomu (Leica RM 2145, Leica Microsystems, Švicarska). Koštani blokovi rezani su horizontalno u odnosu prema uzdužnoj osi zuba na $5 \mu\text{m}$, u intervalima od $140 \mu\text{m}$. Nakon jednosatnog sušenja u termostatu na temperaturi od 50°C , nastavljeno je s bojenjem prema standardnoj henatoksilin-eozinskoj metodi. Od svakog korijena analizirana su po 4 reza, a pozornost se posvećivala resorpciji kosti i vrsti spoja između korijena i okolnog tkiva. Patohistološka prosudba obavljena je na osnovi modificirane verzije klasifikacije prema Andersenu: cijeljenje s normalnim parodontnim ligamentom, cijeljenje ankilozom i cijeljenje uz upalnu resorpciju (24). Svaki rez korijenske strukture bio je podijeljen u osam dijelova te označen slovima (od A do H) i na svakom je segmentu određena vrsta cijeljenja (Slika 1.).

stability of the replanted teeth, splinting of teeth was determined to be unnecessary. Because of divergence of the roots of mandibular premolar teeth in canine, only sutures for fixation were performed (Mersilk 3-0, 26mm 1/2c, round bodied, Ethicon Inc.USA).

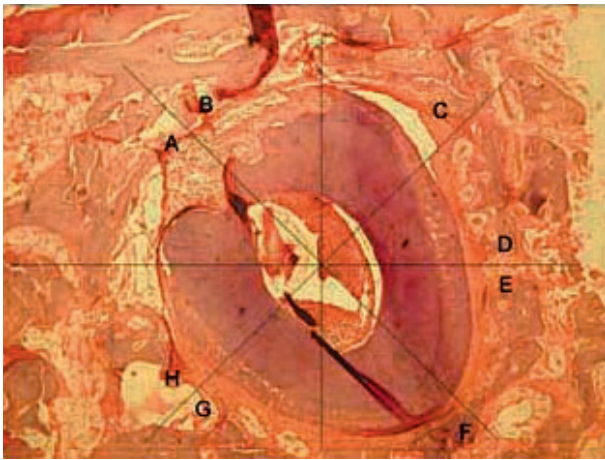
The dogs were maintained on a soft diet for 2 days. They tolerated the treatment procedures well, and their food intake and behavior did not change following the treatment.

After four months, the animals were sacrificed by an overdose of a 6% solution of sodium pentobarbital administered IV, and the bone blocks were prepared for analysis.

Block specimens containing the teeth and surrounding alveolar bone were dissected using diamond separators, and fixed in 10% neutral formalin. After that, bone blocks were placed in 5% nitric acid HNO_3 for seven days and then in 5% sodium sulphate Na_2SO_4 for 24 hours. Bone blocks were placed into tissue processor (Microm STP 120, MICROM International GmbH, Germany) for the process of dehydration through various percentages of alcohol, from 70% (changing the solution every 60 minutes for three times I, II, III,) 96% and absolute alcohol (changing the solution every 60 minutes for two times I, II), then in organic solvent Xylol (changing the solution every 60 minutes for two times I, II), and finally to tissue impregnation in liquid paraffin at the temperature of 56°C . After the process of impregnation was completed, the specimens were embedded in paraffin blocks sized $2 \times 2 \times 2$ cm and sectioned on rotary microtome (Leica RM 2145, Leica Microsystems, Swiss).

Bone blocks were sectioned horizontally in reference to the longitudinal axis of the teeth to the thickness of $5 \mu\text{m}$, at $140 \mu\text{m}$ intervals. After drying in thermostat at the temperature of $50^\circ\text{C}/1\text{h}$, we proceeded with staining by haematoxylin-eosin method in digester. Five sections per tooth were analyzed for the extent of root resorption and type of interface between the root and surrounding tissue. Pathohistological evaluation was done based on modified version of the classification by Andreasen: healing with a normal periodontal ligament, healing with ankylosis, and healing with inflammatory resorption (24).

Every section of the root structure was divided in 8 parts labeled with letters (A-H), and in every segment, we evaluated the healing models, Figure 1.



Slika 1. Poprečni presjek histološkog izgleda procijenjene površine korijena - Hematoksilin i eozin (X 10)
Figure 1 Cross-section of the histological appearance of an evaluated root surface Hematoxylin and eosin (X 10)

Histološko ocjenjivanje resorpcije korijena

Na svakom je korijenu bilo pod svjetlosnim mikroskopom (10 x povećanje) ocijenjeno pet rezova kako bi se odredio opseg resorpcije korijena i vrsta spoja između korijena i okolnog tkiva. Za ocjenjivanje gubitka strukture korijena određen je originalni opseg korijena. Polumjer preostale strukture stupnjevan je na linearnoj ljestvici od 0 do 6 za svaku od osam točaka na preslikanoj mrežici - vrijednost šest dana je za nepromijenjen polumjer, a vrijednost 0 za točku bez preostale strukture korijena. (Slika 2.)

Gubitak korjenske mase bio je raščlanjen multiprim usporednim testom Scheffe F kako bi se identificirala razlika među skupinama.

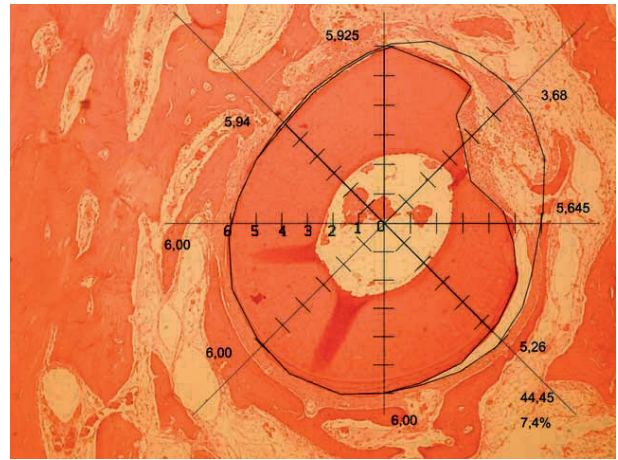
Za ocjenu statističke znatnosti u skupinama s različitim modalitetima cijeljenja korišten je T-test za neovisne uzorke. Statistički izračuni obavljani su Windowsovim programom SPSS®.

Rezultati

Rezultati histomorfometrijske analize modela cijeljenja za svaku skupinu terapijskog protokola bili su sljedeći: u prvoj skupini (n=17) bilo je najčešće normalno cijeljenje - 50,97 posto, ankiloza se pojavila u 27,09 posto slučajeva, a upalne resorpcije u 21,94 posto.

U drugoj skupini (n=18) najčešće je bilo normalno cijeljenje - 47,94 posto, ankiloze je bilo 25 posto, a upalnih resorpcija 27,06 posto.

Statistička analiza T-testom pokazala je veliku razliku između prve i druge skupine u upalnoj resorpciji ($p < 0,005$), ali nije bilo znatne razlike u odnosu prema ostalim tipovima cijeljenja, Tablica 1.



Slika 2. Preklapanje mrežice s osam točaka na histološki rez - Hematoksilin i eozin (X10)
Figure 2 Superimposed eight point grid on histologic section Hematoxylin and eosin (X10)

Histological evaluation of root resorption

Five sections per tooth were analyzed with light microscopy (10 x magnifications) for the extent of root resorption and type of interface between the root and surrounding tissue. To evaluate the extent of root structure loss due to resorption, the original circumference of the root was determined. The radius of the remaining root structure was ranked on a linear, integer 0–6 scale at each of the eight points of the superimposed grid, with the value of 6 given to an unaffected radius and the value of 0 given to a point without any remaining root structure, Figure 2.

The root mass loss data were subjected to Scheffe F multiple comparison tests to identify differences between groups. The T-test for two independent samples was used to evaluate significance of differences in groups with different healing modalities. Statistical calculations were carried out with statistic software programme SPSS® for Windows®.

Results

The results of the histomorphometric analysis of healing models for each treatment group are as follows: In the group I (n=17): there was a highest occurrence of normal healing 50.97%, ankylosis 27.09%, inflammatory resorption 21.94%. In the group II (n=18) there was a highest occurrence of normal healing 47.94%, occurrence of ankylosis was 25% and inflammatory resorption 27.06% .

Statistical analysis performed with the T-test revealed significant differences among group I and group II in inflammatory resorption ($p < 0.005$), but there were no significant differences between groups in other healing models, Table 1.

Tablica 1. Statistička analiza T-testom za načine cijeljenja

Table 1 Statistical analysis performed with the T-test for healing modalities

	skupina • Group	aritmet. sredina • Mean	stand. devijacija • Standard deviation	stand. pogreška • Standard Error	T – TEST
normalno cijeljenje • Normal Healing	Alendronat 1 mmol	3,84	1,38	0,15	t=-,927; df=157; p=,360 p>0.05
	Alendronat intrakanalno • intracanal	4,08	2,03	0,21	
upalna resorpcija • Inflammatory resorption	Alendronat 1 mmol	2,16	1,35	0,15	t=-2,001; df=173; p=,047 p<0.05
	Alendronat intrakanalno • intracanal	1,76	1,35	0,14	
ankiloza • Ankylosis	Alendronat 1 mmol	2,00	1,53	0,17	t=-,670; df=171; p=,506 p>0.05
	Alendronat intrakanalno • intracanal	2,17	1,76	0,19	

Gubitak strukture korijena u različitim skupinama

Rezultati histomorfometrijske analize gubitka strukture korijena za svaku skupinu iznose: u prvoj skupini gubitak strukture zuba bio je 11,59 posto; u drugoj skupini gubitak strukture zuba iznosio je 17,21 posto. Nakon obrade multiplim usporednim testom Scheffe F, dobivena je statistički velika razlika među skupinama ($p < 0,005$), Tablica 2.

Root structure loss in different groups

The results of the histomorphometric analysis of root structure loss for each treatment group are as follows: In the group I the root structure loss was 11.59%. In the group II the root structure loss was registered at a value of 17.21%.

Statistical analysis performed with the Scheffe F multiple comparison test revealed significant differences among treatment groups ($p < 0.005$). (table 2)

Tablica 2. Scheffeoov test za gubitak strukture korijena

Table 2 Scheffe test for root structure loss

skupina • Group	skupina • Group	srednja razlika • Mean differences	stand.pogreška • Standard error	P		
Alendronat 1mmol	Alendronat intrakanalno • intracanal	5,6229	1,02163	,000	2,7524	8,4934

Rasprava

Histološki je dokazano da je zapravo upalni odgovor inicijalna reakcija nakon zakašnjele reimplantacije zuba. U mnogim istraživanjima stručnjaci su pokušali identificirati lijek koji bi djelovao kao inhibitor resorpcije reimplantiranog korijena (12,13,27-30). Unatoč mnogobrojnim testiranim tvarima, ni jedna nije u cijelosti spriječila resorpciju. Dosadašnja istraživanja upućuju na to da je glavni oblik djelovanja bifosfanata u prevenciji gubitka koštane strukture inhibicija funkcije osteoklasta preko prekidanja prometa enzima koji su iznimno važni za djelovanje i preživljavanje osteoklasta (31). Na temelju tih svojstava bifosfanatne tvari korištene su kao topikalne u istraživanjima zuba i dale su zadovoljavajuće rezultate (24, 25, 32). Na temelju istraživanja su Peshley i njegovi kolege te Ozok dokazali permeabilnost dentinskih kanalića, a Kum i suradnici predložili su tu otopinju kao intrakanal-

Discussion

It is histological proven that the initial reaction after delayed replantation of teeth is essentially an inflammatory response. Many studies have tried to identify a medicament that would act in the manner of inhibiting root resorption of replanted teeth (12,13,27-30).

Despite the great number of substances tested for treatment of the radicular surface resorption, none has yet completely avoided the occurrence of resorptions.

To date research indicates that the main mode of action of bisphosphonates in the prevention of bone structure loss is inhibition of osteoclast function through the breakdown of enzyme passages, which are very important for osteoclast activity and survival (31).

Based on the aforementioned characteristics, bisphosphonated compounds have been used as top-

no terapijsko sredstvo (33-36). Mori i njegovi kolege otkrili su difuzijsko svojstvo otopine alendronata da kroz dentinske tubule proдре do vanjske površine korijena (9).

Kada se Alendronat postavlja kao intrakanalni medikament, djeluje kroz dentinske kanale kao proturesorptivna tvar za zaštitu od osteoklastne penetracije u strukturu dentina. Proturesorptivan učinak bifosfanata je dvosmjerni: unutarnji i vanjski. Na temelju navedenih istraživanja ispitali smo Alendronat kao intrakanalni terapijski uložak i usporedili podatke sa studijom Levina i suradnika, te smo dobili bolje rezultate u gubitku strukture korijena. U Levinovu istraživanju govori se o 30 posto gubitka strukture korijena u skupini podvrgnutoj topikalnoj aplikaciji 1mMolom Alendronata, a u našem istraživanju imali smo 17,21 posto gubitka u 1mMol skupini. Mi smo također statistički usporedili skupinu s intrakanalno postavljenim Alendronatom s rezultatima 1 mMol skupine, no uzeli smo u obzir da smo ih rehidrirali u istoj koncentraciji, te smo dobili statistički mnogo manji gubitak strukture korijena kod skupine s intrakanalnom primjenom Alendronata od 11,59 posto, u odnosu prema skupini od 1mMola Alendronata i 17,21 posto gubitka ($p < 0,005$). Na kraju su sve skupine bile podvrgnute usporednom testu ($p < 0,005$) i na temelju tih rezultata možemo pretpostaviti da razlika među skupinama nije slučajna nego se temelji na različitim koncentracijama i oblicima lokalne aplikacije Alendronata. Kada se govori o različitom načinu cijeljenja kod skupine od 1mMola Alendronata, Levin je u svojem istraživanju zabilježio 29 posto normalnog cijeljenja, 30 posto ankiloze i najveći postotak upalne resorpcije – čak 41 posto. U našem smo istraživanju u istoj skupini zabilježili 48 posto normalnog cijeljenja, 25 posto ankiloze i 28 posto upalne resorpcije. Takve rezultate u našem istraživanju kronološki prate i postoci gubitka strukture korijena, što se može jasno vidjeti u usporednom pregledu statističkih trendova u vrijednosti normalnog cijeljenja i postocima gubitka strukture korijena. Ekstremno visoke vrijednosti upalnih resorpcija iznenadile su stručnjake, jer su očekivali da će endodontska terapija spriječiti bakterijsku penetraciju iz korijenskog kanala četiri mjeseca nakon reimplantacije. Bez ikakva vidljivog poticaja produžene upale, većina područja bi normalno cijelila ili bi pokazivala ankiлозу, a na tim bi mjestima aktivna resorpcija bila iznimka (24). Hipoteza da Alendronat djeluje protuupalno nije bila uopće postavljena - govorilo se samo o proturesorptivnom učinku koji u statističkim

ical agent in dental research and demonstrated satisfactory results (24,25,32).

Based on the previously published research of Peshley et al. and Ozok which thoroughly clarifies the permeability of dentine canals, Kum et al. suggested utilization of this solution as intracanal therapeutic agent (33-36). Mori et al. revealed the diffusion capacity of the solution of alendronate through the dentinal tubules, reaching the external root surface (9).

When alendronat is placed as an intracanal medicament, it acts through dentine canals as an anti-resorption substance for the protection of osteoclast penetration into dentine structure. Thus, the anti-resorption activity of bisphosphonate is focussed in two directions: internal and external.

Based on previously mentioned studies we tested alendronate as an intracanal therapeutic agent, and compared with study published by Levin et al. We obtained better results in root structure loss. Levin study registered 30% root structure loss in the group treated with topical application of 1mmol alendronate. In our research, we registered 17.21% root structure loss in the 1mmol group. We also statistically compared the intracanal alendronate group with the 1 mmol group results, given that we hydrated the roots in the same concentration, and obtained a statistically significantly smaller loss of root structure in the intracanal alendronate group 11.59%, than in 1mmol alendronate group 17.21% ($p < 0.005$). Finally, all groups were subjected to joint comparative tests ($p < 0.005$), based on this results we can assume that differences between groups are not coincidence but the result of different concentrations and forms of local alendronate applications. When it comes to the results of different healing modalities in the alendronate 1mmol group, the above mentioned Levin study registered 29% normal healings, 30% ankylosis, and the highest percentage of inflammatory resorptions, 41%. In our study, in the same group, we registered 48% normal healings, 25% ankylosis, and 28% inflammatory resorptions. Such results of different healing modalities in our study are chronologically followed by the results of root mass loss percentages, which can clearly be seen in the comparison review of statistical trends in the values of normal healing and percentages of root structure loss. Extremely high values of the registered inflammatory resorptions surprised the previous researchers, who expected that endodontic treatment would stop the penetration of bacteria from the root canal 4

okvirima pokazuje znatno više normalnih cijeljenja u eksperimentalnoj skupini negoli u kontrolnoj. U vrijednostima različitih načina cijeljenja, mnogo je više upalnih resorpcija u skupini od 1mMola kada se uspoređi sa skupinom s intrakanalnom aplikacijom Alendronata ($p < 0,005$). Rezultate tog istraživanja možemo predložiti kao znanstveni pristup za buduća istraživanja u području traumatskih ozljeđa dento-alveolarnog područja. Možemo zaključiti da intrakanalna i topikalna aplikacija Alendronata u koncentraciji od 1mMola pokazuje jači proturesorptivni učinak nego topikalna aplikacija Alendronata bez intrakanalnog postavljanja. Aplikacija Alendronata u obliku intrakanalnog uložka reimplantiranog zuba uz topikalnu aplikaciju hidracije, omogućuje učinak lijeka u dvama smjerovima: vanjskom i unutarnjem.

months after replantation. Without any evident stimulus of prolonged inflammation, most areas would normally heal or display ankylosis, and these places of active resorption would be an exception (24). However, not even a hypothesis was made that alendronate has anti-inflammatory effects, only an anti-resorptive effect was hypothesized, which in terms of statistics, certainly shows a significantly higher number of normal healings in experimental than in control groups. In terms of the values of different healing modalities there is significantly higher number of inflammatory resorptions in the 1 mMol-group when compared to the group with intracanal application of alendronate ($p < 0.005$).

According to the results of this study we can propose this scientific approach for the future researches in the area of traumatic injuries of dento-alveolar.

We can conclude that the intracanal and topical alendronate application of 1mMol concentration shows stronger antiresorptive effects than topical application of alendronate without intracanal placement. Application of alendronate in the form of intracanal placement of the replanted tooth with topical hydration enables the effect of medicament in the two directions: external and internal.

Abstract

Use of root resorption-inhibiting substances may be an alternative in cases of unsuccessful reimplantation of the teeth. **Objective:** The aim of this study was to test a solution of Alendronate (ALN), as an intracanal therapeutic agent for late replanted teeth. **Material and methods:** Mandibular premolar mature roots of four dogs were used in the study. The roots were endodontically treated, then extracted and bench dried for 45 minutes. Thereafter, the teeth were divided into two groups: Group I: intracanal dressing with solution of 1mMol ALN and topical application of ALN 1mMol, Group II: topical application of ALN 1mMol. Teeth were then replanted in their respective sockets. The animals were sacrificed four months after replantation and the samples processed for morphometric and microscopic analysis. The residual root mass was measured to determine the extent of root structure loss. **Results:** Statistical analysis performed with the Scheffe F multiple comparison test revealed significant differences among treatment groups ($p < 0.005$). **Conclusion:** The results indicate that alendronate was able to reduce root resorption when applied as intracanal solution and topical solution more efficiently than when applied solely as topical solution.

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Key words

Tooth Replantation; Tooth Root; Tooth Avulsion; Alendronate

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