Učinak CO_2 i Nd:YAG lasera na parodontno tkivo u furkaciji molara

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Sažetak

CO_2 i Nd:YAG laseri najčešće su rabljeni laseri u stomatologiji i u medicini. Zbog atraumatskog i djelotvornoga rezanja mekih tkiva laser se može, između ostalog, rabiti za pulpotomiju mliječnih i trajnih mladih zuba. Svrha rada bila je istražiti učinak tih dvaju lasera, čije su energetske vrijednosti dostatne za ablaciju mekih tkiva, na parodontno tkivo tijekom pulpotomije laserom.

Eksperiment je izvršen na psima mješancima teškim oko 25 kg. Životinje su anestezirane pentobarbitalom, trepanirane su pulpne komore molara, a pulpno tkivo je uklonjeno ekskavatorom te učinjena namjerna trepanacija dna pulpne komore. Parodontno tkivo u furkacijskom području obasjano je CO_2 (2 W / 10 ms / 5x/s) i Nd:YAG (2W / 20 pps) laserskom zrakom tijekom 3 s. Na histološkim pripravcima, učinjenim 30 odnosno 45 dana nakon obradbe, vidi se površinska nekroza kosti, upalna infiltracija, fragmentacija koštanih lamela. Prvi znakovi cijeljenja koštane strukture javljaju se kod CO_2 lasera 45 dana nakon obasjavanja stvaranjem novoga koštanog matriksa, osteoida. Nd:YAG laser dublje prodire u tkivo i uzrokuje opsežnije promjene te i 45 dana nakon obradbe tim laserom nije opaženo da parodontna rana zacjeljuje.

Ključne riječi: *parodontno tkiivo, CO_2 laser, Nd:YAG laser, histološke promjene*

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Uvod

Prva istraživanja o utjecaju lasera na zubna tkiva počeli su Goldman i sur. (1), te Stern i sur. (2), ubrzo nakon Maimanova izuma lasera 1960. godine, a slijedio ih je čitav niz autora u laboratorijskim i kliničkim ispitivanjima. Danas postoji više od 600 vrsta laserskih aktivnih sredstava koja određuju valne dužine laserskoga svjetla, a u stomatologiji su najčešće upotrebljavaju CO_2 i Nd:YAG laseri (3,4).

Uporaba lasera u somatologiji može se u grubo podijeliti na postupak na tvrdim tkivima i na kirurgiju mekih tkiva (5), premda se laser također može

rabiti za dijagnostiku (1), analgeziju (6), biostimulaciju (7) i za polimerizaciju kompozita (8).

Uspješna uporaba lasera u parodontologiji, kojom prednjači pred drugim tehnikama, očituje se u gingivoplastici i u uklanjanju hiperplastičnoga gingivnog tkiva (9). Osim toga, laser se može upotrijebiti za čišćenje kamenca (10), za kiretažu mekih tkiva (11) i zaglađivanje površine korijena (12).

S obzirom na činjenicu da laser reže tkivo djelotvorno i atraumatski, može se uporabiti za pulpotomiju mliječnih i trajnih mladih zuba (13, 14). Tijekom rada svrdlima u pulpnoj komorici incidentno se može probiti dno pulpne komore i nenamjerno obasjati furkacijsko područje parodonta. Zato smo u ovome radu htjeli ispitati učinak CO_2 i Nd:YAG laserskih zraka, čije su vrijednosti energije dostatne za ablaciju mekih tkiva, na parodontno tkivo furkacije psećih molara nakon neposrednog obasjavanja te na histološkim pripravcima pronaći znakove zacjeljivanja tkiva.

Materijal i metoda

Eeksperiment smo proveli na dvama psima mješancima teškim oko 25 kg. Anesteziranisu s 30 mg pentobarbitala po kg tjelesne težine, a ako se pokazala potreba dodatno je injiciran anestetik intraperitonealno. Za vrijeme zahvata, glave životinja bile su omotane sterilnim kompresama. Na tretirane je zube postavljen rubber dam. Usta životinja držana su otvorena držačem čeljusti postavljenim na strani suprotnoj od radne. Pulpne komore gornjih molara trepanirane su visokoturažnom vrtaljkom uz hlađenje sterilnom destiliranom vodom, a pulpno je tkivo uklonjeno oštrim ekskavatorom. Namjerno je izvršena trepanacija dna pulpne komore sa sterilnim čeličnim svrdlom kako bi se prikazalo područje furkacije. Rana je isprana s 0,9 postotnom otopinom natrij klorida. Područja furkacije desne strane gornje čeljusti obasjavana su s CO_2 LX-20D (Luxar Corp., Bothel, WA, USA) laserom (2W / 5x10 ms/ s) tijekom 3 sekunde. Furkacijska područja lijeve strane čeljusti obasjavana su s Nd:YAG dLase-300 (American Dental Laser, Fremont, CA, USA) laserom (2W/20 pps) isto vrijeme.

Zraka CO_2 lasera provedena je savitljivim šupljim vodičem zraka koji se završava metalnim nastavkom promjera 0,8 mm, a zraka Nd:YAG lasera provedena je do tkiva savitljivim optičkim vlaknom promjera 300 μm.

Zube tretirane u eksperimentu, nakon obasjavanja, ispunili smo polikarboksilatnim cementom "My-Bond Carbo" (Shofu, Inc., Kyoto Japan). Cement je miješan na sterilnoj staklenoj pločici.

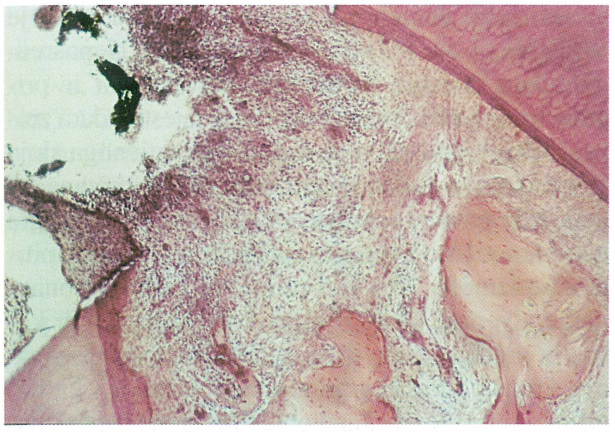
Kod oba se je lasera radilo kontaktnim i statičnim načinom rada. Pri radu s CO_2 laserom za svaki je zub rabljen sterilan kovinski završetak. Nakon obasjavanja svakoga zuba Nd:YAG laserom, vrh optičkog vlakna odrezan je sterilnim skalpelom. Preostali dio vrha optičkoga vlakna, između dvaju obasjavanja, bio je uronjen u 8% otopinu natrij hipoklorita (NaOCl).

Petnaest dana nakon prvog dijela pokusa životinje su ponovno anestezirane pentobarbitalom, a postupak je ponovljen na molarima donje čeljusti.

Životinje su usmrćene 45 dana nakon zadnjeg postupka prekomjernom intravenoznom dozom pentobarbitala. Uklonjeno je meko tkivo i čeljusti su resecirane. Prije nego što su čeljusti uronjene u 10 postotnu otopinu formalina, uklonjen je cement iz kaviteta da bi fiksativ lakše prodro u preostalo tkivo. Fiksacija u formalinu trajala je 7 dana, nakon čega su čeljusti dekalcinirane dnevnim promjenama demineralizacijske otopine (dušična kiselina) raznih koncentracija. Nakon demineralizacije, od čeljusti su odvojeni mikrotom nožem blokovi tkiva koji su sadržavali tretirane zube i njihov parodont. Za vrijeme dok su zubi bili uronjeni u Celloidin (Cekudol + 30 postotni etanol, Merck, Darrmstad; Germany), orijentirani su tako da je bilo moguće napraviti sekcije usporedne s okomitom osi zuba. Kad se masa stvrdnula, napravljeno je 6 sekcija središnjega dijela područja furkacije debljine 3 μm. Pripravci su obojeni hematoxylinom i eosinom i histološki su analizirani mikroskopom (AMPLIVAL, Carl Zeiss Jena, Germany).

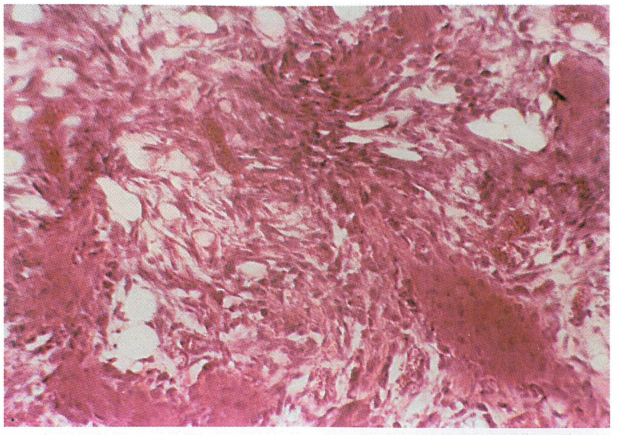
Rezultati

Na površini mjesta trepanacije u furkaciji molara obasjavanih CO_2 laserom nalaze se čestice karbonificiranog odnosno nekrotičnog tkiva. Postoje limfociti i jaka upalna reakcija. Koštano tkivo pokazuje promjene od destrukcije kortikalnog dijela do fragmentacija lamela (Slika 1). Postoji razrješenje kosti, koštane gredice su stanjene i nepravilne, a na



Slika 1. *Upala, nepravilne koštane gredice te resorpcija cementa HE pov. x 40*

Figure 1. *Inflamation, irregular bone trabeculae and cement resorption HE mag. x 40*



Slika 3. *Stvaranje nove kosti - osteoid s osteoblastima HE pov. x 200*

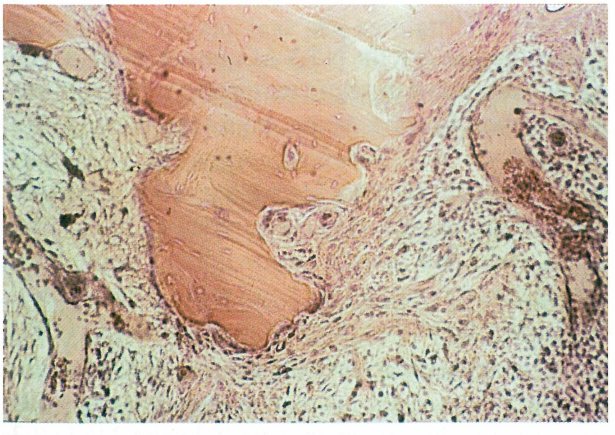
Figure 3. *New bone formation - osteoid with osteoblasts HE mag. x 200*

njima se pomnijim pregledom pod većim povećanjem opažaju multinuklearne orijaške stanice, osteoklasti te resorpcija kosti (Slika 2). Koštane su lakune neposredno ispod nekrotičnoga pojasa prazne, bez osteocita. Krvne su žile proširene i uočljiva je ekstravazacija eritrocita.

Parodontna pukotina je proširena, a ligamentna su vlakna naglašene celularnosti. Cement je neravan i nazubljen, a postoji resorpcija cementa s urastanjem granulacijskoga tkiva.

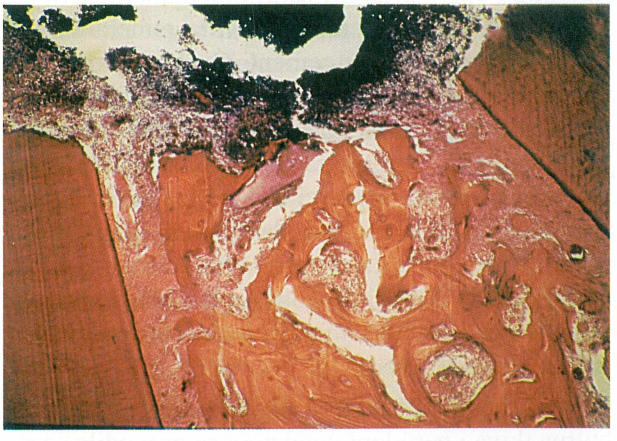
Na pripravcima zuba gornje čeljusti, koji su obasjavani 15 dana prije zuba donje čeljusti, u dubljim dijelovima kosti, u području pregradnje, vidi se granulacijsko tkivo (novostvorene kapilare, fibroblasti, vezivna vlakna i limfociti). Ono se isprepliće s novostvorenim koštanim matriksom tj. osteoidom u kojemu još uvijek nije nastala mineralizacija (Slika 3).

Promjene u interradikularnom tkivu uzrokovane djelovanjem Nd:YAG lasera razlikuju se svojim opsegom i zahvaćanjem dubljih područja od onih koje su dobivene CO_2 laserom. Nekroza na površini parodontne rane je veća. Na nju se nadovezuje upala s resorpcijom koštanih gredica, a nekrotične, karbonificirane čestice moguće je pronaći i u dubljim slo-



Slika 2. *Resorpcija kosti s umočenim octeoklastima HE pov. x 200*

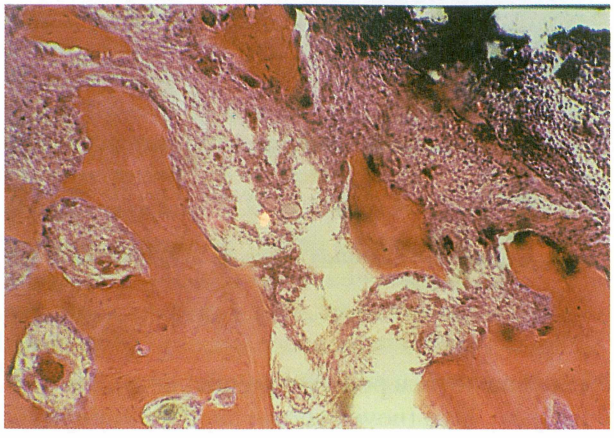
Figure 2. *Resorption of bone with multiplied osteoclasts HE mag. x 200*



Slika 4. *Karbonificirano tkivo na površini, krvarenje u kosti te upalna infiltracija HE pov. x 40*

Figure 4. *Carbonized surface, bleeding in bone and inflammatory infiltration HE mag. x 40*

jevima tkiva (Slika 4). Izražena je rarefikacija: koštane gredice su stanjene, a na mjestu spongioze nalaze se mononuklearne upalne stanice (Slika 5). Jaka fragmentacija kosti seže u dublja područja, a multinuklearne orijaške stanice okružuju fragmente kosti.



Slika 5. *Destrukcija kosti: na mjestu spongioze nalaze se upalne stanice HE pov. x 100*

Figure 5. *Bone destruction: inflammatory cells on the place of spongiosis HE mag. x 100*

Rasprava

Rabeći laser za uklanjanje bolesnoga dijela pulpe iz komore, kako bi se sačuvala vitalnost njezina radikularnoga dijela, treba imati na umu mogućnost jatrogenog obasjavanja u parodontnog tkiva. Parodont u svojoj strukturi sadrži meka tkiva primjerice vezivno tkivo i epitel, te tvrda mineralizirana tkiva kao što su kost i cement. Osobitost kronične upale koja zahvaća parodontna tkiva jest stvaranje granulacijskoga tkiva koje se sastoji od proliferirajućih kapilara, nezreloga kolagena te različitoga stupnja celularne infiltracije. Potreba da se ukloni granulacijsko tkivo ili pak da se preoblikuju meka tkiva, te njihova uska povezanost s tvrdim tkivima, također često dovodi do doticaja laserske zrake parametara dostatnih za ablaciju mekih tkiva s tvrdim parodontnim tkivima.

Istraživanja utjecaja laserskih zraka na mineralizirna tkiva i parodont uglavnom se provodila s vrijednostima energije većim od dostatnih za ablaciju mekih tkiva (15, 16). Vrijednost gustoće energije CO_2 lasera (41,28 J/cm^2), dostatne za ablaciju granulacijskoga tkiva iz parodontnih džepova, rabio je Williams sa sur. (17) u pokusu na psima. Neposredno nakon laserske obradbe, na histološkim se pripravcima opažala nekroza kosti na mjestu udara zrake, široka 40 do 120 µm. Ispod nekroztiènoga sloja bio je pojas avitalne kosti u kojemu su koštane lakune bile prazne, bez osteocita ili su njihove jezgre bile piknotične. Taj pojas toplinskog oštećenja proteže se između 30 i 100 µm. Prvi znakovi obnavljanja alveolne kosti, u obliku stvaranja novih koštanih gredica, pojavili su se 14 dana po obasjavanju, i to u području gdje nije bilo doticaja s karbonificiranim tkivom. I nakon 28 dana nekrotični pojas kosti i termičćki oštećen sloj su perzistirali premda su se unutar makrofaga opažale čestice karbonificiranoga tkiva. U termički oštećenom sloju celularna aktivnost bila je minimalna ili je uopće nije bilo.

U ovom istraživanju rabljene su najviše vrijednosti gustoće energije prethodno upotrebljene za pulpotomiju. Prve znakove obnove koštanoga tkiva u smislu stvaranja osteoida, koji se još uvijek nije mineralizirao, pronašli smo tek 45 dana nakon obradbe CO_2 laserom. U ostalim slučajevima nađena je površinska nekroza uz jaku upalnu infiltraciju, prazne koštane lakune bez osteocita, fragmentacija koštanih gredica, te osteoklastična resorpcija bez znakova regeneracije kosti. Granulacijsko tkivo, nastalo kao posljedica upale, urasta u cement i resorbira ga. Manja upalna infiltracija i manje izražena fragmentacija opažene su pri uporabi CO_2 lasera u usporedbi s uporabom Nd:YAG lasera čije zrake, očito je, prodiru dublje u koštano tkivo i izazivaju opsežnije promjene.

Apsorpcija svjetla Nd:YAG lasera ovisi o boji tkiva. Kada tkivo dosegne temperaturu na kojoj nastaje karbonifikacija, tamna boja toga tkiva dodatno apsorbira veću količinu energije Nd:YAG lasera. Uporabom pulsnoga lasera, teoretski, u razdoblju između impulsa, tkivo se površinski hladi. Međutim, Nd:YAG laser, s nepravilnom i nepredvidljivom apsorpcijom u bilo kojoj točki pojasa prodiranja, gubi taj učinak površinskoga hlađenja (18).

Spektrofotometrijom odrelene najviše vrijednosti apsorpcije svjetla nedekalcinirane isušene kosti kreću se između vrijednosti valnih dužina 2,9 do 3,3 µm. U tome području spektra emitira Erb:YAG laser, što znači da se njegova energija apsorbira u vrlo malom obujmu koštanoga tkiva. Voda je jak apsor-

ber CO_2 laserskih zraka (10,6 μm), a ni jedan osnovni sastavni dio kosti ne apsorbira u većoj mjeri zrake Nd:YAG lasera. Zbog toga će, dok god zraka Nd:YAG lasera ne udari u kromofornu tvar u kosti, ona prodirati dublje u tkivo (15). To objašnjava oštećenje dubljih slojeva koštanoga tkiva te više karbonifikacija na površini i u dubini rane koje smo opazili u našem istraživanju pri uporabi Nd:YAG lasera.

Friedman i sur. (19) ozračivali su alveolarnu kost resecirajući vršak korijena fokusiranom CO_2 laserskom zrakom (15 W). Nakon 6 mjeseci opazili su u periapikalnom području upalnu infiltraciju, pretežito mononuklearima. Rubno od infiltrata nalazile su se novostvorene koštane gredice s osteoblastičnim šupljinama, a u upalnom infiltratu nalaze se čestice karbonificiranoga tkiva, često u dodiru s multinuklearnim orijaškim stanicama tipa stranoga tijela. Citoplazma ponekih orijaških stanica sadržavala je čestice nalik na razgrađeno karbonificirano tkivo. Ponegdje su koštane karbonifikacije bile prekrivene lamelarnom kosti s osteoblastima na površini. U eozinofilnom sloju kosti bile su prazne koštane lakune.

Karbonificirane čestice Friedman (19) je opazio unutar tkiva čak 6 mjeseci nakon zahvata, a u našem su istraživanju one postojale 45 dana nakon obradbe. Njihova nazočnost pokazuje da su, premda izazivaju imuni odgovor, otporne na biološke postupke čišćenja.

O sporijem cijeljenju koštane rane, uzrokovane CO_2 laserom, pisali su Clayman (20) i Small sa suradnicima (21). Razlika u brzini cijeljena skalpela i lasera nestaje ukloni li se sloj karbinificiranoga tkiva s rane. Suprotno tome, u Williamsovoj studiji (17) cijeljenje laserski obrađivanih područja kosti ne razlikuje se od onih obrađivanih kiretom, iako karbonifikacije nisu uklonjene.

McKee (22) je svjetlosnim i elektronskim mikroskopom proučavao kratkoročne i dugoročne učinke osteotomije mandibule štakora CO_2 laserom koja je rađena u svrhu pristupa neizniklome zubnome tkivu. Znakovi obnavljanja kosti, i to najviše novostvorene kosti na rubu lezije uz periost, javljaju se 10 dana od izvršene laserske osteotomije. Činjenica da se je McKee služio štakorima za pokusne životinje a ne psima, kao mi u našem istraživanju može donekle objasniti povoljnije nalaze o vremenu potrebnom za oporavak koštane rane, premda su vrijednosti gustoće energije u njegovu pokusu više nego u našem istraživanju.

Nedostatak stvaranja nove kosti neposredno uz karbonificiranu površinu McKee (22) objašnjava potrebom prethodnog uklanjanja ili preobrazbe takva tkiva bilo samostalnom aktivnošću fagocitnih stanica ili u suradnji s izvanstaničnim čimbenicama mikrookoliša. Nazočnost mnogobrojnih multinuklearnih orijaških stanica s fagosomima unutar kojih postoje čestice karbonificiranog tkiva, te naboranost stanične membrane u dodiru s karbonificiranom površinom, govori u prilog staničnoj resorpciji laserom ozračene kosti, a enzimtski je mehanizam izvanstanične tekućine u drugome planu. Stvaranje nove kosti u dodiru s avitalnim dijelom stare kosti, u čijim lakunama nema osteocita, kako se čini, ne ovisi o nazočnosti zdravih osteocita nego o stanju koštanoga matriksa. Novostvorenu kost od stare kosti dijeli, vremenski i prostorno, cementna linija čiji je organski ustroj nakupina staničnog adheziva, koštanoga fosfoproteina, tj. osteopontina. Pretpostavlja se da taj spoj nastaje staničnom aktivnošću i da predstavlja jedan način obradbe površine kosti koja prethodi osteogenezi na mjestu ozljede.

Zaključak

Vrijeme potrebno da se kost počne obnavljati pri uporabi CO_2 lasera iznosi 45 dana, a obradba Nd:YAG laserom uzokuje opsežnije promjene i biološkim mehanizmima čišćenja treba vjerojatno više vremena nego što je trajao ovaj eksperiment. S obzirom na nepovoljan učinak lasera na parodontno tkivo, potrebno je tijekom pulpotomije lasersku zraku usmjeriti u korijenski kanal i izbjegavati slučajno obasjavanje parodontnoga tkiva.

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The Effects of CO_2 and Nd:YAG Lasers on Periodontal Tissue in the Furcations of Molars

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Summary

CO_2 and Nd:YAG lasers are the most commonly used in medicine and dentistry. Due to atraumatic and efficient cutting of the soft tissue they can be used for pulpotomy of deciduous and young permanent teeth. The aim of this study was to evaluate the effect of these two lasers with energy parameters used for abblation of soft tissues on the incidently irradiated periodontal tissue during pulpotomy.

Mixed breed dogs weighing 25 kg were used for experiments. The animals were anesthetized with pentobarbital. Pulp chambers of molars were opened and pulp tissues removed with a sharp spoon. The floor of the pulp chamber was penetrated intentionally with a water cooling low speed steel bur. Periodontal tissue of the furcation region was irradiated with CO_2 (2 W / 10 ms / 5x/s) i Nd:YAG (2W / 20 pps) lasers for 3 s. Histological analysis made 30 and 45 days after treatment revealed superficial necrosis of bone, inflammatory infiltration and fragmentation of bone trabeculae. The first signs of bone healing appeared 45 days after treatment with the CO_2 laser as formation of new bone matrix, osteoid. Nd:YAG laser penetrates deeper into bone tissue and causes more severe changes. Thus 45 days after lasing the bone with it there were no signs of healing.

Key words: *periodontal tissue, CO_2 laser, Nd:YAG laser, histological analysis*

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The first studies on the laser influence on hard dental tissue were carried out by Goldman et al. (1) and Stern et al. (2) soon after Mainman's discovery of the laser. They were followed by the clinical and laboratory investigations of many authors. Today more than 600 laser active substances exist that determine the wave length of the laser beam. CO_2 and Nd:YAG lasers are the most commonly used lasers in dentistry (3,4).

Application of lasers in dentistry can be roughly classified as procedures on hard and soft tissues (5). Although the laser can also be used for diagnostics

(1), analgesia (6), biostimulation (7) and resin composite polymerization (8).

Useful application of the laser in periodontology, which is superior to other techniques, is gingivoplastic and removal of hyperplastic gingival tissue (9). The laser can also be used for calculus removal (10), soft tissue curettage (11) and root planing (12).

Because of atraumatic and efficient cutting of soft tissue the laser can be used for pulpotomy of deciduous and young permanent teeth (13,14). During drilling in the pulp chamber the floor can be accidentally penetrated and periodontal tissue in furcation can be subsequently unintentionally lased. In this study our intention was to examine the effect of CO_2 and Nd:YAG laser beams on the periodontal tissue of molar furcation in dogs and to detect signs of healing by histological analysis. The energetic parameters of laser beams were adequate for soft tissue ablation and the lasing was performed in contact mode.

Materials and methods

Two mixed breed dogs each weighing 25 kg, were used in this experiment. They were anesthetized with 30 mg sodium pentobarbitone per kg/wt and if necessary the anesthetic was added intraperitoneal. During the procedure the head of each animal was covered with sterile sheet. A rubber dam was placed on the tooth to be treated. The jaws of the animals were held open with a self-locking canine gag placed on the opposite side to that being operated. The pulp chambers of the upper molars were penetrated using a high speed diamond drill with sterile distilled water coolant. Pulp tissue was removed from the pulp chamber with a sharp excavator. In order to expose the periodontal tissue of the furcation region the floors of the pulp chamber were penetrated with sterile steel bur. The wounds were rinsed with 0.9% solution of natrium chloride. The furcation regions of the right side of the upper jaw were lased with CO_2 LX-20D (Luxar Corp., Bothel, WA, USA) laser (2W / 5x10 ms/s) for 3s. Furcations of the left side of the upper jaw were irradiated with Nd:YAG dLase-300 (American Dental Laser, Fremont, CA, USA) laser (2W/20 pps) for 3 s.

The CO_2 laser beam was delivered through a flexible hollow fibre which ends with a fine metal tube, 0.8 mm in diameter. Nd:YAG laser beam was delivered to the tissue through flexible fibre 300 μm in diameter.

After lasing, the teeth treated in the experiment were filled with polycarboxylat cement "My-Bond Carbo" (Shofu, Inc., Kyoto Japan). The cement was mixed on a sterile glass plate.

In both lasers, the procedure was performed in contact and static mode. In the case of the CO_2 laser, a sterile metal tube was used for each tooth. After lasing of each tooth with Nd:YAG laser, the fibre tip was cut with a sterile scalpel. Between the lasing procedures the tip of the remaining fibre was kept in 8% NaOCl solution.

Fifteen days after the first treatment, the animals were anesthetized again and the procedure repeated on the molars of the lower jaws.

The animals were killed 45 days after the last treatment by an intravenous overdose of pentobarbitone sodium. The soft tissues over the jaws were removed and the jaws resected. Before submerging the jaws in 10% formalin solution, the cement from the cavities was removed to allow the fixative better penetration into the remaining tissue. Fixation lasted for 7 days. After which demineralization followed, with daily changes of demineralizing solutions (nitric acid) of different concentrations. After demineralization, the tissue blocks, consisting of individual teeth and surrounding periodontal tissue, were separated from the jaws using a microtome blade. When embedding into Celloidin (Cekudol + 30% ethanol, Merck, Darrmstad; Germany), the teeth were positioned to allow subsequent sectioning parallel to the longitudinal axis of the teeth. After hardening, serial sections at 3 μm were obtained and six sections from the central area of the furcation. The sections were stained with hematoxylin and eosin, and histologically evaluated under the microscope (AMPLIVAL, Carl Zeiss Jena, Germany) by a pathologist.

Results

In the superficial zone of the bur the penetration area of the teeth lased with CO_2 laser carbonized, respectively, necrotic tissue was seen. Marleed inflammation and lymphocytes were present. In bone tissue gradients of changes from destruction of corticalis to fragmentation of lamellae could be seen

(Figure 1). Rarefaction of bone was present. Trabeculae were tiny and irregular and under higher magnification multinuclerar giant cells and osteoclasts could be seen on their borders. Bone resorption was present (Figure 2). Bone lacunae below the zone of necrosis were empty, without osteocytes. Blood vessels were dilated and extravasation of erythrocytes was noted.

The periodontal ligament was widened, and cellularity emphasized. Cementum was rough and scalloped, and resorption of cementum with penetrating granulation tissue was observed.

On the histological sections of the upper jaws, which were lased 15 days prior to the lower jaws, in deeper parts of the bone, on the area of rebuilding bone granulation tissue could be seen (newborn capillaries, fibroblasts, connective fibers and lymphocytes). It was interwoven with newborn bone matrix i.e. osteoid, which was not jet mineralized.

Changes in interradicular tissue caused by the Nd:YAG laser differed from these of the CO_2 laser with regard to the severity and deep tissue reaction. Necrosis on the surface of the periodontal wound was more prominent and so was inflammation and resorption of bone trabeculae. Necrotic, carbonized particles could be found in deeper bone layers (Figure 4). Rarefaction of bone was marked: bone trabeculae were tiny and mononuclear inflammation cells replaced the spongiosis (Figure 5). Severe fragmentation of the bone reached deeper portions and multinuclear giant cells surrounded the bone fragments.

Discussion

When using the laser to remove pulp tissue from the pulp chamber, the possibility of iatrogenic irradiation of periodontal tissue should be kept in mind in order to maintain the vital remainder of radicular pulp tissue. Periodontal tissue consists of soft tissue: connective and epithelial tissue, and hard mineralized tissue such as bone and cement. A characteristic of chronic inflammation of periodontal tissue is proliferation of granulation tissue which consists of proliferating capillaries, immature collagen fibers and various degrees of cell infiltration. The removal of granulation tissue or reshaping of soft tissue by laser also leads to irradiation of hard tissue with a laser beam of energy parameters for soft tissue ablation.

Studies on the effects of the laser beam on mineralized and periodontal tissue were mostly performed with energy parameters higher than those sufficient for soft tissue ablation (15,16). Williams et al. (17) used CO_2 laser with energy parameters (41,28 J/cm^2) sufficient for ablation of granulation tissue from the periodontal pocket when performing experiments on dogs. Immediately after lasing, at the place where the beam strikes 40 - 120 μm wide bone necrosis could be seen on histological sections. Below the necrotic zone there was a zone of devitalised bone, where the osseous lacunae were empty, with or without osteocytes, althgough their nuclei were picnotic. This zone of thermic injury extended from 30 to 100 μm. The first signs of bone repair as formation of new bone lacunae appeared 14 days after lasing, but only in the area where tissue was not carbonized. After 28 days the necrotic zone of the bone and thermally injured zone persisted although particles of carbonized tissue could be seen inside the macrophages. In the thermally injured zone cellular activity was minimal or there was no activity at all.

In this experiment -lasing of periodontal tissue- the highest energy density was used, as previously used for pulpotomy. The first signs of bone tissue repair, i.e. formation of non mineralized osteoid, was found 45 days after treatment with CO_2 laser. Superficial necrosis with marked inflammatory infiltration was found in other conditions of lasing. Bone lacunae were devoid of osteocytes. Fragments of bone trabeculae and osteoclastic resorption with no sign of bone reparation were also seen in these conditions. Granulation tissue, formed due to of inflammation, developed into cementum, causing its resorption. Less prominent inflammatory infiltration and fragmentation were noticed in spaces lased with CO_2 laser in comparision with these irradiated with Nd:YAG laser. Obviously Nd:YAG irradiation penetrated deeper into bone tissue and caused more severe changes.

Absorption of Nd:YAG laser beam depends on the colour of the tissue. When the lased tissue reaches the temperature at which carbonification begins, the dark colour of that tissue contributes to the higher rate of aborption of the Nd:YAG laser light. Theoretically using a pulse laser beam, the lased ti-

ssue cools between the pulses. However, unpredictable and irregular absorbtion of the Nd:YAG laser beam at any point of the zone of penetration make that superficial cooling effect minimal (18).

The broad absorbtion bands of the nondecalcified, dehydrated bone, determined by spectrophotometric method, was in the range 2.7 to 3.3 μm wave length. In that area of the spectrum Erb:YAG laser emits light, signifying that its energy has been absorbed by a very small amount of bone tissue. Water is a strong absorber of the CO_2 laser energy (10,6), while any major constituent of bone tissue does not absorb the Nd:YAG laser irradiation. This explains the penetration of the Nd:YAG laser beams deep into the tissue until they strike the chromophore substance (15). This it is understandable why the Nd:YAG laser caused injury to the deeper layer and more prominent carbonifications on the surface and in the inner zone of the tissue which can be seen in our study.

Friedman et al. (19) irradiated the alveolar bone while resecting the tip of the root by focused CO_2 laser beam (15W). After 6 months they noticed inflammatory infiltration, with predominant mononuclear cells. On the border of the infiltration newborn bone trabeculae with osteoblastic cavities could be seen. Particles of carbonized tissue, often in contact with multinuclear giant cells of foreign body type, were present in the inflammatory infiltration. Inside the cytoplasm of the same giant cells were some particles resembling nondigested carbonizied tissue. In places the bone carbonifications were covered by with lamellar bone with osteoblasts on the surface. Empty bone lacunae could be seen in the eosinophylinic layer.

Friedman (19) noticed carbonized particles in the tissue even 6 months after treatment. In our experiment such particles were present 45 days after laser procedure. Their presence confirms resistance to the biological cleaning mechanism, although they a provoke strong imunological response.

Clayman (20) and Small et al. (21) reported slower healing of the bone wound treated with CO_2 laser. If the carbonized layer was removed from the wound, the difference in healing between the laser and scalpel wound was not noticeable. In contrast, in his study Williams (17) did not differentiate the healing of the laser bone wounds from the bone wounds obtained by the curette, although the carbonifications were not removed.

McKee (22) used a light and electronic microscope to examine the effects of CO_2 laser osteotomy of a rat mandible done performed for access to a nonerupted tooth. The first signs of bone regeneration appeared 10 days after treatment, mostly as newborn bone on the border of the lesion near periosteum. The fact that Friedman used rats as experimental animals and not dogs as in our experiment, could explain the faster healing of the bone wounds, although he performed lasing with higher energy density than us.

The absence of formation of new bone near the carbonized surface was explained by McKee (22). For new bone formation removal or transformation of that tissue by independent activity of phagocyte cells or by their cooperation with extracellular elements of microenviroment is necessary. The presence of many multinuclear giant cells with phagosomes, containing carbonized particles and their ruffled membrane in contact with carbonized tissue corroborates the opinion that the cellular mechanism was the main agent in resorption of lased bone. Enzymes from the extracellular matrix are considered to have had a minor role in that process. The formation of the new bone near the old nonvital bone with lacunae devoid of ostecytes, appeared to be dependent and on the condition of the bone matrix.not on the presence of healthy osteocytes. Newborn bone was separated in space and time from the old bone by cement lines. This organic structure contained the cell-adhesive bone matrix phosphoprotein - osteopontin which is believed to arise from cellular activity at these sites and could represent one form of bone surface modification prior to osteogenesis at these sites of the bone lesions.

Conclusion

The repair of the bone lased with CO_2 laser started after 45 days. Treatment with Nd:YAG laser caused more extensive changes and biological mechanisms needed more time than the duration of this experiment to clean the lased area. Because of the negative influence of the laser on periodontal tissue, it is recommended that the laser beam should be targeted straight into the root canal during pulpotomy, to avoid the incidental irradiation of the periodontal tissue.