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Antimikrobni učinak različitih medikamentoznih uložaka tijekom endodontskog liječenja

Antimicrobial Effect of Different Intracanal Dressings in Endodontic Treatment

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Sažetak

Na četiri hranilišta kontaktnim testom *in vitro* ispitan je utjecaj pet različitih intrakanalnih medikamentoznih uložaka: kalcij-hidroksida, klorheksidin glukonata u obliku gela 1% i otopine 0,2%, paraklorfenolkamfora i 1% metronidazola. Mikroorganizmi kojima se arteficialno inficirao korijenski kanal 40 ispitanih ekstrahiranih zuba bile su: *Pseudomonas aeruginosa*, *Staphylococcus aureus* i *Candida albicans*. Rezultati su pokazali znatne razlike među utjecajima pet ispitanih intrakanalnih uložaka na brojnost mikroorganizama *P. aeruginosa* nakon tri i sedam dana ($p < 0,05$) te *C. albicans* nakon tri dana ($p < 0,05$). Preparati na temelju kalcij-hidroksida i paraklorfenolkamfora imaju veliku antimikrobnu učinkovitost. Paraklorfenolkamfor je znatno reducirao broj svih ispitanih mikroorganizama *P. aeruginosa* i *C. albicans*, nakon tri i nakon sedam dana u usporedbi s ostalim materijalima ($p < 0,05$). Kalcijev hidroksid je pokazao isti učinak na *P. aeruginosa* kao i paraklorfenolkamfor, s obzirom na to da je djelovao nakon tri dana ($p < 0,05$). Klorheksidin gel je imao najslabije antibakterijsko djelovanje na *P. aeruginosa* nakon tri dana u odnosu prema svim ispitanim materijalima, ali njegovo antibakterijsko djelovanje nakon sedam dana bilo je statistički znatno veće u usporedbi s ostalim materijalima ($p < 0,05$), osim paraklorfenolkamfora. Svi testirani materijali imali su dobar antimikotski učinak na *C. albicans*, osim metronidazola koji - kao što se i očekivalo - uopće nije djelovao na gljivu ($p < 0,05$). Može se zaključiti da klorheksidin gel povećava antibakterijsko djelovanje s duljinom primjene, a paraklorfenolkamfor i kalcij-hidroksid pokazuju najbrže i najjače antimikrobno djelovanje.

Zaprimljen: 10. listopada 2006.

Prihvaćen: 25. siječnja 2006.

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Ključne riječi

Terapija korijenskog kanala,
Pseudomonas aeruginosa,
Staphylococcus aureus, *Candida albicans*, anti-mikrobni čimbenici.

Uvod

Svrha čišćenja, širenja, dezinfekcije i punjenja korijenskog kanala jest ukloniti mikroorganizme iz endodontskog prostora i spriječiti ponovnu infekciju. Zbog složene anatomije endodontskog prostora, apikalne delte, lateralnih i akcesornih kanala nemoguće je potpuno ukloniti mikroorganizme. Sjögren

Introduction

The aim of the instrumentation and filling of the endodontic space is the elimination of the microorganisms from that space and prevention of re-infection. Due to the complex anatomy of the apical delta, lateral and accessory canals it is impossible to completely eliminate the microorganisms. Sjögren et al.

i suradnici (1) istaknuli su poteškoće u uklanjanju mikroorganizama iz korijenskog kanala. Glavni korijenski kanal čini najveći dio endodontskog prostora i u njegovu je području lokalizirano najviše mikroorganizama te se tijekom čišćenja uklanja njihov najveći broj. No, da bi se potpuno uklonili, potrebno je uporabiti dezinficijense. Radovi u kojima nisu rabljena kemijska sredstva pokazali su da dolazi do znatne redukcije bakterija, ali potpuna eliminacija nije moguća. Ingle i Zeldow (2) su nakon instrumentacije s destiliranom vodom utvrdili 80% pozitivnih kultura inicijalne flore i porast broja na 95,4% nakon 48 sati. Kemijska sredstva koja se danas najčešće koriste za dezinfekciju endodontskog prostora su vodena otopina natrijeva hipoklorita (NaOCl) i klorheksidin. Za NaOCl je dokazano fungicidno, baktericidno i virucidno djelovanje, a učinkovito otapa i organsko tkivo unutar korijenskog kanala.

Orstavik i Haapasalo (3) su utvrdili da nakon instrumentacije i dezinfekcije korijenskog kanala mikroorganizmi još ostaju u dentinskim tubulusima, gdje se mogu razmnožavati. Istraživanja su pokazala da bakterije mogu prodirjeti 10 do 300 μm u dentinske kanaliće. U slučajevima takve infekcije korijenski kanal bi trebalo proširiti prosječno 0,4 do 0,6 mm od njegova početnog promjera. No, to je teško učiniti, posebice u apikalnoj trećini. Najčešće rabljeno sredstvo kao medikamentozni uložak tijekom endodontskog zahvata jest kalcijev hidroksid. Zbog alkaličnosti (pH 12,5) ima antimikrobni učinak, a hidroksilni ioni prodiru u dentinske tubuluse (4-7).

Svrha rada bila je: ustanoviti antimikrobni učinak medikamentoznih uložaka klorheksidin glukonat otopine i gela, paraklorfenola, metronidazol kreme te kalcijeva hidroksida na mikroorganizme *Pseudomonas aeruginosa*, *Staphylococcus aureus* i *Candida albicans* u uvjetima *in vitro*.

Materijal i postupci

Istraživanje je provedeno na uzorku od 46 ekstrahiranih maksilarnih inciziva. Zubi su dobiveni od Zavoda za oralnu kirurgiju Stomatološkog fakulteta Sveučilišta u Zagrebu, a ekstrahirani su zbog parodontoloških razloga. Nakon mehaničkog čišćenja sterilizirani su u autoklavu na temperaturi od 134°C i tlaku od 120 kPa. Krune zuba su uklonjene na caklinsko-cementnom spojištu, a korijenski kanali su na apeksu obrađeni do #40. Zatim je cijeli kanal obrađen do #80 standardnom tehnikom "step-back" uz završno proširivanje kanala Pесо proširivačem br. 4 na mikromotoru. Tijekom instrumen-

(1) have pointed out the difficulties in removing microorganisms from the root canal. The main root canal constitutes the main part of the endodontic space and it is the location of most of the microorganisms that are eliminated during instrumentation. However, for the complete elimination one must use disinfectants. Publications that did not use chemical agents have shown that the substantial reduction can be confirmed, but complete elimination is not feasible. Ingle and Zeldow (2) have confirmed 80% of initial flora positive cultures together with an increase to 95.4% after 48 hours, when using distilled water as an irrigation solution. Chemical agents that are nowadays most frequently used for disinfection of the endodontic space are sodium hypochlorite solution (NaOCl) and chlorhexidine. NaOCl has fungicidal, bactericidal and virucidal effect, and can be used as tissue diluting agent inside root canal as well.

Orstavik and Haapasalo (3) have reported that it is possible to find microorganisms inside dentinal tubules that are capable of multiplying even after instrumentation and disinfection of the root canal. Research has shown that bacteria are able to penetrate 10 to 300 μm into the dentinal tubules. In such cases the root canal should be on average widened 0.4-0.6 mm more than the initial root canal diameter. However, this is difficult to perform, especially in the apical third. Most frequently used agent as a dressing during endodontic treatment is calcium hydroxide. Due to its alkalinity (pH 12.5) it possesses antimicrobial effect, and hydroxide ions penetrate in the dentinal tubules (4-7).

The aim of this study was to establish the *in vitro* antimicrobial effect of intracanal dressings containing chlorhexidine gluconate solution and gel, camphorated parachlorophenole, metronidazole cream and calcium hydroxide against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans*.

Material and Methods

The study was performed on a sample of 46 extracted maxillary incisors, obtained from the Department of Oral Surgery, School of Dental Medicine, University of Zagreb, Croatia. All teeth were extracted due to periodontal reasons. Following mechanical cleaning, the teeth were autoclaved at 134°C and 120 kPa. The crowns were removed at cemento-enamel junction, and the root canals were instrumented up to #40 at the apex. The whole of the canal was instrumented up to #80 by means of the standard Step-back technique with final widening by means of Pесо widener Nr. 4 on a hand-

tacije kanala rabljena je 2,5% vodena otopina natrij hipoklorita u količini 10 mL po kanalu. Korijenski kanali su inficirani s dva standardna bakterijska soja: *Pseudomonas aeruginosa* ATCC 27853 i *Staphylococcus aureus* ATCC 25923 te jednim klinički izoliranim nepoznatim sojem *Candida albicans* HK 2324/03 (izoliran iz krvne kulture na Odjelu za kliničku mikrobiologiju Klinike za infektivne bolesti "Dr. Fran Mihaljević" u Zagrebu). Sojevi su inokulirani u 5 mL Brain Heart Infusion (BHI) bujona (Oxoid, UK) i inkubirani na 37°C tijekom 24 sata. Kulture su sadržavale 3×10^8 cfu/ml *P. aeruginosa*, 9×10^8 cfu/ml *S. aureus* i 9×10^7 cfu/ml *C. albicans*. Uzorci su inficirani s 10 µl suspenzije, zatvoreni materijalom za privremene ispune (Cavit, Espe, Seefeld - Njemačka) i inkubirani u sterilnoj fiziološkoj otopini na 37°C tijekom tri i sedam dana. Nakon toga su slučajnim odabirom podijeljeni u pet skupina po osam zuba, uz četiri zuba kontrolne skupine i tretirani ispitanim materijalima koji su uneseni u korijenski kanal i privremeno zatvoreni Cavitom:

- 1. skupina: klorheksidin glukonat gel (Corsodyl gel 1%, Glaxo Smith Kline - Velika Britanija)
- 2. skupina: paraklorfenol (Cresophene, Septodont - Francuska)
- 3. skupina: metronidazol krema (Rozamet krema 1%, Jadran-Galenski laboratorij Rijeka - Hrvatska)
- 4. skupina: klorheksidin glukonat otopina (Corsodyl mint 0,2%, Glaxo Smith Kline - Velika Britanija)
- 5. skupina: kalcijev hidroksid u vodenoj suspenziji (Calasept, Speiko - Njemačka).

Korijenski kanali zuba iz kontrolne skupine tretirani su samo fiziološkom otopinom.

Korijenski kanal svakog uzorka ispran je standardnim volumenom od 2 mL BHI bujona. Nakon ispiranja, 0,1 mL sadržaja svakog kanala inokulirano je na agar pločicu (5% krvni agar) (Columbia agar base, Oxoid - UK), MacConkey agar (Oxoid - UK), salt manitol agar (Oxoid - UK) i Sabouraud agar (Oxoid - UK) u serijskim razrjeđenjima od 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} i 10^{-5} . Antibakterijsko djelovanje ispitanih materijala ustanovljeno je brojenjem mikroorganizama na hranilištima odmah nakon infekcije te nakon tri i sedam dana od infekcije korijenskog kanala. Sva mikrobiološka testiranja obavljena su na Odjelu za kliničku mikrobiologiju Klinike za infektivne bolesti "Dr. Fran Mihaljević" u Zagrebu.

Statistička analiza

Dobiveni rezultati uneseni su u bazu podataka i statistički obrađeni pomoću statističkog paketa

piece. During the instrumentation 2.5% solution of NaOCl was used in the amount of 10 ml per canal. The root canals were infected with two standard bacterial species: *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 25923, as well as with one clinically isolated species *Candida albicans* HK 2324/03 (isolated from a blood culture at the Department of Clinical Microbiology, Clinic for Infectious Diseases "dr. Fran Mihaljević", Zagreb, Croatia). The species were inoculated in 5 ml of Brain Heart Infusion (BHI) bouillon (Oxoid, UK) and incubated at 37°C during 24 hours. The cultures had 3×10^8 cfu/ml of *P. aeruginosa*, 9×10^8 cfu/ml of *S. aureus*, and 9×10^7 cfu/ml of *C. albicans*. The samples were infected with 10 µl of suspension, closed with temporary sealing material (Cavit, 3MESPE, Seefeld, Germany) and incubated in sterile saline at 37°C during three and seven days. Following these time periods, the samples were randomly divided in five groups of eight, with four controls, and treated with test materials that were inserted in the root canal and were temporarily closed with Cavit:

- 1st group: chlorhexidine gluconate gel (Corsodyl gel 1%, GlaxoSmithKline, UK)
- 2nd group: camphorated parachlorophenole (Cresophene, Septodont, France)
- 3rd group: metronidazole cream (Rozamet crema 1%, Jadran Galenski laboratorij Rijeka, Croatia)
- 4th group: chlorhexidine gluconate solution (Corsodyl mint 0.2%, GlaxoSmithKline, UK)
- 5th group: calcium hydroxide in a water suspension (Calasept, Speiko, Germany).

The root canals in the control group were treated only by saline.

The root canal of every sample was rinsed with the standard volume of 2 ml of BHI bouillon. After rinsing, 0.1 ml of the canal content was inoculated on an agar plate (5% blood agar; Columbia agar base, Oxoid, UK), MacConkey agar (Oxoid, UK), salt manitol agar (Oxoid, UK) and Sabouraud agar (Oxoid, UK) in serial dilutions of 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} i 10^{-5} . Antibacterial effect of test materials was determined by counting the microorganisms in the mediums immediately after infection, and three and seven days after infection of the root canal, respectively. All microbiological testings were performed at the Department of Clinical Microbiology, Clinic for infectious diseases "dr. Fran Mihaljević" in Zagreb.

Statistical analysis

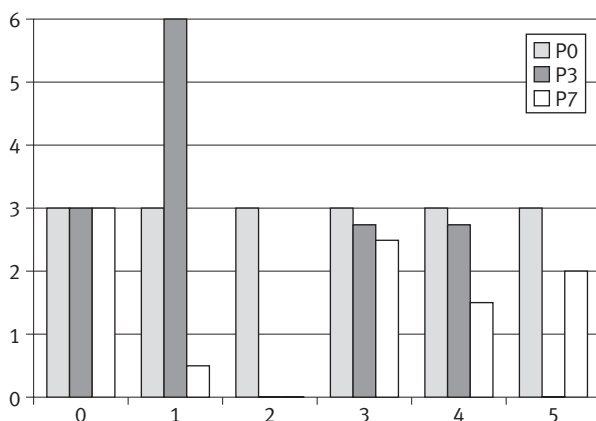
The results were imported in a database and statistically analyzed using a statistical package SPSS

SPSS 12.0 for Windows. Normalnost raspodjele podataka analizirana je testom Kolmogorov-Smirnov. Kako distribucija nije bila standardna, rabio se neparametrijski test Kruskal-Wallis za usporedbu neovisnih uzoraka. Neparametrijski test Mann-Whitney za usporedbu dvaju neovisnih uzoraka koristio se u slučajevima kada je test Kruskal-Wallis pokazao da postoji barem jedna skupina podataka koja se znatno razlikuje od neke druge te se njime preciznije određivalo koja grupa odskače. Svi su testovi rađeni uz razinu značajnosti od 95%.

Rezultati

Bakterija *S. aureus* nije se uspjela održati u laboratorijskom modelu mješovite endodontske infekcije, kako u pozitivnoj kontroli tako i u ispitnim skupinama te se nije dalje mikrobiološki ispitivao učinak medikamentoznih intrakanalnih uložaka na tu bakteriju. Rezultati djelovanja pet intrakanalnih uložaka na brojnost *P. Aeruginosa* i *C. albicans* prikazani su na Slici 1. i 2.

Testovi Kruskal-Wallis i Mann-Whitney pokazali su statistički znatne razlike među utjecajima pet ispitanih intrakanalnih uložaka na brojnost mikroorganizama *P. aeruginosa* nakon tri i sedam dana ($p < 0,05$) te *C. albicans* nakon tri dana ($p < 0,05$). Preparati na temelju kalcijeva hidroksida i paraklorfenolkamfora imaju veliku antimikrobnu učinkovitost. Paraklorfenolkamfor je znatno reducirao broj svih ispitanih mikroorganizama *P. aeruginosa* i *C. albicans* nakon tri i nakon sedam dana, u usporedbi s ostalim materijalima ($p < 0,05$). Kalcijev hidroksid je pokazao isti učinak na *P. aeruginosa* kao i paraklorfenolkamfor, s obzirom na djelovanje nakon tri dana ($p < 0,05$). Klorheksidin gel je imao najsl-



Slika 1. Utjecaj ispitanih materijala na brojnost *Pseudomonas aeruginosa* u nultom testiranju te nakon tri i sedam dana

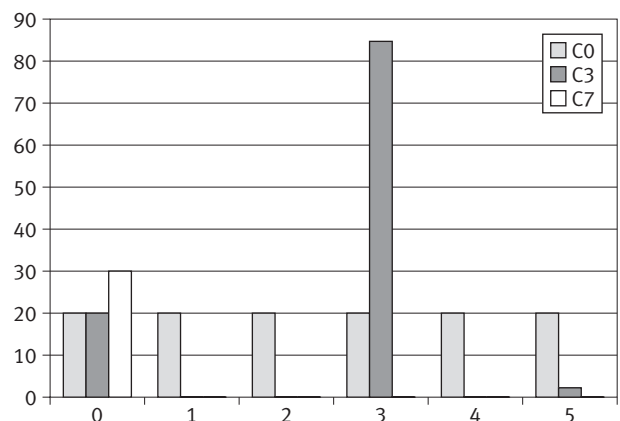
Figure 1. The effect of tested materials on the number of *Pseudomonas aeruginosa* in zero-testing, and after three and seven days.

12.0 for Windows. The normality of distribution was analyzed by means of Kolmogorov-Smirnov test. Since the distribution was not normal, we used non-parametric Kruskal-Wallis test to compare independent samples. Non-parametric Mann-Whitney test for comparing two independent samples was used in situations where Kruskal-Wallis test showed at least one group of data which is significantly different from any other, and it was used for more precise determination of that group. All tests used the significance level at 95%.

Results

S. aureus was unable to survive the laboratory model of mixed endodontic infection in positive controls as well as in test groups, and the effect of intracanal dressings on that microorganism was not tested. The effect of five intracanal dressings on the numbers of *P. aeruginosa* and *C. albicans* are shown in figures 1 and 2.

Kruskal-Wallis and Mann-Whitney tests showed statistically significant differences in the effects of five tested intracanal dressings on number of *P. aeruginosa* after three and seven days ($p < 0.05$), and of *C. albicans* after three days ($p < 0.05$). The samples using calcium hydroxide and camphorated parachlorphenole have significant antibacterial effect. Camphorated parachlorphenole has significantly reduced the number of all microorganisms (*P. aeruginosa* and *C. albicans*) after three and seven days, compared to other tested materials ($p < 0.05$). Calcium hydroxide had similar effect on *P. aeruginosa* as camphorated parachlorphenole after three days ($p < 0.05$). Chlorhexidine gel had the weakest effect on *P. aeruginosa* after three days, but its



Slika 2. Utjecaj ispitanih materijala na brojnost *Candida albicans* u nultom testiranju te nakon tri i sedam dana

Figure 2. The effect of tested materials on the number of *Candida albicans* in zero-testing, and after three and seven days.

bije antibakterijsko djelovanje na *P. aeruginosa* nakon tri dana, no njegovo antibakterijsko djelovanje nakon sedam dana bilo je statistički najveće u usporedbi s preostala tri materijala, osim paraklorfenokamfora ($p < 0,05$). Svi testirani materijali imali su dobro antimikotsko djelovanje na *C. albicans* osim metronidazola – on nije djelovao na gljivu nakon tri dana ($p < 0,05$).

Rasprava

Svrha istraživanja bila je ispitati učinkovitost najčešće rabljenih preparata za dezinfekciju korijenskog kanala (kalcij-hidroksida, klorheksidina, paraklorfenola i metronidazola) na *Pseudomonas aeruginosa*, *Staphylococcus aureus* i *Candida albicans*. *Pseudomonas aeruginosa* je gram-negativna fakultativno anaerobna bakterija. Koristio se u istraživanju, jer su fakultativni anaerobi – posebice *Pseudomonas aeruginosa* – dominantna mikrobnog flora kronično inficiranog endodontskog prostora (8), a na njih slabije djeluju antimikrobni intrakanalni agensi nego na anaerobe. Zato se može očekivati da će te vrste bakterija duže zaostati u korijenskom kanalu nakon neadekvatnog tretmana inficiranoga endodontskog prostora. One mogu perzistirati u korijenskom kanalu duže u latentnoj fazi s niskom metaboličkom aktivnošću, a njihovo ubrzano umnožavanje i rast mogu potaknuti promjene hranidbene okoline, poput curenja biološkog materijala iz usne šupljine kroz neadekvatno zabrtvljenu krunu u endodont i periapiks (9). Upravo *Pseudomonas* je uzrok i sekundarne perzistentne infekcije periradikularnih tkiva (10). *Staphylococcus aureus* ne nalazi se često u endodontski liječenim zubima. Za ovo je istraživanje rabljen zato što se često nalazi u okolini i može biti uzrok sekundarne bakterijske infekcije tijekom endodontskog zahvata (13). No, kako se nije uspio održati na hranilištu, na njemu nisu provedena istraživanja. *Candida albicans* samostalno ne dovodi do infekcije u korijenskom kanalu, ali je ustanovljeno da sudjeluje u stvaranju biofilma na površini korijenskog kanala. *Candida* ima sposobnost adheriranja na epitelne i endotelne stanice. Hapasalo i suradnici (3) utvrdili su da se *Candida* može zadržati u mikropukotinama te da je otporna na djelovanje vodene otopine kalcijeva hidroksida. Sen i suradnici (11) su elektroničkim mikroskopom ustanovili da *C. albicans* može rasti na stijenci korijenskog kanala u različitim oblicima kao blastokonidije, pseudohife i hife te može prodirati u dentinske tubuluse. Kod miješane infekcije *Candida* interferira s ostalim mikroorganizmima, a

antibacterial effect after seven days was statistically greatest compared to other materials, except for camphorated parachlorophenole ($p < 0.05$). All tested materials had good antimycotic effect on *C. albicans* except metronidazole that had no effect on the yeast after three days ($p < 0.05$).

Discussion

The aim of this study was to test the efficacy of most frequently used agents for disinfection of the root canals (calcium hydroxide, chlorhexidine, camphorated parachlorophenole and metronidazole) on *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans*. *Pseudomonas aeruginosa* is a gram-negative, facultative anaerobic microorganism. It has been used due to the fact that facultative anaerobes, especially *Pseudomonas aeruginosa*, dominate the microbial flora of a chronically infected endodontic space (8), and also it is more resistant to intracanal antimicrobial agents than anaerobes. Therefore, one might expect that these bacterial species would remain longer in the root canal after inadequate treatment of an infected endodontic space. These bacteria can persist in the root canal during longer time periods in their latent phase with low metabolic activity, and their multiplication and growth can be triggered by the changes of the nutritive environment, for example by leakage of the biologic material from the oral cavity into the endodontic and periapical space due to the insufficient seal of a crown (9). It is exactly *Pseudomonas* that causes secondary persistent infections of the periradicular tissues (10). *Staphylococcus aureus* cannot be frequently found in endodontically treated teeth. It has been used in this study due to its frequent identification in the environment, and because it can often cause secondary bacterial infection during endodontic treatment (13). Since it was unable to survive the laboratory conditions, it was not tested. *Candida albicans* alone cannot cause infection in the root canal, but it has been established that it is involved in the creation of a biofilm on the surface of the root canal. *Candida* can adhere to epithelial and endothelial cells. Hapasalo et al. (3) have reported that *Candida* can persist in microfractures, and that it is resistant to calcium hydroxide solution. Sen et al. (11) have confirmed by SEM analysis that *C. albicans* can grow on the root canal surfaces in different forms, such as blastoconidia, pseudohyphae and hyphae, and that it can penetrate in dentinal tubules. In mixed infections *Candida* reacts with other microorganisms, and in case of environment

u slučaju promjene uvjeta mijenja fenotip i prilagođava se novonastalim uvjetima.

Rezultati ovoga rada pokazali su statistički veliku antibakterijsku učinkovitost preparata na temelju kalcijeva hidroksida i paraklorfenolkamfora. Paraklorfenolkamfor je, u usporedbi s ostalim materijalima, znatno reducirao broj svih ispitivanih mikroorganizama *Pseudomonas aeruginosa* i *Candida albicans* nakon tri i nakon sedam dana. Paraklorfenolkamfor je iz skupine antiseptika aromatskog reda, pa su se rezultati dobiveni u ovom radu i očekivali. Kalcijev hidroksid je pokazao snažno antibakterijsko djelovanje na *Pseudomonas aeruginosa* kao i paraklorfenolkamfor, s obzirom na učinak nakon tri dana. Slični učinak preparata kalcij-hidroksida dokazali su Estrela i suradnici (12) kod kojih je taj preparat bio djelotvoran na *Pseudomonas* u korijenskom kanalu nakon tri dana, ali ne i nakon sedam dana. Preparati na temelju kalcijeva hidroksida najčešće se preporučuju kao medikamentozni uložak između posjeta tijekom endodontskog liječenja.

Klorheksidin gel je nakon tri dana najslabije antibakterijski djelovao na *Pseudomonas aeruginosa*. No, njegovo antibakterijsko djelovanje na *Pseudomonas* bilo je nakon sedam dana statistički najveće u usporedbi s ostalim materijalima, osim paraklorfenolkamfora. Iz rezultata ovoga rada može se zaključiti da klorheksidin gel povećava antibakterijsko djelovanje s duljinom djelovanja, a paraklorfenolkamfor i kalcijev hidroksid pokazuju najbrže i najjače antibakterijsko djelovanje. Utvrđeno je da klorheksidin ima inhibitorni učinak na bakterije izolirane tijekom endodontske infekcije (13). Barbosa i suradnici (14) proučavali su antibakterijski učinak 0,12% klorheksidin glukonata, paste kalcijeva hidroksida i paramonoklorfenola kao intrakanalne uloške. Rezultati su pokazali da su svi materijali bili učinkoviti i smanjili su broj bakterija, ali u 22 do 31% slučajeva nakon primjene se ustanovio porast bakterija. Autori su utvrdili da je klorheksidin djelotvoran do 72 sata. Rezultati nisu u skladu s ovim radom kojim se pokazalo da se broj bakterija smanjuje nakon sedam dana. Kuruvilla i suradnici (15) u svojem su radu ustanovili veću redukciju mikrobnog flore (84,6%) kada se uz NaOCl rabio klorheksidin glukonat. Razlika je bila statistički znatna u usporedbi s postotkom redukcije mikrobnog flore kada se rabio samo NaOCl (59,4%), a razlika u redukciji nije bila velika kada se koristio samo klorheksidin glukonat (70%). Parson i suradnici (16) zaključili su da klorheksidin izvrsno antibakterijski djeluje kao intrakanalno sredstvo za dezinfekciju.

changes, it changes its phenotype and adapts to the new environment.

The results of this study have shown statistically significant antibacterial efficacy of the dressings based on calcium hydroxide and camphorated parachlorphenole. Camphorated parachlorphenole has significantly reduced the number of all microorganisms after three and seven days, compared to other materials. Camphorated parachlorphenole is a member of a group of aromatic antiseptics, so the obtained results were expected. Calcium hydroxide has shown strong antibacterial effect against *Pseudomonas aeruginosa* as well as camphorated parachlorphenole, after three days. Estrela et al. (12) showed similar effect of calcium hydroxide; it was effective against *Pseudomonas* after three days, but not after seven days. Calcium hydroxide-based materials are most frequently suggested as dressings between sessions during endodontic therapy.

Chlorhexidine gel had the weakest antibacterial efficacy against *Pseudomonas aeruginosa* after three days. However, its effect on *Pseudomonas* was significantly greatest after seven days, compared to other materials, except for camphorated parachlorphenole. The results of this study lead to a conclusion that the antibacterial effects of chlorhexidine gel grows with time, while camphorated parachlorphenole and calcium hydroxide show the fastest and strongest antibacterial effect. It has been established that chlorhexidine possesses an inhibitory effect on bacteria isolated in endodontic infection (13). Barbosa et al. (14) have studied the antibacterial effect of 0.12% chlorhexidine gluconate, calcium hydroxide past and camphorated paramonochlorphenoleas intracanal dressings. Their results have shown that all materials diminish the number of bacteria, but in 22-31% of the cases they could confirm an increase in the number of the bacteria. The authors have concluded that chlorhexidine is efficient during 72 hours, which is not in accordance with our work, where we have shown that the number of bacteria decreases after seven days. Kuruvilla et al. (15) have established a greater reduction in microbial flora (84.6%), when chlorhexidine gluconate was used in combination with sodium hypochlorite. The difference was statistically significant in comparison with the percentage of the reduction of the microbial flora when only NaOCl was used (59.4%), while the difference in reduction was not significant when only chlorhexidine gluconate was used (70%). Parson et al. (16) have concluded that chlorhexidine has excellent antibacterial efficacy as an intracanal disinfection dressing.

Abstract

The effect of five intracanal dressings, calcium hydroxide, chlorhexidine gluconate (1% gel and 0.2% solution), camphorated parachlorophenole and 1% metronidazole was tested *in vitro* in four media. The canals of 40 test teeth were artificially infected with *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans*. Significant differences were observed between five intracanal dressings regarding the number of *P. aeruginosa* after 3 and 7 days ($p < 0.05$) and *C. albicans* after 3 days ($p < 0.05$). Specimens with calcium hydroxide and camphorated parachlorophenole have significant antibacterial efficacy. Camphorated parachlorophenole has significantly reduced the number of all tested microorganisms *P. aeruginosa* and *C. albicans* after 3 and 7 days in comparison with other materials ($p < 0.05$). Calcium hydroxide has shown similar effect on *P. aeruginosa* as camphorated parachlorophenole regarding the efficacy after 3 days ($p < 0.05$). Chlorhexidine gel has shown the weakest efficacy on *P. aeruginosa* after 3 days, compared to all tested materials, but its antibacterial efficacy after 7 days was statistically greater compared to other materials ($p < 0.05$), except for camphorated parachlorophenole. All tested materials had good antimycotic efficacy against *C. albicans* except metronidazole that, as was expected, had no efficacy against yeast ($p < 0.05$). We can conclude that the antibacterial efficacy of chlorhexidine gel is enhanced with time, while camphorated parachlorophenole and calcium hydroxide have fastest and strongest antimicrobial efficacy.

Received: October 10, 2005

Accepted: January 25, 2006

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Key words

Root Canal Therapy, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans*, Anti-Microbial Agents.

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