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IZVORNI ZNANSTVENI RAD
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Krešimir Bašić, Kristina Peroš, Ivana Šutej, Kata Rošin-Grget

Učinak kalcija iz sline i zubne paste s fluorom na stvaranje KOH-topljivih fluorida: studija *in vitro*

The Effect of Salivary Calcium and Fluoride Toothpaste on the Formation of KOH-Soluble Fluoride: In Vitro Study

Katedra za farmakologiju Stomatološkog fakulteta Sveučilišta u Zagrebu, Hrvatska
Department of Pharmacology, School of Dental Medicine, University of Zagreb, Zagreb, Croatia

Sažetak

Svrha: Svrha ovoga preliminarnog istraživanja *in vitro* bila je procijeniti učinak sline pušača (pretpostavlja se da sadržava veću koncentraciju kalcija) u kombinaciji s fluornom pastom za zube na ugradnju alkalno topljivih fluorida na površinu cakline. **Materijali i metode:** Sa svakoga od 14 ekstrahiranih impaktiranih umnjaka odrezana su četiri caklinska bloka koji su nakon toga nasumičnim odabirom svrstani u četiri skupine. Uzorci nestimulirane sline prikupljeni su od dvojice dobrovoljca istoga spola i dobi – jedan je bio pušač, a drugi nepušač. Dva uzorka iz skupine A i B mućkala su se pet minuta u slini (A – u slini pušača, B – u slini nepušača), a zatim tri minute u smjesi paste za zube i deionizirane vode (1 : 3). Uzorci iz skupina C nisu bili tretirani slinom nego su se mućkali samo u smjesi paste za zube i deionizirane vode. Tretman je ponovljen poslije šest sati. Jedna od skupina (D) poslužila je kao kontrolna. **Rezultati:** Koncentracija kalcija u slini pušača bila je veća negoli u slini nepušača. Ugradnja alkalno topljivih fluorida na površinu cakline u skupini A bila je statistički značajno veća nego u ostalim dvjema tretiranim skupinama (B i C). Ugradnja alkalno topljivih fluorida na površinu cakline u sve tri skupine koje su bile tretirane slinom i zubnom pastom bila je statistički značajno veća negoli u kontrolnoj skupini. **Zaključak:** Rezultati pokazuju da slina pušača, koja je imala veću koncentraciju kalcija, povećava ugradnju alkalno topljivih fluorida na površinu cakline, što nas potiče da provedemo opsežniju studiju.

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Adresa za dopisivanje
Krešimir Bašić
Sveučilište u Zagrebu
Stomatološki fakultet
Katedra za farmakologiju
Šalata 11, 10 000 Zagreb, Hrvatska
Tel: +385 1 4590 211
Fax: +385 1 4920 049
basic@sfzg.hr

Ključne riječi
slina; kalcij, fluoridi; poremećaji uzrokovani pušenjem; zubna caklina

Uvod

Karijesni preventivni mehanizam topikalnih fluorida temelji se na formiranju CaF_2 ili *nalik na materijal* CaF_2 na površini tvrdih zubnih tkiva. CaF_2 sudjeluje u procesima demineralizacije i remineralizacije tijekom napada kiseline tako što povećava razinu fluorida tijekom otapanja cakline (1 – 3). Njegova količina ovisi o nekoliko čimbenika, kao što su koncentracija fluorida, vrijeme izloženosti, pH otopine te koncentracija kalcija i fosfata u slini (4 – 9). Dostupnost kalcijevih iona važan je čimbenik za formiranje kalcijeva fluorida (7). Larsen i Richards pokazali su da je slina čak i u malim količinama važna za uspjeh topikalne fluoridacije, vjerojatno zato što sadržava kalcij (6). No malo se zna o učincima pušenja na sastav sline, posebno na njezin anorganski dio. Nedavne studije pokazale su da pušenje duhana znatno utječe na kemijski sastav prirodne sline (10). Svrha ovoga preliminarnog istraživanja bila je ustanoviti razliku u ugradnji alkalno topljivih fluorida na površinu cakline iz sline pušača i nepušača, uz pretpostavku da postoji razlika u koncentraciji kalcija u slini.

Introduction

The caries-preventive mechanism of topical fluoride is based on formation of CaF_2 or "CaF_2-like material" on the surface of hard dental tissues. CaF_2 interferes with de- and remineralization processes during acid attacks by elevating fluoride levels through slow dissolution (1–3). The amount of CaF_2 formed is known to depend on several factors, such as fluoride concentration, the time of exposure, the pH of the solution, phosphate and salivary calcium concentration (4–9). The availability of calcium ions is an important factor for calcium fluoride formation (7). Larsen and Richards showed that presence of saliva, even in small amounts, is important for the success of topical fluoride treatment, presumably because of its calcium content. (6) However, little is known about the effects of smoking on the composition of saliva and, in particular, on its inorganic constituents. Recent studies showed that tobacco smoking has an important impact on the chemical composition of natural saliva (10). The aim of this preliminary study was to assess the difference in fluoride uptake by enamel from a smoker's saliva and a non-smoker's saliva, assuming the difference in salivary calcium concentration.

Materijali i metode

Uzorci cakline

U istraživanju su se koristili kirurški izvađeni umnjaci (Zavod za oralnu kirurgiju Stomatološkog fakulteta Sveučilišta u Zagrebu, Hrvatska) pohranjeni u vlažnom okolišu. Sadržaj fluorida u vodi za piće manji je od 0,1 ppm F/L. Zubi su očišćeni četkicom i upotrebom kamena plovučca bez fluorida. S njih 14 dijamantnim su diskom izrezana četiri caklinska bloka (2 bukalno i 2 lingvalno). Nakon pregleda pod povećalom (10 x) odabrani su samo blokovi bez ikakvih vidljivih nedostataka. Blokovi sa svakog zuba podijeljeni su nasumično u četiri skupine, tako da se svaka sastojala od 14 blokova (ukupno 56 blokova).

Uzorci iz dviju skupina uronjeni su u svježu nestimuliranu slinu pušača ili nepušača prije tretmana smjesom paste za zube i vode. Jedna skupina tretirana je samo smjesom paste za zube i vode, a druga je služila kao kontrolna (tablica 1.). Prije tretmana blokovi su isprani destiliranom vodom i osušeni na sobnoj temperaturi. Sve površine, osim površine cakline, prekrivene su stomatološkim voskom na koji je bila pričvršćena ortodontska ligaturna žica radi lakšeg rukovanja. Caklinske površine mjerene su kaliperom, prenesene na milimetarski papir i izražene su u mm^2. Blokovi su bili pohranjeni u vlažnom okolišu.

Materials and methods

Enamel Slabs

Impacted human third molars were provided by oral surgeons (Department of Oral Surgery, School of Dental Medicine, University of Zagreb, Croatia) and stored in a humid environment until use. The fluoride content in drinking water was under 0.1 ppm F/L. The teeth were brushed with non-fluoride pumice powder and four enamel slabs were cut (2 from buccal and 2 from lingual surfaces) from 14 teeth using a dental diamond disk. Only the slabs without any visible defects were selected after examination under a magnifying glass (10x magnification). The slabs from each tooth were randomly assigned to 4 groups, so that each group consisted of 14 slabs (total of 56 slabs). Two groups were immersed in the fresh unstimulated whole saliva (smoker or non–smoker) before treatment with the toothpaste slurry. One group of slabs was treated only with the toothpaste slurry and one group without treatment served as the control (Table 1). Before treatment, the slabs were rinsed with distilled water and dried at room temperature. All surfaces except the enamel surface were covered with dental wax and orthodontic ligature wire was attached to each slab for easier handling. The enamel surfaces were measured using a caliper, transferred to millimeter paper, and expressed in square millimeters. The slabs were stored in a humid environment.

Tablica 1. Režim tretmana za caklinske blokove
Table 1 Treatment regimen for groups of slabs

	Slina[1] • Saliva[1]	Smjesa zubne paste[2] • Toothpaste slurry[2]
Grupa A • Group A	Pušač • Smoker	+
Grupa B • Group B	Nepušač • Non-smoker	+
Grupa C • Group C	Bez tretmana • No treatment	+
Grupa D • Group D	Bez tretmana • No treatment	-

[1] Blokovi su pet minuta uronjeni u uzorak sline pušača ili nepušača i nakon toga osušeni • Slabs were immersed in one of the saliva samples (Smoker, Non-smoker) for 5 minutes and gently dried.
[2] +: Blokovi su tri minute uronjeni u smjesu zubne paste i vode tijekom, nakon toga su 30 sekundi ispirani destiliranom vodom, pa su osušeni; blokovi nisu tretirani smjesom zubne paste • Slabs were immersed in toothpaste slurry for 3 minutes with gentle agitation, rinsed with distilled water for 30 seconds and gently dried; -: slabs were not immersed in toothpaste slurry

Prikupljanje uzoraka sline

Donatori sline za istraživanje bili su pacijenti iz privatne ordinacije koji su dobrovoljno pristali sudjelovati. Za potrebe ovoga preliminarnog istraživanja koristili smo se samo dvama uzorcima sline. Jedan je bio uzorak pušača koji je u proteklih pet godina pušio 30 cigareta na dan, a drugi nepušača. Kratka povijest bolesti, uključujući i korištenje lijekova i navike pušenja, dobiveni su anketnim upitnikom. Dobrovoljci su dobili informacije o ciljevima i planu istraživanja te su potpisali pristanak. Oba dobrovoljca bili su zdravi muškarci u dobi od 27 i 28 godina, dobre oralne higijene i bez aktivnih karijesa i parodontne bolesti. Bilo im je rečeno da jedan sat prije kliničkoga pregleda i prikupljanja sline ne četkaju zube i ne puše.

Nestimulirana slina skupljala se devet sati. Oba dobrovoljca bila su obaviještena o tijeku prikupljanja sline i zamoljena da usta isperu običnom vodom. Nestimulirani uzorci sline prikupljani su 15 minuta u sterilne plastične čaše u skla-

Saliva Collection

Saliva donors for this study were patients from a private dental clinic, who volunteered for the study. For the purpose of this preliminary study, only two saliva samples were used. One of them was from a smoker, who smoked 30 cigarettes a day for the past 5 years, and the other was from a non-smoker. A brief medical history, including medications and smoking habits, was obtained using a questionnaire filled out by volunteers before screening. The volunteers received written information about the aims and the design of the study, and signed a written informed consent. Both subjects were healthy males, aged 27 and 28, with good oral hygiene and absence of active caries and periodontal disease. They were asked not to eat or drink, and to refrain from brushing the teeth and smoking for 1 hour before clinical examination and saliva collection.

Unstimulated saliva was collected at 9 a.m. Before sampling, each subject was briefed about the procedure and in-

du s objavljenim smjernicama (11). Odmah nakon uzimanja uzorka određen je pH sline. Analiza je obavljena pH elektrodom tipa 91 02 BN (Orion Res Inc., SAD) spojenom na potenciometar Orion EA 940 (Orion Res Inc., SAD). Elektroda je kalibrirana korištenjem standardnih otopina na pH 4,0 i 7,0. Dobiveni uzorci do daljnje obrade bili su pohranjeni na -20° C.

Određivanje kalcija

Agilent 7500 cx (Agilent Technologies, Waldbronn, Njemačka), induktivno spregnuta plazma sa spektrometrijom masa (ICP-MS), korišten je za mjerenje koncentracije kalcija u slini. Uzorci sline (1 ml) digestiraju se dušičnom kiselinom (2 ml 65 % HNO_3 i 1 ml H_2O) s pomoću visokotlačne mikrovalne digestije (UltraCLAVE, Milestone, Italija). Nakon hlađenja razrjeđuju se 1-postotnim (v/v) HNO_3 do ukupnog volumena od 15 ml, a Ca je analiziran ICP-MS-om. Sve standardne otopine pripravljene su od 1 g/l PlasmaCAL standarda (SCP Science, Kanada). Seronorm® Trace Elements Serum Control Level I i Level II (Sero AS, Billingstad, Norveška) korišteni su za kontrolu točnosti mjerenja. Uzorci seruma rekonstituiranog od referentne tvari pripremljeni su istim postupkom kao i kod uzoraka sline.

Ljudski materijal

Protokol studije odobrilo je Etičko povjerenstvo Stomatološkog fakulteta Sveučilišta u Zagrebu.

Tretman fluorom

Pasta za zube korištena u ovom istraživanju bila je Elmex®, GABA International AG, Münchenstein Švicarska (baza paste je silikagel, 1400 ppm F^-, aminofluorid (Olaflur), pH = 4,6). Za uporabu u tretmanu pripremljena je smjesta paste za zube i deionizirane vode (1 : 3). Smjesa paste za zube pripremala se 15 minuta prije svakoga tretmana. Postupak je ponovljen nakon šest sati za svaku skupinu.

Analiza fluora

Alkalno topljivi fluoridi stvoreni na površini cakline određeni su prema metodi Časlavske i suradnika (12). Svaki uzorak uronjen je u 2 ml 1M KOH-a tijekom 24 sata uz laganu trešnju na sobnoj temperaturi. Otopine su neutralizirane s 2 ml HNO_3 i puferirane s 0,5 ml TISAB-a III® (Orion Research Inc., Cambridge, MA, SAD) kako bi se podesio pH konačne otopine uzorka na približno 6,0. Fluor je analiziran fluoridnom elektrodom (Orion 96 – 09, Boston, MA., SAD). Količina alkalno topljivog fluorida izračunata je kao što su opisali Dijkman i Arends (13).

Statistička analiza

Pretpostavka o približno normalnoj distribuciji podataka potvrđena je Kolmogorov-Smirnovim testom s Lilliefor korekcijom te Shapiro-Wilkovim testom. Friedmanov test korišten je za ispitivanje ukupne razine značajnosti za podatke.

structed to wash his mouth with plain water. Unstimulated whole saliva samples were collected over a 15 minute period into sterile plastic cups following the guidelines published previously (11). pH of the saliva was determined immediately after collection. The analysis was made using a pH electrode type 91 02 BN (Orion Res Inc., USA) connected to a potentiometer Orion EA 940 (Orion Res Inc., USA). The electrode was calibrated using standard solutions at pH of 4.0 and 7.0. The collected samples were stored at -20° C until further processing.

Calcium Determination

Agilent 7500cx (Agilent Technologies, Waldbronn, Germany) inductively coupled plasma mass spectrometer (ICP-MS) with a collision cell was used for the measurements of saliva calcium concentration. Saliva samples (1 ml) were digested with nitric acid (2 ml of 65% HNO_3 and 1 ml of H_2O), using a high pressure microwave digestion system (UltraCLAVE, Milestone, Italy). After cooling down, samples were diluted with 1% (v/v) HNO_3 to a total volume of 15 ml and Ca was analyzed by ICP-MS. All standard solutions were prepared from a single 1 g/l PlasmaCAL standard (SCP Science, Canada). Seronorm® Trace Elements Serum Control Level I and Level II (Sero AS, Billingstad, Norway) were used to control the accuracy of measurements. Samples of reconstituted serum reference material were prepared using the same procedure as saliva samples.

Human Subjects

The Ethics Committee of the School of Dental Medicine University of Zagreb, Croatia, approved the study protocol.

Fluoride treatment

The toothpaste used in this experiment was Elmex®, GABA International AG, Münchenstein, Switzerland (Silica based toothpaste, 1400 ppm F^-, Amine fluoride (Olaflur), pH=4.6). The toothpaste/deionized water slurry (1:3 w/w) was made for use in treatment. Toothpaste slurries were prepared fresh, 15 min before each treatment. The procedure was repeated after a 6-hour overnight period for each of the groups.

Fluoride Analysis

The amount of KOH-soluble fluoride was determined by the method of Caslavska et al. (12). Each slab was exposed to 2 ml of 1 M KOH for 24 h with gentle agitation at room temperature. The solutions were neutralized with 2 ml of HNO_3 and buffered with 0.5 ml of TISAB III® (Total Ionic strength Adjusting Buffer, Orion Research Inc., Cambridge, Mass., USA), adjusting the final pH of the sample solution to approximately 6.0. Fluoride was analyzed using a fluoride electrode (Orion 96-09, Boston, Mass., USA). The amount of KOH-soluble fluoride was calculated as described by Dijkman and Arends (13).

Statistical analysis

The assumption of approximate normal distribution of the data was verified by Kolmogorov–Smirnov test with Lilliefors correction, as well as by the Shapiro-Wilk test. The Friedman test procedure was used to test the overall level

Neparametrijski Wilcoxonov test parova korišten je za određivanje značenja promjena u količini alkalno topljivih fluorida stvorenih na površini cakline između ispitnih skupina. P < 0,05 smatrao se statistički značajnim. Sve statističke analize obavljene su programskim paketom Statistica (verzija 7.1, StatSoft, Inc).

Rezultati

Koncentracija kalcija u slini pušača bila je veća (52,68 mg/L) u odnosu prema slini nepušača (23,95 mg/L). Koncentracija fluorida u uzorcima sline bila je 0,05 ± 0,02 mg /L. pH sline pušača bio je 6,55, a sline nepušača 6,48.

Rezultati Kolmogorov-Smirnova i Shapiro-Wilkova testa nisu pokazali statističku značajnost za skupine A i B, što znači da pretpostavka o normalnoj distribuciji podataka nije pobijena. Rezultati tih testova bili su značajni za skupine C i D, što upućuje na to da je pretpostavka o normalnoj raspodjeli podataka pobijena. Budući da podatci nisu imali normalnu distribuciju, korišten je neparametrijski test.

Ukupna razina značajnosti za podatke prethodno je analizirana Friedmanovim postupkom (n = 14, df = 3, χ^2 = 34.71, p < 0,015) i dala je značajan rezultat.

Količina alkalno topljivih fluorida na površini cakline u skupini koja je tretirana slinom pušača i smjesom paste za zube (skupina A) bila je značajno viša od onih u kontrolnoj skupini (skupina D), skupini koja je tretirana samo smjesom paste za zube (skupina C) i skupini koja je tretirana smjesom paste za zube i slinom nepušača (skupina B) (P < 0,015). Količina alkalno topljivog fluorida u skupini B bila je značajno viša od one u kontrolnoj skupini (P < 0,015), ali nije bila statistički značajno različita u odnosu prema skupini C (P> 0,015). Podatci o količini ugradnje alkalno topljivih fluorida na površinu cakline nalaze se u tablici 2.

of significance for the data. The non-parametric Wilcoxon matched-pairs test, following a significant Friedman result, was used to determine the significance of changes in the enamel uptake of KOH-soluble fluoride concentration between the groups. P < 0.05 was considered statistically significant. All statistical analyses were performed using the Statistica software package (version 7.1, StatSoft, Inc.).

Results

Calcium concentration in the saliva of the smoker was higher (52.68 mg/L) than in that of the non-smoker (23.95 mg/L). The fluoride concentration of saliva samples was 0.05±0.02 mg/L. pHs of the smoker's and nonsmoker's saliva samples were 6.55 and 6.48, respectively.

The results of the Kolmogorov–Smirnov test and the Shapiro-Wilk test were non-significant for groups A and B, indicating that the assumption of the normal distribution of the data was not violated. The results of the Kolmogorov–Smirnov test and the Shapiro-Wilk test were significant for groups C and D, indicating that the assumption of the normal distribution of the data was violated. Since the data did not have a normal distribution, a non-parametric test was used.

The overall level of significance for the data was previously analyzed using the Friedman procedure (n=14, d.f.=3, χ^2 =34.71, P<0.015), and it yielded a significant result.

The amount of KOH-soluble fluoride on the enamel in the group treated with the smoker's saliva and toothpaste was significantly higher than those of control group, toothpaste only group and non-smoker's saliva and toothpaste group (P<0.015). The amount of KOH-soluble fluoride in the group treated with the non-smoker's saliva and toothpaste was significantly higher than those of control group (P<0.015) but was not significantly different when compared to toothpaste only group (P>0.015). Summary data for the enamel uptake of KOH-soluble fluoride concentrations are presented in Table 2.

Tablica 2. Podatci o količini ugradnje alkalno topljivih fluorida na površinu cakline nakon tretmana slinom i smjesom zubne paste (0,14 % F) n = 14
Table 2 Summary data for the enamel uptake of KOH-soluble fluoride after treatment with saliva and fluoride toothpaste (0.14% F). n=14

Skupina • Group	Tretman • Treatment	KOH-topljivi fluoridi µg/cm² • KOH-soluble fluoride µg/cm² Srednja vrijednost ± SD • Mean±SD	Značajnost[#, a, b, c, d] • Significance[#, a, b, c, d]
A	Slina pušača + smjesa zubne paste • Smoker's saliva + toothpaste	1.43±0.48	*b, c, d
B	Slina nepušača + smjesa zubne paste • Non-smoker's saliva + toothpaste	0.76±0.26	*a, d
C	Samo smjesa zubne paste • Toothpaste only	0.96±0.27	*a, d
D	Bez tretmana • No treatment (control)	0.10±0.12	*a, b, c

[#] Friedmanov ANOVA test pokazuje značajnost (P < 0,05) • Friedman's ANOVA test yielded a significant result (P<0.05)
* Wilcoxonov test parova pokazuje značajnost (P < 0,015):[a] kada je vrijednost uspoređena s vrijednosti iz skupine A, [b] kada je vrijednost uspoređena s vrijednosti iz skupine B, [c] kada je vrijednost uspoređena s vrijednosti iz skupine C, [d] kada je vrijednost uspoređena s vrijednosti iz skupine D • the Wilcoxon matched-pairs test significant (P<0.015): [a]when value is compared with the A group data, [b]when value is compared with the B group data, [c]when value is compared with the C group data, [d]when value is compared with the D group data.

Rasprava

Rezultati ovog istraživanja daju nove spoznaje o povezanosti kalcija u slini i ugradnje fluorida na površinu cakline. Koncentracija kalcija u slini u ovom istraživanju bila je dva puta veća u uzorku sline pušača negoli u uzorku sline nepušača. Kalcij u slini najčešće je istraživan u kontekstu parodontnog zdravlja pušača. U mnogobrojnim istraživanjima istaknuto je da pušenje znatno utječe na razvoj parodontne bolesti te je identificirano kao jedan od rizičnih čimbenika za nastanak parodontitisa [pregledni članak Johnsona i Hilla (14)]. U dosad objavljenim istraživanjima dobiveni su suprotni rezultati kad je riječ o razlici u koncentraciji kalcija u slini pušača i nepušača. Sewón i suradnici pronašli su veću koncentraciju kalcija u slini osoba koje puše više od deset cigareta dnevno negoli kod nepušača (15). Otkrili su također da pušači imaju manju mineralnu gustoću kostiju pa pretpostavljaju da je veća koncentracija kalcija u slini povezana sa skeletnim poremećajem kalcija. Khan i suradnici pronašli su, nakon stimulacije nikotinom, veću razinu kalcija u slini korisnika duhanskih proizvoda negoli kod onih koji se njima ne koriste (16). Pretpostavljaju da povišena koncentracija kalcija u slini pušača može nastati od različitih duhanskih proizvoda. U drugoj studiji pronađene su značajno više razine kalcija u slini pušača negoli nepušača, a češće četkanje zuba bilo je povezano sa smanjenim sadržajem kalcija i fosfora u slini (17). Kiss i suradnici istraživali su razliku u koncentraciji kalcija u slini žena pušača i nepušača s parodontitisom ili bez njega, te su pokazali da su bolesnice s parodontitisom, koje su pušile, imale veću koncentraciju kalcija u slini negoli one koje nisu pušile (18). Postoji istraživanje kojim je utvrđena povećana, ali ne statistički značajna koncentracija kalcija u slini mladih, umjerenih pušača (19), a u drugom istraživanju pronađena je smanjena koncentracija kalcija u slini pušača prije parodontne terapije i poslije nje (20). Ovi podatci podupiru jasnu povezanost između pušenja i koncentracije kalcija u slini, iako taj odnos nije potpuno razjašnjen.

Naši rezultati pokazuju da su sve tretirane skupine imale statistički značajno veću koncentraciju alkalno topljivih fluorida u odnosu prema kontrolnoj skupini koja je bila bez tretmana. U nekim studijama podupire se ideja da slina u kombinaciji s topikalnom fluoridacijom ne pospješuje stvaranje kalcijeva fluorida (21,22), što je u suprotnosti s našim rezultatima. Jedan od razloga za to može biti dulja ekspozicija (60 min.) u otopini fluorida, što je možda potaknulo otpuštanje kalcija iz cakline. S druge strane, u nekoliko je istraživanja (6,8) istaknuto da slina povećava koncentraciju kalcijeva fluorida. Sugerirano je da slina može povećati količinu kalcijeva fluorida zbog sadržaja kalcija i zbog svoje mucinozne građe (6). Naše istraživanje pokazalo je da je skupina tretirana pastom za zube i slinom pušača imala značajno veći unos alkalno topljivih fluorida na površinu cakline u odnosu prema skupini tretiranoj pastom za zube i slinom nepušača. Istodobno, nema značajne razlike između skupine tretirane pastom za zube i slinom nepušača te one tretirane samo smjesom zubne paste. Ovaj rezultat sugerira da na količinu unosa alkalno topljivih fluorida na površinu cakline ne utječe samo kalcij u slini, nego i njegova koncentracija. Budući da su ovo

Discussion

The results of this study provide some novel observations regarding the association between salivary calcium and fluoride uptake by enamel. Salivary calcium concentration in this study was two times higher in the smoker's saliva sample compared to the nonsmoker's saliva sample. Salivary calcium is usually investigated in relation to periodontal health of tobacco smokers. A large number of studies have shown that smoking has unquestionable influence on the development of periodontal disease and has been identified as one of the risk factors for periodontitis (review by Johnson and Hill (14)). There are conflicting results regarding the difference in salivary calcium concentration of smokers and nonsmokers in the literature. Sewón found higher salivary calcium in subjects who smoke more than ten cigarettes per day than in non-smokers (15). They found that smokers have lower mineral density of bones and suggested that higher salivary calcium concentration related to skeletal calcium disturbances. Khan et al. found a higher level of salivary calcium in tobacco users than in non-users under resting conditions and following stimulation with nicotine (16). They suggested that higher salivary calcium concentration in smokers could come from different tobacco products. In another study, significantly higher salivary calcium levels were observed in smokers than non-smokers, but more frequent tooth-brushing was associated with reduced salivary content of calcium and phosphorus (17). Kiss et al. conducted a study with female smokers and non-smokers with or without periodontitis and their findings showed that patients with periodontitis who smoke exhibit higher salivary calcium levels than those in non-smokers (18). A separate study found higher, but not statistically significant, salivary calcium in young, moderate smokers (19), while yet another one found reduced calcium concentrations in saliva of smokers before and after periodontal therapy (20). These data support a clear association between smoking and salivary calcium concentrations, although the relationship remains to be fully elucidated.

Our results showed that all treatment groups had statistically significant higher concentrations of KOH-soluble fluoride compared to the control group with no treatment. There are studies supporting the idea that saliva combined with exposure to low fluoride topical treatment does not enhance the formation of calcium fluoride (21,22), which is not supported by our findings. One reason for those findings could be longer exposure time (60 min) to the fluoride solution, which may have allowed the release of calcium from enamel in those studies. On the other hand, there are several different studies (6, 8) which reported that saliva increased the concentration of calcium fluoride. It has been suggested that the presence of saliva may increase the amounts of calcium fluoride because of its calcium content and mucinous nature (6). Our study demonstrated that the group treated with toothpaste and smoker's saliva had significantly higher enamel uptake of KOH-soluble fluoride compared to the group with the toothpaste and nonsmoker's saliva treatment. On the other hand, there was no significant difference between the group with toothpaste and nonsmok-

novi rezultati, potrebna su daljnja istraživanja kako bi se razjasnilo zašto je u slini pušača veća koncentracija kalcija. Važno je i razjasniti koja koncentracija kalcija u slini čini razliku u količini unosa fluorida na površinu cakline.

Pozitivnu korelaciju između visoke razine kalcija u slini i broja intaktnih zuba utvrdili su Sewon i Mäkelä (23). Prije njih su Ashley (24) i Shaw (25) uspostavili suprotni odnos između karijesa i mineralnog sadržaja plaka i sline (23, 24). Sewon i suradnici također su otkrili da kod pacijenata s parodontitisom postoji više intaktnih zuba negoli kod ispitanika koji nemaju parodontitis (26). Dakle, iako su nužna daljnja istraživanja u području mineralnog sadržaja sline pušača, naši rezultati mogu dati mehanističko objašnjenje za postojanje više intaktnih zuba kod osoba s parodontitisom.

Zaključno, ovo istraživanje pokazuje da je viša razina kalcija u slini, nađena kod pušača, povećala količinu unosa alkalno topljivih fluorida na površinu cakline. Ovaj nas rezultat ohrabruje da provedemo istraživanje većih razmjera u koje ćemo uključiti veći broj uzoraka sline.

er's saliva treatment and the group with the toothpaste but no saliva treatment. This result suggests that the amount of enamel uptake of KOH-soluble fluoride is affected not only by the presence, but the concentration of calcium in saliva, as well. Since these are novel findings, further research is needed to elucidate where salivary calcium in smokers' saliva comes from. Another question that remains to be answered is which concentration of salivary calcium makes a difference in the amount of fluoride uptake by enamel.

A positive correlation between high salivary calcium levels and the number of intact teeth was established by Sewon and Mäkelä (23). Prior to their study, Ashley (24) and Shaw (25) established an inverse relationship between caries and mineral content of plaque and saliva (23, 24). Sewon et al. also found that periodontitis-affected subjects have more intact teeth than subjects who are free of the disease (26). Therefore, while further investigation remains necessary in the field of smokers' saliva mineral content, our results provide a possible mechanistic explanation of existence of more intact teeth in subjects with periodontitis.

In conclusion, this study demonstrates that higher levels of salivary calcium found in smokers enhance the amount of KOH-soluble fluoride uptake. This result encourages us to conduct a larger scale study with more sample donors.

Zahvale

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Sukob interesa

Nije ga bilo.

Conflict of interest

None declared

Abstract

Objective: The aim of this *in vitro* preliminary study was to assess the effect of smokers' saliva (assuming their higher calcium concentration) in combination with fluoridated toothpaste on the enamel uptake of alkali-soluble (KOH-soluble) fluoride. **Materials and methods:** Four enamel slabs were cut from each of 14 impacted third molars and randomly assigned into 4 groups. Unstimulated saliva samples were collected from two age and sex matched volunteers. One of the samples was taken from a heavy smoker and the other sample was taken from a non-smoker. Two groups (A and B) were shaken in saliva (A in smoker's saliva, B in nonsmoker's saliva) for 5 min and then shaken for 3 min in a toothpaste/deionized water slurry (1:3 w/w). One of the groups (group C) received no saliva treatment and was only shaken in toothpaste slurry for 3 min. The treatment was repeated after a 6-hour period. One of the groups (D) served as a control group with no treatment. **Results:** Calcium concentration in the smoker's saliva was higher than in the nonsmoker's saliva. The enamel uptake of KOH-soluble fluoride in group A was significantly higher than that in the other two treatment groups, B and C. The enamel uptake of KOH-soluble fluoride in all 3 groups was statistically different from that in the control group. **Conclusion:** These results demonstrate that saliva collected from a heavy smoker, which had higher salivary calcium concentration, enhances enamel uptake of alkali-soluble fluoride and encourages us to conduct a large-scale study.

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Address for correspondence
Krešimir Bašić
University of Zagreb
School of Dental Medicine
Department of Pharmacology
Šalata 11, 10 000 Zagreb, Croatia
Tel: +385 1 4590 211
Fax: +385 1 4920 049
basic@sfzg.hr

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