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IZVORNI ZNANSTVENI RAD

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A Pilot Study on the Deposition of Drugs in Dental Tissues as Alternative Matrices for Forensic Toxicology

Pilot-studija o taloženju lijekova u zubnim tkivima kao alternativnim matricama za forenzičku toksikologiju

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Abstract

Background: Teeth may serve as alternative matrices for post-mortem toxicological analysis. Although the mechanism of drug incorporation into dental tissues remains unclear, it is hypothesized that orally ingested substances can permeate dental enamel and reach dentin and the pulp. **Objectives:** This study investigated enamel permeability to commonly ingested drugs, considering molecular characteristics (methadone - MET and dextromethorphan hydrobromide – DXM), pH, sugar content, and tooth conditions (intact enamel, cement-enamel junction-CEJ exposure, enamel wear). **Materials and Methods:** Sixteen clinically extracted teeth were divided into three groups and immersed in different substances, including MET with sugar, DXM with and without sugar (pH 4.5, 1 mg/ml), and acidified saliva (pH 5–6), simulating an addicted individual's oral environment. Cyclic immersions (substance-saliva-substance) mimicked chronic drug intake for about six weeks. After treatment, each tooth was separated into enamel, dentin, and pulp, and analyzed using liquid-liquid extraction and LC-MS/MS. **Results:** Preliminary results showed that both MET and DXM can penetrate both the enamel and dentin, reaching the pulp regardless of tooth integrity or molecule type. Acidity and sugar content significantly increased the diffusion. **Conclusions:** These findings suggest that other substances orally assumed might be endowed with similar effects on dental tissues, especially when combined with sugar or acid solutions, supporting teeth as viable matrices in post-mortem toxicology. However, pulp concentrations may reflect oral contamination rather than systemic levels.

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Introduction

In recent years, some authors have proposed teeth as alternative matrices for post-mortem forensic toxicology due to their high resistance to environmental factors, making them among the most frequently found remains in severely decomposed bodies, alongside hair and nails (1 – 15). However, the current results do not enable pathologists to correlate the positivity of toxicological analyses on dental substrates with the quantity of the presumed substance, its frequency or period of intake, or the mechanisms of xenobiotic deposition within the hard tissues of the tooth (enamel and dentin).

Uvod

Posljednjih godina neki su autori predložili zube kao alternativne matrice za postmortalnu forenzičku toksikologiju zbog njihove visoke otpornosti na čimbenike u okolišu zato što su, uz kosu i nokte, među najčešće pronađenim ostatcima u teško raspadnutim tijelima, (1 – 15). No trenutačni rezultati ne omogućuju patolozima da koreliraju pozitivnost toksikoloških analiza na zubnim supstratima s količinom pretpostavljene tvari, njezinom učestalošću ili razdobljem unosa, te mehanizmima taloženja ksenobiotika unutar tvrdih tkiva zuba (caklina i dentin).

In 2024, our research group developed an innovative protocol for the separation and analysis of dental tissues for forensic investigation in the distribution and behavior of substances of abuse in teeth (16). Dental enamel, primary and secondary dentin, and pulp have distinct embryogenetic origins and histology (17-20). Separate analysis of these tissues could provide additional and specific information regarding the mechanism of substance deposition into teeth.

A pilot study showed that dental pulp could serve as an indicator of acute intoxication and may be associated with peripheral blood circulation. Conversely, the detection of substances in dental hard tissues (enamel and dentin) appears to suggest chronic intake, thus correlating findings in the secondary dentin with those in hair (16). However, these results exhibited variability depending on the substance detected and, particularly, the method of intake. For instance, the presence of cocaine, typically administered intravenously or inhaled (21), within the dental pulp suggests absorption from peripheral blood, representing an optimal substrate for defining the psycho-physical conditions of the subject at the time of death. In the case of methadone (MET), typically administered orally as syrup solutions in conjunction with sugars for medical purposes in heroin-addicted individuals (22), the substance can be detected in almost all the various dental tissues even when absent in blood and hair, suggesting a potential external contamination through the enamel itself.

On the other hand, Klima et al (23) observed that the deposition of substances of abuse in hard dental tissues, particularly in the dentin, is significantly influenced not only by the chemical-physical properties of the substance analyzed but also by the duration of contact with the dental structures. Consequently, it can be hypothesized that oral intake, resulting in prolonged contact between the solution and the dental surface in the oral cavity, facilitates the deposition of substances of abuse in dental hard tissues and pulp through the enamel, potentially in addition to the concentrations originating from blood circulation. This implies that the method of substance intake, chemical characteristics of xenobiotics, variations in pH within the oral cavity, and the health status of teeth influence the quantitative results of toxicological analyses on the dental matrix and must be considered in the interpretation of post-mortem data.

Given that the external contamination of inner dental tissues (dentin and pulp) can solely be determined by an increase in enamel permeability, as observed in cases of tooth wear or decay, or the exposure to the oral cavity of the cement-enamel junction (CEJ), as observed in cases of periodontal disease, we developed an *in vitro* study to simulate these passive processes in chronic substance abuse that were taken orally. The aim of this study was to investigate the permeability of the enamel from the external (oral cavity) to the inner dental tissues (dentin and pulp) to oral-administered opioids, such as MET and dextromethorphan (DXM), to the presence or absence of cariogenic sugars, to pH variability, and to different healthy conditions of teeth in contact with the administered solution (sound enamel, worn enamel, or CEJ exposure). For this research, a new analytical method was fully validated and applied to dental tissues.

Godine 2024. naša je istraživačka skupina predložila inovativni protokol za odvajanje i analizu zubnih tkiva za forenzičku istragu distribucije i ponašanja tvari zlouporabe u zubima (16). Zubna caklina, primarni i sekundarni dentin te pulpa imaju različito embriogenetsko podrijetlo i histologiju (17 – 20). Odvojena analiza tih tkiva mogla bi dati dodatne i specifične informacije o mehanizmu taloženja tvari u zubima.

U pilot-studiji istaknuto je da zubna pulpa može poslužiti kao pokazatelj akutne intoksikacije i može biti povezana s perifernom cirkulacijom krvi. Suprotno tomu, otkrivanje tvari u tvrdim zubnim tkivima (caklina i dentin) čini se da sugerira kronični unos, što korelira nalaze u sekundarnom dentinu s onima u kosi (16). No ti su rezultati pokazali varijabilnost ovisno o otkrivenoj tvari i, posebno, o načinu unosa. Primjerice, kokain, koji se obično primjenjuje intravenski ili inhalacijom (21), unutar zubne pulpe sugerira apsorpciju iz periferne krvi, što je optimalna podloga za definiranje psihofizičkog stanja ispitanika u trenutku smrti. U slučaju metadona (MET), koji se obično primjenjuje oralno kao otopina sirupa u kombinaciji sa šećerima u medicinske svrhe kod osoba ovisnih o heroinu (22), tvar se može otkriti u gotovo svim različitim zubnim tkivima čak i kada je nema u krvi i kosi, što sugerira potencijalnu vanjsku kontaminaciju kroz samu caklinu.

S druge strane, Klima i suradnici (23) uočili su da na taloženje tvari zlouporabe u tvrdim zubnim tkivima, posebno u dentinu, značajno utječu ne samo kemijsko-fizička svojstva analizirane tvari, nego i trajanje kontakta sa zubnim strukturama. Posljedično, može se pretpostaviti da oralni unos, koji rezultira produljenim kontaktom između otopine i zubne površine u usnoj šupljini, olakšava taloženje tvari zlouporabe u tvrdim zubnim tkivima i pulpi kroz caklinu, potencijalno uz koncentracije koje potječu iz cirkulacije krvi. To implicira da način unosa tvari, kemijske karakteristike ksenobiotika, varijacije pH vrijednosti unutar usne šupljine i zdravstveno stanje zuba utječu na kvantitativne rezultate toksikoloških analiza zubne matrice te se moraju uzeti u obzir pri tumačenju podataka obdukcije.

S obzirom na to da se vanjska kontaminacija unutarnjih zubnih tkiva (dentin i pulpa) može ustanoviti isključivo povećanjem propusnosti cakline, kao što je uočeno u slučajevima trošenja ili karijesa zuba, ili izloženošću usne šupljine spoju cementa i cakline (CEJ), kao što je uočeno u slučajevima parodontne bolesti, odabrali smo studiju *in vitro* kako bismo simulirali te pasivne procese u slučaju kronične zlouporabe tvari koje su uzimane oralno. Cilj ovog istraživanja bio je istražiti propusnost cakline od vanjskoga zubnog tkiva (usna šupljina) do unutarnjega (dentin i pulpa) na oralno primijenjene opioide, poput MET-a i dekstrometorfana (DXM), na prisutnost ili odsutnost kariogenih šećera, na varijabilnost pH i na različita zdrava stanja zuba u kontaktu s primijenjenom otopinom (zdrava caklina, istrošena caklina ili izloženost CEJ-u). Za ovo istraživanje nova je analitička metoda u cijelosti validirana i primijenjena na zubna tkiva.

Materials and Methods

Chemicals and reagents

Hydrochloric acid (HCl), methanol (MeOH), sodium hydroxide (NaOH), monobasic potassium phosphate (KH-2PO4), and dipotassium phosphate (K2HPO4) were purchased from Panreac Quimica S.L.U. (Castellar del Vallès, Spain). Water (H2O), acetonitrile (ACN) and a MeOH for LC-MS/MS were acquired from Biosolve Chimie SARL (Dieuze, France). Fentanyl (internal standard, IS), DXM and MET standards were purchased from LGC standards (Milan, Italy). MET and DXM pharmaceutical preparations (Aricodil® and Bisolvon®) were provided by the hospital pharmacy. The distributed preparations exhibited a pH of 4.5. Saliva for the rinsing phase was provided by the laboratory personnel (a single healthy female donor aged 33 years, approximately 3 hours after finishing the last meal and oral hygiene practices, non-consumer), and the pH was adjusted to 5-6 with acetic acid.

Teeth collection

Sound permanent teeth (n = 16) were collected from living subjects who were non-consumers of any drugs of abuse. These subjects underwent dental extraction for clinical reasons, including impacted third molars, orthodontic treatment, and periodontal disease. Informed consent for research was obtained from each participant. The inclusion criteria were sound dental elements, free from tooth wear or decay, absence of conservative, prosthetic, or endodontic treatments, and subjects negative for the history of substance abuse. Teeth that did not fulfil the inclusion criteria were excluded from the study.

Teeth were divided into two groups: the experimental group (n = 9) and the validation phase (n = 7).

Experimental design

Collected teeth were immersed in different solutions of opioids using a custom-made device. MET and DXM were widely distributed and orally administered therapeutic opioids with molecular weight (MW), pKa (Acid dissociation constant), and lipophilicity variations among their constituent molecules (24,25). Thick and malleable metallic wire and self-adhesive mesh were used to build a steady support that allowed maintaining teeth at the correct immersion level into a silicon well plate containing the different 1-mg/mL opioids solutions.

For MET, this concentration was chosen according to the Guidelines for the Psycho-socially Assisted Pharmacological Treatment of Opioid Dependence (26), which suggests a daily consumption of 15-20 mg. Considering the surface of the oral cavity, tongue, and teeth, and also the volume of fluid, we approximated in about 1 mg (1 mL of a 1 mg/mL solution) the available MET amount for each tooth. The same amount was then applied also for the DXM preparations (DXM+sugar, Aricodil®, and DXM without sugar Bisolvon®), diluted with H2O. In this way, any influence of the concentrations on the permeability was excluded.

Since opioids chronic consumers are usually affected by periodontal diseases with exposition of CEJ, caries, and

Materijali i metode

Kemikalije i reagensi

Klorovodična kiselina (HC_l), metanol (MeOH), natrijev hidroksid (NaOH), monobazni kalijev fosfat (KH_2PO_4) i dikalijev fosfat (K_2HPO_4) nabavljeni su u tvrtki Panreac Quimica S.L.U. (Castellar del Vallès, Španjolska). Voda (H_2O), acetonitril (ACN) i MeOH za LC-MS/MS nabavljeni su kod proizvođača Biosolve Chimie SARL (Dieuze, Francuska). Fentanil (interni standard, IS), standardi DXM-a i MET-a kupljeni su od tvrtke LGC standards (Milano, Italija). Farmaceutske pripravke MET-a i DXM-a (Aricodil® i Bisolvon®) osigurala je bolnička ljekarna. Distribuirani pripravci pokazali su pH od 4,5. Slinu za fazu ispiranja osiguralo je laboratorijsko osoblje (jedna zdrava donorica u dobi od 33 godine, otprilike 3 sata poslije završetka posljednjeg obroka i obavljanja oralne higijene, nekonzument), a pH je octenom kiselinom podešen na 5 do 6.

Prikupljanje zuba

Zdravi trajni zubi (n = 16) prikupljeni su od živih ispitanika koji nisu konzumirali drogu. Svi su bili podvrgnuti vađenju zuba iz kliničkih razloga, uključujući impaktirane treće kutnjake, ortodontski tretman i parodontnu bolest. Od svakog sudionika dobiven je informirani pristanak za istraživanje. Kriteriji za uključivanje bili su zdravi zubni elementi bez trošenja ili karijesa, odsutnost konzervativnih, protetičkih ili endodontskih tretmana te ispitanici bez povijesti zlouporabe droga. Zubi koji nisu ispunjavali te kriterije isključeni su iz studije.

Zubi su podijeljeni u dvije skupine: eksperimentalnu skupinu (n = 9) i fazu validacije (n = 7).

Eksperimentalni dizajn

Prikupljeni zubi uronjeni su u različite otopine opioida s pomoću prilagođenog uređaja. MET i DXM bili su široko rasprostranjeni i oralno primijenjeni terapijski opioidi s varijacijama molekularne težine (MW), pKa (konstanta disocijacije kiseline) i lipofilnosti među njihovim sastavnim molekulama (24, 25). Debela i savitljiva metalna žica i samoljepljiva mrežica korištene su za izgradnju stabilnog nosača koji je omogućio održavanje zuba na ispravnoj razini uranjanja u silikonsku pločicu s različitim otopinama opioida od 1 mg/mL.

Za MET je ta koncentracija odabrana prema Smjernicama za psihosocijalno potpomognuto farmakološko liječenje ovisnosti o opioidima (26) u kojima se predlaže dnevna konzumacija od 15 do 20 mg. Uzimajući u obzir površinu usne šupljine, jezika i zuba te volumen tekućine, procijenili smo dostupnu količinu MET-a za svaki zub na oko 1 mg (1 mL otopine od 1 mg/mL). Jednaka je količina zatim primijenjena i za pripravke DXM-a (DXM + šećer, Aricodil® i DXM bez šećera Bisolvon®), razrijeđene s H_2O. Na taj je način isključen svaki utjecaj koncentracija na propusnost.

Budući da kronični konzumenti opioida obično pate od parodontne bolesti s izloženošću CEJ-u, karijesu i bruksizmu (21, 27 – 30), prikupljeni zubi podijeljeni su u tri sku-

bruxism (21, 27-30), the collected teeth were divided in three groups: group 1 (G1), sound teeth (n=3) immersed in the substance up to dental crown (excluding the CEJ); group 2 (G2), sound teeth (n=3) immersed in the substance up to the CEJ; group 3 (G3), worn teeth (n=3) immersed in the substance up to the dental crown (excluding the CEJ) (Figure 1). Worn teeth were obtained treating sound teeth with a diamond bur mounted on a turbine up to expose an approximate 3-mm2 area of dentine. Teeth were immersed 40 times for 30 min in order to simulate a consumption of 1 month. Each diving phase was followed by a rinsing step with saliva for 30 min. The saliva was acidified since chronic consumption of opioids is related to xerostomia (21, 27-30) and a sugar-rich diet (29) leading to a decrease of the saliva pH (31). Opioids and saliva solutions were replaced every 8 immersion-rinsing cycles.

pine: skupina 1 (G1): zdravi zubi (n = 3) uronjeni u tvar do zubne krune (isključujući CEJ); skupina 2 (G2): zdravi zubi (n = 3) uronjeni u tvar do CEJ-a; skupina 3 (G3): istrošeni zubi (n = 3) uronjeni u tvar do zubne krunice (isključujući CEJ) (slika 1.).

Istrošeni zubi dobiveni su tretiranjem zdravih zuba dijamantnim svrdlom postavljenim na turbinu kako bi se otkrila površina dentina od približno 3 mm^2. Zubi su uronjeni 40 puta po 30 minuta da bi se simulirala konzumacija od 1 mjeseca. Nakon svake faze uranjanja slijedilo je ispiranje slinom tijekom 30 minuta. Slina je zakiseljena jer je kronična konzumacija opioida povezana s kserostomijom (21, 27 – 30) i prehranom bogatom šećerom (29), što smanjuje pH sline (31). Opioidi i otopine sline mijenjani su poslije svakih 8 ciklusa uranjanja i ispiranja.

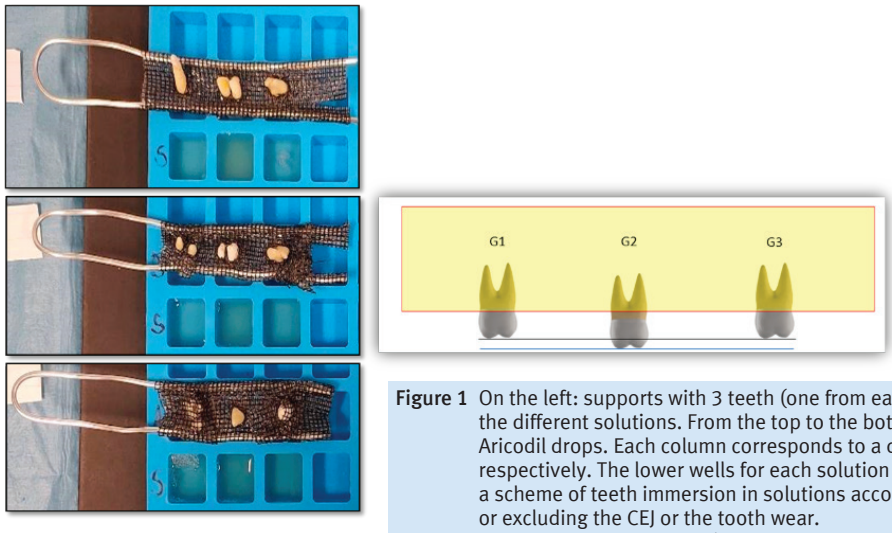

Figure 1 On the left: supports with 3 teeth (one from each group G1, G2, and G3) immersed into the different solutions. From the top to the bottom, Methadone syrup, Bisolvon syrup, and Aricodil drops. Each column corresponds to a dental element from group G1, G2, and G3, respectively. The lower wells for each solution line contain the rinsing saliva. On the right: a scheme of teeth immersion in solutions according to the different groups, thus including or excluding the CEJ or the tooth wear.

Slika 1 Lijevo: nosači s triju zuba (po jedan iz svake skupine G1, G2 i G3) uronjeni u različite otopine; od vrha do dna, metadonski sirup, sirup Bisolvon i kapi Aricodil; svaki stupac odgovara jednom zubnom elementu iz skupine G1, G2 i G3; donja udubljenja za svaku liniju otopine sadrže slinu za ispiranje. S desne strane: shema uranjanja zuba u otopine prema različitim skupinama, uključujući ili isključujući CEJ ili trošenje zuba

Dental tissue separation

Once the diving and contamination phases have been completed, each tooth underwent meticulous washing and preparation according to the "dental tissue separation protocol" previously developed from the Laboratory of Personal Identification and Forensic Morphology at the University of Florence (16). This protocol employs a diamond bur mounted on the turbine of a dental unit. Initially, teeth were divided into two halves to facilitate the extraction of pulp. Subsequently, the separation of mineralized tissues, namely enamel and dentin, was achieved through pulverization at the dental unit.

Distinctly from the original protocol, the dentin was not separated into secondary and primary dentin since the study investigated a passive contamination process (external contamination) for which the deposition of secondary dentin is negligible.

A standard quantity of enamel and dentin powder was analyzed. The pulp amount varied depending on the specific tooth sample.

Odvajanje zubnog tkiva

Nakon što su završene faze uranjanja i kontaminacije, svaki je zub podvrgnut detaljnom pranju i pripremi prema protokolu odvajanja zubnoga tkiva koji je prije toga razvio Laboratorij za osobnu identifikaciju i forenzičku morfologiju Sveučilišta u Firenci (16). Prema tome protokolu, koristi se dijamantno svrdlo postavljeno na turbinu stomatološke jedinice. Na početku zubi su podijeljeni na dvije polovine da bi se olakšalo vađenje pulpe. Nakon toga slijedi odvajanje mineraliziranog tkiva, odnosno cakline i dentina, a postiže se usitnjavanjem u stomatološkoj jedinici.

Za razliku od izvornog protokola, dentin nije odvojen na sekundarni i primarni jer se istraživao proces pasivne kontaminacije (vanjska kontaminacija) za koji je taloženje sekundarnog dentina zanemarivo.

Analizirana je standardna količina praha cakline i dentina. Količina pulpe varirala je ovisno o specifičnom uzorku zuba.

Sample treatment

A 10-mg aliquot of each dental tissue was sonicated for 45 min in the presence of HCl (1 mL, 1 M) and IS (200 ng). The mixture was added with 310 µL of NaOH (1 M), 1 mL of phosphate buffer (pH 8) and then extracted with 6 mL of a DCM: MeOH solution (8:2). After vortexing and centrifugation (4000 rpm, 5 min), the organic layer was dried and a gentle nitrogen steam at 40 °C. The residue was reconstituted with 1 mL of MeOH for LC-MS/MS and then 1:10 diluted.

LC-MS/MS

An analysis was performed using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), employing an Agilent 1290 Infinity HPLC system interfaced with an Agilent 6460 triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source operating in positive mode. Operating conditions included a gas temperature of 325 °C, flow rate of 10 L/min, nebulizer pressure of 20 psi, and capillary voltage of 4000 V. Chromatographic separation was achieved using a Zorbax Eclipse Plus C18 column (2.1 × 50 mm, 1.8 µm), with a mobile phase composed of H2O with 5 mM formic acid (A) and ACN (B). The gradient of elution was carried out as follows: from 0–5 min, linear ramp from 1 to 95%B; isocratic hold to 7 min. Post-time was set at 2 min. The flow rate was 0.4 mL/min. The analysis was carried out in multiple reaction monitoring (MRM) mode, acquiring two transitions per analyte. For MET: 310 → 105, 265 m/z; for DXM: 272 → 147, 171 m/z; for fentanyl: 337 → 188, 132 m/z (Table 1). Data processing was performed using Agilent MassHunter Workstation software.

Validation parameters

The analytical method was validated according to the guidelines of the American Academy of Forensic Sciences (AAFS), covering the main parameters required to ensure reliability and reproducibility (32). The evaluated parameters included: specificity/selectivity to exclude interference from endogenous components; linearity, with coefficients of determination (R^2) >0.99 across multiple concentration levels; sensitivity, through determination of the limits of detection (LOD) and quantification (LOQ); accuracy and precision, assessed both intra- and inter-day; recovery, by comparing pre- and post-extraction fortified samples; matrix effect, to evaluate possible endogenous interferences; and carry-over, to verify the absence of contamination between injections.

Obrada uzorka

Alikvot od 10 mg svakoga zubnog tkiva soniciran je 45 minuta u prisutnosti HC_1 (1 mL, 1 M) i IS-a (200 ng). Smjesi je dodano 310 µL NaOH (1 M), 1 mL fosfatnog pufera (pH 8) i zatim ekstrahirano sa 6 mL otopine DCM : MeOH-a (8 : 2). Nakon vrtloženja i centrifugiranja (4000 rpm, 5 min.), organski sloj je osušen i lagano propušten kroz paru dušika na 40 °C. Ostatak je rekonstituiran s 1 mL MeOH-a za LC-MS/MS, a zatim razrijeđen 1 : 10.

LC-MS/MS

Analiza je provedena tekućinskom kromatografijom u kombinaciji s tandemskom spektrometrijom masa (LC-MS/MS), a koristio se Agilent 1290 Infinity HPLC sustav povezan s trostrukim kvadrupolnim spektrometrom masa Agilent 6460 opremljenim izvorom ionizacije elektrosprejem (ESI) u pozitivnom načinu rada. Radni uvjeti uključivali su temperaturu plina od 325 °C, brzinu protoka od 10 L/min., tlak raspršivača od 20 psi i kapilarni napon od 4000 V. Kromatografsko odvajanje postignuto je korištenjem Zorbax Eclipse Plus C18 kolone (2,1 × 50 mm, 1,8 µm), s mobilnom fazom sastavljenom od H_2O s 5 mM mravlje kiseline (A) i ACN-a (B). Gradijent eluiranja proveden je na sljedeći način: od 0 do 5 minuta, linearni porast od 1 do 95 % B; izokratsko zadržavanje do 7 minuta. Vrijeme nakon reakcije postavljeno je na 2 minute. Brzina protoka bila je 0,4 mL/min. Analiza je provedena u načinu višestrukog praćenja reakcija (MRM), uz dva prijelaza po analitu. Za MET: 310 → 105, 265 m/z; za DXM: 272 → 147, 171 m/z; za fentanil: 337 → 188, 132 m/z (tablica 1.). Obrada podataka obavljena je u softveru Agilent MassHunter Workstation.

Parametri validacije

Analitička metoda validirana je prema smjernicama Američke akademije forenzičkih znanosti (AAFS), a obuhvaća glavne parametre potrebne za osiguranje pouzdanosti i ponovljivosti (32). Procijenjeni parametri uključivali su: specifičnost/selektivnost kako bi se isključile interferencije endogenih komponenti, linearnost s koeficijentima određivanja (R^2) > 0,99 na više razina koncentracije, osjetljivost određivanjem granica detekcije (LOD) i kvantifikacije (LOQ), točnost i preciznost tzv. procjene tijekom dana i međudnevne, oporavak usporedbom uzoraka obogaćenih prije i poslije ekstrakcije, učinak matrice za procjenu mogućih endogenih interferencija i prijenos kako bi se provjerila odsutnost kontaminacije između injekcija.

Table 1 MRM transitions and retention times for MET, DXM and fentanyl. In bold the quantitative fragment.

Compound	Fragmentor (V)	$[M+H]^+$	Production (m/z)	Collision energy (V)	Retention time (min)
MET	88	310	**265** 105	13 29	11.05
DXM	144	272	**171** 147	42 30	9.36
Fentanyl	143	337	**188** 132	21 33	9.56

Results

Method validation

The method proved to be highly sensitive with LOD and LOQ values of 0.1 and 1.0 pg/mg for both the substances in all matrices. However, according to the observed concentration ranges in the real samples, the working calibration curves were set at higher concentrations (1.0 – 1000.00 ng/mg from enamel and dentin; 10 – 5,000.00 ng/mg for pulp). The average R^2 was 0.9994 for MET and 0.9997 for DXM. Ion suppression was observed for both the compounds (average values: -22.3% for MET; -24.3% for DXM); recovery was above 75 % (average values: 77.1% for MET; 78.4% for DXM). The threshold of the acceptance rate was calculated at 20%, an all the measurements resulted within the acceptance criteria (≤ 20%). Carry-over was not observed.

Distribution of MET and DXM in dental tissues

The application of the separation technique employed by Bianchi et al. (16), in conjunction with the novel analytical procedure, facilitated the investigation of molecular deposits within various dental tissues, underscoring even the internal contamination of xenobiotics through the structures of dental crowns. Consequently, this enabled the evaluation of the permeability of the dental enamel. DXM and MET were detected in all dental tissues (Table 2), irrespective of the dental health conditions or the solution applied. Furthermore, dental pulps exhibited significantly higher concentrations of opioids compared to those detected in both enamel and dentin, particularly in all G1 groups of each solution (sound teeth without CEJ exposure).

The lowest concentrations of opioids were observed for DXM in the absence of sugar (Bisolvon®), for all dental tissues, and in all three healthy groups (Figure 2).

MET+sugar levels were higher in G2 (42.45 ng/mg in enamel; 94.78 ng/mg in dentin) and lower in G1 (13.52 ng/mg in enamel; 28.79 ng/mg for dentin), apart from the pulp where they were higher in G1 (2,800.66 ng/mg) and lower in G3 (403.45 ng/mg). DXM+sugar was constantly more concentrated in G3 (121.78 ng/mg in enamel; 186.13 ng/mg in dentin; 902.38 ng/mg in pulp). DXM was always less concentrated in G3 (3.57 ng/mg in enamel; 3.41 ng/mg in dentin; 15.18 ng/mg in pulp), while the highest amounts were observed in G2 for enamel and dentin (28.27 and 14.73 ng/

Rezultati

Validacija metode

Metoda se pokazala vrlo osjetljivom s vrijednostima LOD i LOQ od 0,1 i 1,0 pg/mg za obje tvari u svim matricama. No prema uočenim rasponima koncentracija u stvarnim uzorcima, radne kalibracijske krivulje postavljene su na više koncentracije (1,0 – 1000,00 ng/mg iz cakline i dentina; 10 – 5000,00 ng/mg za pulpu). Prosječni R^2 bio je 0,9994 za MET i 0,9997 za DXM. Potiskivanje iona uočeno je u oba spoja (prosječne vrijednosti: -22,3 % za MET; -24,3 % za DXM); oporavak je bio iznad 75 % (prosječne vrijednosti: 77,1 % za MET; 78,4 % za DXM). Prag stope prihvaćanja izračunat je na 20 %, a sva mjerenja bila su unutar kriterija prihvaćanja (≤ 20 %). Nije uočen prijenos.

Raspodjela MET-a i DXM-a u zubnim tkivima

Primjena tehnike separacije kojom su se koristili Bianchi i suradnici (16), zajedno s novim analitičkim postupkom, olakšala je istraživanje molekularnih naslaga unutar različitih zubnih tkiva i istaknula čak i unutarnju kontaminaciju ksenobiotika kroz strukture zubnih krunica. Posljedično, to je omogućilo procjenu propusnosti zubne cakline. DXM i MET otkriveni su u svim zubnim tkivima (tablica 2.), bez obzira na stanje zubnoga zdravlja ili primijenjenu otopinu. Nadalje, zubna pulpa pokazala je značajno veću koncentraciju opioida u usporedbi s onima otkrivenim i u caklini i u dentinu, posebno u svim G1 skupinama svake otopine (zdravi zubi bez izloženosti CEJ-u).

Najniže koncentracije opioida uočene su za DXM u odsutnosti šećera (Bisolvon®), za sva zubna tkiva i u svim trima zdravim skupinama (slika 2.).

Razine MET-a + šećer bile su više u G2 (42,45 ng/mg u caklini; 94,78 ng/mg u dentinu) i niže u G1 (13,52 ng/mg u caklini; 28,79 ng/mg za dentin), osim pulpe gdje su bile više u G1 (2800,66 ng/mg) i niže u G3 (403,45 ng/mg). DXM + šećer bio je konstantno koncentriraniji u G3 (121,78 ng/mg u caklini; 186,13 ng/mg u dentinu; 902,38 ng/mg u pulpi). DXM je uvijek bio manje koncentriran u G3 (3,57 ng/mg u caklini; 3,41 ng/mg u dentinu; 15,18 ng/mg u pulpi), a najveće količine uočene su u G2 za caklinu i dentin (28,27, odnosno 14,73 ng/mg), te u G1 za pulpu (136,08 ng/mg). U caklinama su razine MET-a + šećer i DXM-a + šećer bile pri-

Table 2 Concentrations of MET and DXM in the different dental tissues for the three groups of teeth.

Dental tissue	Substance	Group		
		G1	G2	G3
Enamel	MET+sugar (ng/mg)	13.52	42.45	30.92
	DXM+sugar (ng/mg)	20.91	29.71	121.78
	DXM (ng/mg)	7.23	28.27	3.57
Dentin	MET+sugar (ng/mg)	28.79	94.78	29.70
	DXM+sugar (ng/mg)	133.88	35.68	186.13
	DXM (ng/mg)	12.77	14.73	3.41
Pulp	MET+sugar (ng/mg)	2,800.66	506.12	403.45
	DXM+sugar (ng/mg)	723.20	200.26	902.38
	DXM (ng/mg)	136.08	48.24	15.18

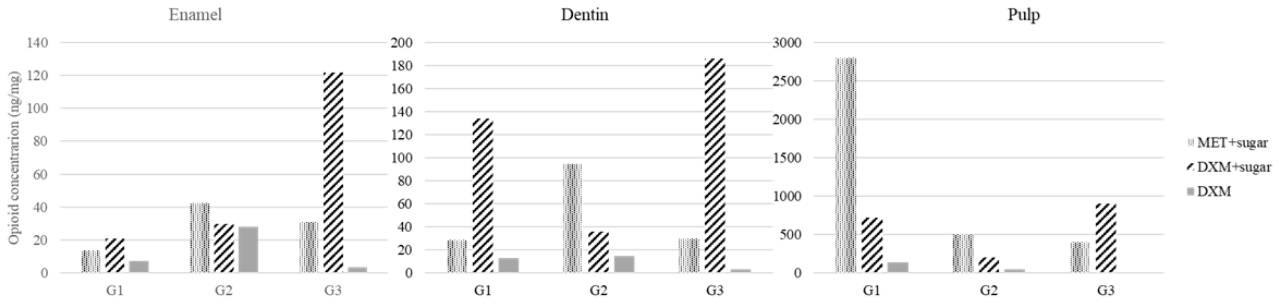

Figure 2 Distribution of MET and DXM in the different dental tissues for the three groups of teeth.
Slika 2. Raspodjela MET-a i DXM-a u različitim zubnim tkivima za tri skupine zuba

mg, respectively), and in G1 for the pulp (136.08 ng/mg). In enamels, MET+sugar and DXM+sugar levels were quite similar (13.52 vs 20.91 ng/mg for G1, 42.45 vs 29.71 ng/mg for G2, respectively), except for G3 (30.92 vs 121.78 ng/mg). A similar trend was also observed for dentin, even if the differences between MET+sugar and DXM+sugar were wider (28.79 vs 133.88 ng/mg for G1; 94.78 vs 35.68 ng/mg for G2; 29.70 vs 186.13 ng/mg for G3). In both dental tissues, MET+sugar concentration was lower in G1 and G3, and higher in G2. In pulps, a higher MET+sugar concentration was observed in G1 (2,800.66 vs 723.20 ng/mg), whilst DXM+sugar was still more concentrated in G3 (403.45 vs 902.38 ng/mg).

Discussion

This study investigated whether orally administered substances of abuse could penetrate from the external dental surface (enamel) into deeper layers (dentin and pulp), resulting in the deposition of xenobiotics within various regions of the tooth. Previous autopsy-based research on drug-related fatalities (16) indicated that dental tissue exhibits distinct characteristics compared to conventional post-mortem toxicological matrices such as fluids or keratinized substrates. Teeth may serve as valuable tools in post-mortem toxicology, particularly in distinguishing acute intoxication (through pulp analysis) from chronic substance exposure (through enamel and dentin). Preliminary data comparing cocaine and MET users revealed that, in cocaine addiction, xenobiotic deposition in dentin and pulp correlates with concentrations detected in hair and blood, respectively, suggesting a deposition through the bloodstream of dental pulp vessels corresponding to circulating blood levels. In contrast, MET appears capable of penetrating enamel and reaching inner dental layers, implying a potential secondary incorporation pathway from the oral cavity. This distinction may be attributed not only to the distinct chemical properties of the two substances but also to their different way of intake (inhalation/injection for cocaine and oral for MET) and duration of contact with oral tissues (22). Given the limited scientific evidence regarding the reliability of teeth as post-mortem toxicological matrices, it is imperative to differentiate between the active (from the bloodstream) and passive (from the oral fluid) incorporation of xenobiotics. The absence of this piece of information may result in misinterpretation, particularly regarding

lično slične (13,52 prema 20,91 ng/mg za G1, 42,45 prema 29,71 ng/mg za G2), osim za G3 (30,92 prema 121,78 ng/mg). Sličan trend uočen je i za dentin, čak i ako su razlike između MET-a + šećer i DXM-a + šećer bile veće (28,79 prema 133,88 ng/mg za G1; 94,78 prema 35,68 ng/mg za G2; 29,70 prema 186,13 ng/mg za G3). U oba zubna tkiva bila je koncentracija MET-a + šećer niža u G1 i G3, a viša u G2. U pulpi je uočena viša koncentracija MET-a + šećer u G1 (2800,66 prema 723,20 ng/mg), dok je DXM + šećer bio još uvijek koncentriraniji u G3 (403,45 prema 902,38 ng/mg).

Rasprava

Autori ove studije istraživali su mogu li oralno primijenjene tvari zlouporabe prodrijeti s vanjske zubne površine (cakline) u dublje slojeve (dentin i pulpa), što rezultira taloženjem ksenobiotika unutar različitih područja zuba. U dosadašnjim istraživanjima smrtnih slučajeva povezanih s drogom temeljenih na autopsiji (16) pokazano je da zubno tkivo pokazuje različite karakteristike u usporedbi s konvencionalnim postmortem toksikološkim matricama poput tekućina ili keratiniziranih supstrata. Zubi mogu poslužiti kao vrijedni alati u postmortem toksikologiji, posebno u razlikovanju akutne intoksikacije (analiza pulpe) od kronične izloženosti tvarima (kroz caklinu i dentin). Preliminarni podatci kojima se uspoređuju korisnici kokaina i MET-a otkrili su da kod ovisnosti o kokainu taloženje ksenobiotika u dentinu i pulpi korelira s koncentracijama otkrivenima u kosi, odnosno u krvi, što sugerira taloženje putem krvotoka žila zubne pulpe koje odgovara razinama u cirkulirajućoj krvi. Suprotno tomu, čini se da MET može prodrijeti u caklinu i dosegnuti unutarnje zubne slojeve, što implicira potencijalni sekundarni put ugradnje iz usne šupljine. Ta se razlika može pripisati ne samo različitim kemijskim svojstvima dviju tvari, nego i njihovu različitom načinu unosa (udisanje / injektiranje za kokain i oralno za MET) i trajanju kontakta s oralnim tkivima (22). S obzirom na ograničene znanstvene dokaze o pouzdanosti zuba kao postmortalnih toksikoloških matrica, nužno je razlikovati aktivno (iz krvotoka) i pasivno (iz oralne tekućine) unošenje ksenobiotika. Nedostatak te informacije može rezultirati pogrešnim tumačenjem, posebno ako je riječ o zubnoj pulpi. Dokazi o ugradnji ksenobiotika iz oral-

findings in dental pulp. Evidence of xenobiotic incorporation from oral fluid could compromise the reliability of comparisons between pulp and blood concentrations. Elevated levels in the pulp may reflect local contamination from substances present in the oral cavity, rather than systemic distribution, potentially leading to an erroneous determination of the cause of death.

An *in vitro* study was conducted to simulate the passive oral contamination of teeth in subjects who are addicted to chronic oral intake of substances. The investigation focused on two compounds, MET and DXM, which are commonly administered in oral syrup or drop formulations. Test solutions reflected commercially available preparations: MET in sugar-containing syrup, and DXM either in sugar-containing drops or sugar-free syrup.

Since oral health of substance-dependent individuals is often compromised by an acidic salivary pH (<6–7), a reduced flow, and an increased risk of dental and periodontal diseases (27-31), these conditions were simulated using teeth in varying healthy states (intact, worn, and periodontally affected). The relevant literature reports higher rates of decay, periodontal disease, bruxism, and cervical lesions in opioid-dependent patients, particularly those undergoing methadone maintenance therapy (27-31).

The primary objective was to identify key factors influencing dental tissue (enamel and dentin) permeability to orally administered substances, including sugar presence, molecular properties, and conditions of the oral-dental cavity.

The previously developed method for tooth pulverization (16) proved to be reliable for isolating enamel, dentin, and pulp tissues, thus enabling a detailed assessment of xenobiotic distribution and the dynamics of passive diffusion in cases of chronic exposure.

The new analytical method proved to be reliable and effective. In addition, the extraction procedure was less time-consuming than previously published methods. To the best of our knowledge, only two procedures have been published for detection of opioids in hard dental tissues. Klima et al. (6) reported a multi-class analysis consisting of a triple extraction with MeOH in ultrasonic bath for 60 min; the supernatant was then reduced, added with a mixture of 2-propanol/HCl 3/1 (v/v) and dried before being reconstituted with LC mobile phase. In a previous pilot study (16), dental tissues were incubated with HCl (0.1 N) for 72 h at 55°C; the mixture was then dried and reconstituted with MeOH. Both the procedures used LC-MS/MS systems. Thus, the method here described demonstrated to be faster, especially for its shorter incubation time (45 min). However, this less time-consumption did not negatively affect the sensitivity since it was in line with the other procedures.

This study demonstrates that both MET and DXM, contrary to previous research by Klima et al. (23) and Riedel et al. (33), not only contaminate dental enamel and dentine, but also penetrate into the innermost dental layers, including the pulp, irrespective of the administered solution, the presence or absence of sugar, or the state of oral-dental health, particularly the intact or compromised enamel as external

ne tekućine mogli bi ugroziti pouzdanost usporedbi između koncentracija pulpe i krvi. Povišene razine u pulpi mogu odražavati lokalnu kontaminaciju tvarima prisutnim u usnoj šupljini, a ne sistemsku distribuciju, što može dovesti do pogrešnog određivanja uzroka smrti.

Provedena studija je *in vitro* kako bi se simulirala pasivna oralna kontaminacija zuba kod ispitanika ovisnih o kroničnom oralnom unosu tvari. Istraživanje se usredotočilo na dva spoja – MET i DXM – koji se obično primjenjuju u obliku oralnoga sirupa ili kapi. Testne otopine sadržavale su komercijalno dostupne pripravke: MET u sirupu koji sadržava šećer i DXM u kapima ili sirupu sa šećerom ili bez šećera.

Budući da je oralno zdravlje osoba ovisnih o supstancijama često ugroženo kiselim pH-om sline (< 6 – 7), smanjenim protokom i povećanim rizikom od zubnih i parodontnih bolesti (27 – 31), ta su stanja simulirana korištenjem zuba u različitim zdravim stanjima (netaknuti, istrošeni i parodontno zahvaćeni). U relevantnoj literaturi izvješćuje se o većim stopama karijesa, parodontnih bolesti, bruksizma i cervikalnih lezija kod pacijenata ovisnih o opioidima, posebno onih koji se primaju na terapiju održavanja metadonom (27 – 31).

Primarni cilj bio je identificirati ključne čimbenike koji utječu na propusnost zubnog tkiva (caklina i dentin) za oralno primijenjene tvari, uključujući prisutnost šećera, molekularna svojstva i stanja oralno-zubne šupljine.

Dosadašnja metoda za pulverizaciju zuba (16) pokazala se pouzdanom za izolaciju tkiva cakline, dentina i pulpe, zato što omogućuje detaljnu procjenu distribucije ksenobiotika i dinamike pasivne difuzije u slučajevima kronične izloženosti. Nova analitička metoda pokazala se pouzdanom i učinkovitom. Uz to, postupak ekstrakcije bio je vremenski manje zahtjevan od dosad objavljenih metoda. Koliko znamo, objavljena su samo dva postupka za detekciju opioida u tvrdim zubnim tkivima. Klima i suradnici (6) izvijestili su o višeklasnoj analizi koja se sastojala od trostruke ekstrakcije s MeOH-om u ultrazvučnoj kupelji tijekom 60 minuta; supernatant je zatim reduciran, dodana mu je smjesa 2-propanola/HC_l 3/1 (v/v) i osušen je prije rekonstitucije mobilnom fazom LC. U prethodnoj pilot-studiji (16) zubna tkiva su inkubirana klorovodičnom kiselinom (HC_l - 0,1 N) tijekom 72 sata na 55 °C; smjesa je zatim osušena i rekonstituirana metanolom (MeOH). U oba postupka korišteni su sustavi LC-MS/MS. Dakle, ovdje opisana metoda pokazala se bržom, posebno zbog kraće inkubacije (45 min.). Međutim, ovaj manji utrošak vremena nije negativno utjecao na osjetljivost jer je bio u skladu s drugim postupcima.

Autori ove studije pokazali su da i MET i DXM, suprotno prethodnim istraživanjima Klime i suradnika (23) i Riedela i suradnika (33), ne samo da kontaminiraju zubnu caklinu i dentin, nego prodiru i u najdublje slojeve zuba, uključujući pulpu, neovisno o primijenjenoj otopini, prisutnosti ili odsutnosti šećera ili stanju oralno-dentalnog zdravlja, posebno netaknute ili narušene cakline kao vanjske zaštite (tablica 2.).

Posljedično, buduća istraživanja trebala bi se usredotočiti na procjenu kinetike ugradnje ksenobiotika u tvrda zubna tkiva pri različitim simuliranim trajanjima izloženosti lijeku kako bi se istražila njihova prikladnost kao biomarkera kro-

protection (Table 2). Consequently, future research should focus on evaluating the incorporation kinetics of xenobiotics in hard dental tissues at various simulated durations of drug exposure, to explore their suitability as biomarkers of chronic drug consumption. However, the values found in the dental pulp may not accurately reflect the concentrations circulating in the peripheral blood during the acute intoxication phase of orally administered solutions. This phenomenon can primarily be attributed to the acidity value to which prepared teeth were exposed (34-38). A pH of 4.5 is the sole characteristic shared by all the considered solutions, enabling the diffusion of molecules from the external enamel towards the internal pulp.

Furthermore, a substantial quantitative disparity can be discerned between the deposits of substances in solution with or without sugar for all the various dental tissues and oral health conditions. The concentrations of DXM detected in samples treated with Bisolvon (sugar-free) consistently exhibit significantly lower values compared to those detected in samples treated with Aricodil (DXM+sugar) and MET+sugar. For instance, examining the group G3 (worn teeth) in Table 2, the concentration of Aricodil in enamel reaches a peak of 121.78 ng/mg, while Bisolvon achieves a minimum of 3.57 ng/mg. This observation is noteworthy since both solutions are composed of the same molecule (DXM), unequivocally highlighting the influence of sugar on dental permeability since the incorporation rate cannot be attributed to the chemical-physical properties of substances or enamel health, as both samples originated from worn teeth. Similar findings were observed for dentin and pulp tissues. Consequently, it is possible to conclude that sugar has facilitated the molecular diffusion through dental tissues, resulting in an enhanced permeability of the enamel to xenobiotics, which is consistent with the results obtained by Berggren et al. (39). These authors established a correlation between the permeability of the enamel both to dyes (methylene blue) and bacterial products (tetanus toxin) and the increased concentration of sugar added to the solution. Furthermore, the heightened enamel permeability induced by sugar can be linked to the elevated bacterial activity observed in our study, as saliva samples were incorporated into solutions during the diving cycles to simulate a realistic oral environment. Therefore, the presence of sugar in orally ingested solutions or the practice of adding products with high sugar content in conjunction with substances of abuse should be considered in toxicological analyses conducted on dental matrices (40).

In contrast, dental health conditions appear to influence the amount of substances deposited in specific dental tissues. Notably, intact enamel (G1) exhibits lower concentrations of xenobiotics compared to samples with CEJ exposure or worn enamel (G2 and G3) for almost all substances (Table 2, Figure 2). Pulps, on the other hand, demonstrate the highest concentrations. Consequently, sound enamel, even when subjected to acidity, sugars, and bacteria, partially maintains its structural integrity by incorporating only a small amount of xenobiotics in contact. However, it loses its protective function, allowing for greater diffusion into the innermost layers, such as dentin and pulp. This process appears to mirnične konzumacije lijekova. Međutim, vrijednosti pronađene u zubnoj pulpi možda ne odražavaju točno koncentracije koje cirkuliraju u perifernoj krvi tijekom faze akutne intoksikacije oralno primijenjenih otopina. Taj fenomen može se ponajprije pripisati vrijednosti kiselosti kojoj su bili izloženi pripremljeni zubi (34 – 38). pH od 4,5 jedina je karakteristika koju dijele sve razmatrane otopine, omogućujući difuziju molekula iz vanjske cakline prema unutarnjoj pulpi.

Nadalje, može se uočiti značajna kvantitativna razlika između naslaga tvari u otopini sa šećerom ili bez njega za sva različita zubna tkiva i stanja oralnog zdravlja. Koncentracije DXM-a otkrivene u uzorcima tretiranima Bisolvonom (bez šećera) dosljedno pokazuju značajno niže vrijednosti u usporedbi s onima otkrivenim u uzorcima tretiranim Aricodilom (DXM + šećer) i MET-om + šećer. Primjerice, ispitivanjem skupine G3 (istrošeni zubi) u tablici 2., koncentracija Aricodila u caklini doseže vrhunac od 121,78 ng/mg, a Bisolvon postiže minimum od 3,57 ng/mg. To opažanje je značajno jer se obje otopine sastoje od iste molekule (DXM), što nedvosmisleno ističe utjecaj šećera na propusnost zuba zato što se brzina ugradnje ne može pripisati kemijsko-fizikalnim svojstvima tvari ili zdravlju cakline, jer oba uzorka potječu od istrošenih zuba. Slični nalazi uočeni su za dentin i pulpno tkivo. Posljedično, može se zaključiti da je šećer olakšao molekularnu difuziju kroz zubna tkiva, što je rezultiralo poboljšanom propusnošću cakline za ksenobiotike, što je u skladu s rezultatima Berggrena i suradnika (39). Ti autori utvrdili su korelaciju između propusnosti cakline i za boje (metilensko plavo) i za bakterijske produkte (tetanusni toksin) te povećane koncentracije šećera dodanog u otopinu. Nadalje, povećana propusnost cakline izazvana šećerom može se povezati s povišenom bakterijskom aktivnošću uočenom u našoj studiji, zato što su uzorci sline uključeni u otopine tijekom ciklusa uranjanja kako bi se simuliralo realno oralno okružje. Stoga šećer u oralno unesenim otopinama ili praksa dodavanja proizvoda s visokim udjelom šećera u kombinaciji s tvarima zlouporabe, treba uzeti u obzir u toksikološkim analizama provedenim na zubnim matricama (40).

Suprotno tomu, čini se da stanje zubnog zdravlja utječe na količinu tvari nataloženih u specifičnim zubnim tkivima. Značajno je da intaktna caklina (G1) pokazuje nižu koncentraciju ksenobiotika u usporedbi s uzorcima s izloženošću CEJ-u ili s istrošenom caklinom (G2 i G3) za gotovo sve tvari (tablica 2., slika 2,). Pulpa, s druge strane, pokazuje najveću koncentraciju. Posljedično, zdrava caklina, čak i kada je izložena kiselosti, šećerima i bakterijama, djelomično održava svoju strukturnu cjelovitost uključivanjem samo male količine ksenobiotika u kontakt. No, omogućujući veću difuziju u najdublje slojeve poput dentina i pulpe, gubi svoju zaštitnu funkciju. Čini se da taj proces odražava tipičnu evoluciju zubnog karijesa i bakterijske invazije u zubna tkiva (41). Klima i suradnici (6) uočili su veće količine ksenobiotika u karijesnom tkivu ekstrahiranom ovisnim ispitanicima u usporedbi s mineraliziranim zubnim tkivima. Slični rezultati dobiveni su za zube s intaktnom caklinom, ali izložene CEJ-u (G2), gdje su veće koncentracije otkrivene u dentinu u usporedbi sa skupinom G1 (bez izloženosti CEJ-u). CEJ se fiziološki sastoji od tanjeg sloja cakline, što je put smanjenog

ror the typical evolution of dental caries and bacterial invasion into dental tissues (41).

Klima et al. (6) observed higher quantities of xenobiotics in carious tissue extracted from addicted subjects compared to mineralized dental tissues. Similar results were obtained for teeth with intact enamel but CEJ exposure (G2), where higher concentrations were detected in the dentin compared to group G1 (without CEJ exposure). The CEJ is physiologically composed of a thinner layer of enamel, representing a path of reduced resistance against the diffusion of xenobiotics within dental tissues, particularly dentin, which is more peripheral compared to the rest of the dental crown (41). Conversely, worn teeth of group G3 exhibited similar concentrations between enamel and dentin (Table 2, Figure 2) due to the compromised integrity of the enamel by mechanical wear, which significantly diminishes its barrier function.

The main limitation of the study is an insufficient sample size for statistical measurements, that is, a low number of tested teeth and in the limited variability of the molecules analyzed. Consequently, it is not feasible to ascertain whether and how the distinct chemical-physical characteristics of the substances of abuse can indeed influence the contamination of dental tissues. Additionally, the substances employed exhibited identical concentrations, and were applied for the same duration and number of cycles, rendering it impossible to establish a correlation between the volume of solution in contact with the teeth and the quantity accumulated in various tissues. The significance of preliminary results, given the current limited knowledge in the literature regarding the mechanisms of substance accumulation in teeth, underscores the necessity of further research to expand the scope of substances, concentrations, dosages, and times of intake. Microbiological investigations may prove instrumental in elucidating the mechanisms of contamination.

Conclusions

This pilot study revealed that the presence of sugar in oral solutions (e.g., syrups) significantly enhances the penetration of molecules into the dentin and pulp, regardless of the dental health conditions, probably favoring bacterial proliferation with the same mechanism and progression of dental caries. Considering the demineralizing action of acids on the enamel, combined with sugars in the present study, the passive accumulation of substances into teeth can be considered regardless of the nature of the ingested molecule. Although the results are limited by a small sample size and the pilot nature of the study, they suggest a generalization of the phenomenon of the external contamination in all oral administrations under the same conditions and contact times (repeated and chronic intake), which must be acknowledged in post-mortem toxicology. Additionally, useful information for forensics is that, in the case of oral intake of acid solutions, the concentration of xenobiotics detectable in the dental pulp may not accurately reflect the circulating values in blood during the acute intoxication phase, as the oral cavity can also be contaminated.

otpora difuziji ksenobiotika unutar zubnih tkiva, posebno dentina koji je periferniji u usporedbi s ostatkom zubne krune (41). Suprotno tomu, istrošeni zubi u skupini G3 pokazali su slične koncentracije između cakline i dentina (tablica 2., slika 2.) zbog narušenog integriteta cakline mehaničkim trošenjem, što značajno smanjuje njezinu zaštitnu funkciju.

Glavno ograničenje studije jest nedovoljna veličina uzorka za statistička mjerenja, odnosno mali broj testiranih zuba i ograničena varijabilnost analiziranih molekula. Posljedično, nije moguće utvrditi mogu li i kako različite kemijsko-fizikalne karakteristike tvari zlouporabe doista utjecati na kontaminaciju zubnih tkiva. Uz to, korištene tvari pokazale su jednaku koncentraciju i primjenjivale su se za isti broj ciklusa, što onemogućuje uspostavljanje korelacije između volumena otopine u kontaktu sa zubima i količine akumulirane u različitim tkivima. Značenje preliminarnih rezultata, s obzirom na trenutačno ograničeno znanje u literaturi o mehanizmima akumulacije tvari u zubima, ističe potrebu za daljnjim istraživanjem kako bi se proširio opseg tvari, koncentracija, doza i vremena uzimanja. Mikrobiološka istraživanja mogu se pokazati ključnima u razjašnjavanju mehanizama kontaminacije.

Zaključci

U ovoj pilot-studiji otkriveno je da šećer u oralnim otopinama (npr. u sirupu) značajno poboljšava prodiranje molekula u dentin i pulpu, bez obzira na stanje zuba, vjerojatno pogodujući proliferaciji bakterija s istim mehanizmom i napredovanjem zubnog karijesa. Uzimajući u obzir demineralizirajuće djelovanje kiselina na caklinu, u ovoj studiji u kombinaciji sa šećerima, pasivno nakupljanje tvari u zubima može se smatrati neovisnim o prirodi unesene molekule. Iako su rezultati ograničeni malom veličinom uzorka i prirodom pilot-studije, oni sugeriraju generalizaciju fenomena vanjske kontaminacije kod svih oralnih primjena u jednakim uvjetima i vremenom kontakta (ponovljeni i kronični unos), što se mora uzeti u obzir u postmortem toksikologiji. Osim toga, korisna informacija za forenziku jest da, u slučaju oralnoga unosa kiselih otopina, koncentracija ksenobiotika koja se može otkriti u zubnoj pulpi možda ne pokazuje točno vrijednosti u cirkulaciji u krvi tijekom faze akutne intoksikacije, jer i usna šupljina može biti kontaminirana.

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Sažetak

Zubi mogu poslužiti kao alternativne matrice za postmortalnu toksikološku analizu. Iako mehanizam prodiranja lijekova u zubna tkiva ostaje nejasan, pretpostavlja se da oralno unesene tvari mogu prodrijeti u zubnu caklinu i doprijeti do dentina i pulpe. **Ciljevi:** Uzimajući u obzir molekularne karakteristike (metadon – MET i dekstrometorfan hidrobromid – DXM), pH, sadržaj šećera i stanje zuba (intaktna caklina, izloženost spoju cement-caklina-CEJ, trošenje cakline), autori ove studije istraživali su propusnost cakline kad je riječ o uobičajeno unesenim lijekovima. **Materijali i metode:** Šesnaest kliničkih izvađenih zuba podijeljeno je u tri skupine i uronjeno u različite tvari, uključujući MET sa šećerom, DXM sa šećerom i bez njega (pH 4,5, 1 mg/mL) i zakiseljenu slinu (pH 5 – 6), simulirajući oralno okruženje ovisne osobe. Cikličke uronjene tvari (tvar-slina-tvar) oponašale su oko šest tjedana kronični unos droge. Poslije tretmana svaki zub odvojen je na caklinu, dentin i pulpu te analiziran s pomoću tekuće ekstrakcije i LC-MS/MS-a. **Rezultati:** Preliminarni rezultati pokazali su da i MET i DXM mogu prodrijeti i u caklinu i u dentin, te dosegnuti pulpu bez obzira na integritet zuba ili vrstu molekule. Kiselost i sadržaj šećera značajno su povećali difuziju. **Zaključci:** Dobiveni nalazi upućuju na to da bi i druge tvari koje se uzimaju oralno mogle slično djelovati na zubna tkiva, posebno u kombinaciji sa šećerom ili kiselim otopinama, podržavajući zube kao održive matrice u postmortalnoj toksikologiji. No koncentracije pulpe mogu odražavati oralnu kontaminaciju, a ne sistemsku razinu.

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