Antimikrobni proteini pljuvačke
Antimicrobial peptides of human saliva

SGS YU ISSN 0039-1743-
COBISS. SR-ID 8417026

Ivan Dožić, Tatjana Todorović

Stomatološki fakultet u Beogradu, OJ Institutski predmeti, Opšta i oralna biohemija

School of Dentistry Belgrade, Institute of Oral Biohemia

INFORMATIVNI RAD (IR)
INFORMATIVE ARTICLE

KRATAK SADRŽAJ

Pored uloge pljuvačke u samočišćenju oralne sredine, održavanju fizioloških vrednosti Ph, održavanju integriteta čvrstih i mekih tkiva oralne sredine, ovaj sekret ispoljava i značajnu antimikrobnu zaštitu oralnih tkiva. Pljuvačka sadrži brojne fizičke, fizičko-hemijske i hemijske agense koji učestvuju u zaštiti oralnih tkiva od mikroorganizama i njihovih produkata, s obzirom na činjenicu da usna duplja predstavlja „ulazna vrata" organizma za patogene.

Antimikrobni proteini pljuvačke predstavljaju glavne faktore nespecifične humoralne antimikrobne zaštite oralne sredine. Poreklom su iz pljuvačnih žlezda, epitelnih ćelija oralne mukoze i leukocita. Najznačajniji antimikrobni proteini pljuvačke su: histatini, defenzini, lakoferin, katelicidini, mucini, kalprotektin, lizozim, oralna peroksidaza.

Pored toga što sprečavaju kolonizaciju oralne mukoze patogenim mikroorganizmima i održavanje normalne oralne bakterijske flore, ovi sastojci pljuvačke imaju i značaj u održavanju opšteg zdravlja organizma, jer sprečavaju transmisiju patogena u unutrašnju sredinu domaćina.

Ključne reči: pljuvačka, antimikrobni peptidi, nespecifična humoralna antimikrobna zaštita

SUMMARY

Among the many functions of human saliva, its digestive and protective properties have attracted the most interest. Human saliva contains a number of physical, physicochemical and chemical agents that protect oral tissues against by various microorganisms and their metabolic products. Among such protective factors, the flushing effect of saliva flow is the most important one, not only because it so effectively removes exogenous and endogenous microorganisms and their products into the gut but also because a steady supply of saliva guarantees continuous presence of both non-immune and immune factors in the mouth.

The most important antimicrobial peptides in saliva are: histatins, defensins, lactoferrin, cathelicidins, mucins, calprotectin, lysozyme, oral peroxidase.

Antimicrobial peptides are components of complex host secretions, acting synergisticaly with other innate defence molecules to combat infection and control resident microbial populations throughout the oral cavity.

Key words. Saliva, antimicrobial peptides, innate defences

U zaštiti oralnih tkiva značajnu ulogu ima pljuvačka, kao sekret koji je u neprekidnom kontaktu sa svim oralnim strukturama. Značaj pljuvačke je održavanje oralne homeostaze, što ovaj sekret realizuje svojim brojnim organskim i neorganskim sastojcima. Uloge pljuvačke su: samočišćenje oralne sredine od zaostalih čestica hrane, održavanje fizioloških vrednosti pH, održavanje integriteta čvrstih i mekih tkiva, specifična i nespecifična antimikrobna zaštita, antioksidativna zaštita oralne sredine. Pljuvačka je značajna i kao mogući biološki materijal za nove dijagnostičke testove koji bi mogli doprineti postavljanju dijagnoze i razjašnjavanju patogeneze mnogih sistemskih i oboljenja lokalizovanih u oralnoj sredini.[1]

S obzirom na to da usna duplja predstavlja „ulazna vrata" organizma za mikroorganizme iz spoljašnje sredine,

In the protection of oral tissues saliva has a very important role, as a secret, which has continuous/constant contact with each and every oral structure . The importance of the saliva and preservation of oral homeostasis, which this secret realises with its numerous organic and inorganic components. The functions of the saliva are: self-cleaning of the oral environment from the food particles left, preservation of the physiological pH values, preservation of the integrity of the hard and soft tissues, specific and unspecific antimicrobial protection, antioxidant protection of the oral environment. Saliva is important as a possible biological matter for the new diagnostic tests which could contribute to diagnostics and to explanation of pathogenesis of great number of systemic diseases and diseases located in the oral environment.[1]

According to the fact that the oral cavity presents "the front door " of the organism for microorganisms from

pljuvačka sa svojim antimikrobnim proteinima predstavlja prvu liniju odbrane domaćina. Na ovaj način se oralna mukoza štiti od kolonizacije mikroorganizmima, a takođe se sprečava poremećaj ravnoteže normalne oralne flore. Antimikrobna zaštita pljuvačke može biti specifična i nespecifična. Nosioci specifične antimikrobne zaštite oralne sredine su imunoglobulini, dok su brojni antimikrobni peptidi odnosno, proteini, glavni faktori nespecifične zaštite oralne sredine.

U antimikrobne peptide, odnosno, proteine pljuvačke ubrajaju se: histatini, defenzini, laktoferin, katelicidini, mucini, kalprotektin, lizozim, oralna peroksidaza.[2,3,4]

Histatini

Histatini su familija proteina pljuvačke male molekulske mase koji ispoljavaju katodnu elektroforetsku pokretljivost. Zbog visokog procenta u sadržaju aminokiseline histidina, poznati su i kao *histidinom-bogati proteini.* Glavni izvori histatina su pljuvačne žlezde, pa pripadaju proteinima pljuvačke lokalnog porekla. Predstavnici proteina ove familije su: histatin 1, histatin 3 i histatin 5.

Histatini su uključeni u različite biološke procese u usnoj duplji, a u cilju održavanja *oralne homeostaze.* Pored uloge u formiranju stečene zubne pelikle, vezivanju pojedinih jona metala u pljuvački, vezivanju toksičnih polifenola u oralnoj sredini, ovi proteini ispoljavaju i antimikrobnu aktivnost, odnosno, antifungicidno i antibakterijsko dejstvo. U ″in vitro″ uslovima posebno izraženo antifungicidno dejstvo ispoljava histatin 5 protiv različitih vrsta gljivica (*Candida albicans, Candida glabrata, Candida krusei, Saccharomyces cerevisiae, Cryptococcus neoformans*). Ovaj protein ne deluje kao klasični antibiotik, formirajući pore ili jonske kanale u membrani C. albicans, već u njegovom mehanizmu antifungicidnog delovanja postoji nekoliko etapa: vezivanje za specifične receptore na membrani, transport kroz membranu i ulazak u ćeliju, mobilizacija jona K^+, Mg^{2+} i ATP-a iz ćelije. Ciljno mesto za dejstvo histatina unutar C. abicans su mitohondrije, gde inhibira respiratorni lanac. [5,6,7]

Na ovaj način se štiti oralna mukoza od kolonizacije gljivicama. Zbog navedene sposobnosti, kao i zbog toga što nije toksičan za humana tkiva, ispituje se mogućnost upotrebe histatina kao potencijalnog antifungicidnog sredstva u tretmanu oralne kandidoze.[6]

Defenzini

Defenzini pripadaju niskomolekularnim peptidima (3-5 kDa), bogatim cisteinom, β-naborane strukture,

the outside , the saliva, with its anti microbial peptides represents the first line of the defence of the host. In this way oral mycosis is protected from the colonisation of the microorganisms, and it prevents the balance loosing of normal oral flora. Antimicrobial saliva protection can be specific and unspecific. The carriers of the specific antimicrobial protection of the oral environment are immunoglobulin products of cell plasma, while the numerous antimicrobial peptides, in other words, proteins, the main factors of unspecific oral environment protection.

Antimicrobial peptides, in other words, saliva proteins include: histatines, defensines, lactoferrin, cathelicidins, mucines, calprotectin, lysozymes, oral peroxidase.

Histatines

Histatines are saliva protein family of saliva, which have small molecule mass and show cathode electrophoresis mobility. Because of the high percentage in content of amino acids of histidine, they are known as *histidine rich proteins*. The main sources of histatine are salivary glands, so they belong to the group of local origin saliva proteins. The representatives of this family proteins are: histatine 1, histatine 3, and histatine 5.

Histatines are included into different biological processes in oral cavity, and their aim is to keep *oral homeostasis* in order. Besides their role in forming acquired tooth pellicles, bonding of some metal ions in saliva, tying toxic poly phenols in oral environment, those proteins show antimicrobial activity, in other words, anti fungal and antibacterial effect. The specially articulated anti fungal effect , in “In vitro” conditionals, has histatine 5 against different kinds of fungus (*Candida albicans, Candida glabrata, Candida krusei, Saccharmyces cerevisiae, Cryptococcus neoformans*). This protein doesn’t behave like classic antibiotic, by forming pores or ionic channels in C. albicans membrane, but in its mechanism of anti fungal effect, there are a couple of phases: bonding for the specific receptors on membrane, transport through membrane and entering the cell, mobilisation of ions K^+, Mg^{2+} I ATP from the cell. The target for histatine to act inside C. albicans is mitochondria, where it inhibits respiratory chain.[5, 6,7]

This is the way that oral mucosa is protected from fungus colonisation. Because of the previously mentioned ability, and because it is non-toxic for human tissues, the possibility of histatine usage as a potential antifungal measure in oral candida treatment is examining.[6]

Defensives

Defensives belong to low molecular peptides (3-5 kDa), which are cysteine-rich beta-wrinkled structure, cat-

katjonske elektroforetske pokretljivosti. Podeljeni su u dve grupe*: α-defensini i β-defensini.* [8]

U humanom tkivu je identifikovano šest vrsta α-defenzina. U neutrofilima su dokazana četiri α-defensina, pa se i nazivaju *humani neutrofilni defenzini (HNP-1-4),* dok su preostala dva *HNP-5 i HNP-6* izolovana iz Panethovih ćelija tankog creva i epitelnih ćelija urogenitalnog trakta žena.[8,9]

U mešovitoj pljuvački zdravih pacijenata su detektovani HNP-1, HNP-2, HNP-3. Za razliku od pljuvačke, nije dokazano prisusutvo HNP-1 u pljuvačnim žlezdama. Koncentracija HNP-1 u pljuvački pacijenata sa različitim oralnim oboljenjima (lichen planus, leukoplakija, inflamacija, skvamozni celularni karcinom) je mnogo veća u odnosu na pljuvačku zdravih pacijenata.[10,11,12]

Dokazano je šest tipova *humanih β-defenzina (HBD-1-6).* Proizvode ih epitelne ćelije mnogih organa, uključujući kožu, pluća, bubreg, pankreas, uterus i oko. U epitelnim ćelijama oralne mukoze (keratinocitima), pljuvačnim žlezdama (epitelne ćelije kanala) dokazano je prisustvo tri β-defensina (HBD-1, HBD-2, HBD-3). Za razliku od HBD-1, za ekspresiju HBD-2 i HBD-3 u epitelnim ćelijama su potrebni induktivni faktori, kao što su proinflamatorni citokini (I*L-1β, tumor nekrozis-faktor (TNF-α), γ- interferon (IFN-γ)* i mikroorganizmi.

Defenzini ispoljavaju antimikrobnu aktivnost, jer su u stanju da „ubijaju" razne Gram-pozitivne i Gram-negativne bakterije, gljivice (*Candida albicans*), kao i neke viruse (*Herpes simplex).* [9,13]

Mehanizam antimikrobnog delovanja defenzina može se podeliti u nekoliko faza:

1. Elektrostatsko povezivanje između defenzina kao katjona i površine ćelijske membrane bakterije koja ima anjonske karakteristike.
2. Povećanje permeabilnosti membrane bakterije se ostvaruje na dva načina: prvi način je formiranje jonskih kanala, čije dimenzije zavise od tipa ćelija ili drugi način koji se naziva "*carpet model"* koji podrazumeva agregaciju ovih peptida sa pozitivno naelektrisanim delovima membrane formirajući tranzitni put za njihov prolaz.
3. Poremećaj u sintezi proteina u bakterijskoj ćeliji.[9]

Zbog velikog potencijala u "ubijanju" bakterija, defenzini se popularno nazivaju i "prirodnim antibioticima". U pojedinim radovima se sugeriše o mogućnostima njihove upotrebe u terapiji oralnih oboljenja. Međutim, njihova eventualna primena bi bila dosta ograničena, jer visoka koncentracija β-defenzina ispoljava citotoksičan efekat na mnoge eukariotske ćelije. [9]

ionic electrophoresis mobility. They are divided into two groups; *alpha-defensives* and *beta-defensives*.[8]

The six kinds of alpha -defensives are identified in human tissue. In neutrophil there are proved four of alpha -defensives so they are called *human neutrophil defensives* (HNP-1-4), while the other two HNP-5 and HNP-6 are isolated from Paneth's cells of the small intestine and from epithelia cells of women urinogenital tract.[8,9]

In mixed saliva of healthy patients HNP-1, HNP-2, HNP-3 are detected. In contrast to saliva, the presence of HNP-1 is not proved in saliva glands. The concentration of HNP-1 in saliva of the patients with different oral diseases (lichen planus, leukoplaqia, inflammation, squamous cell carcinoma) is much larger then in saliva of the healthy patients.[10,11,12]

The six types of *humane beta-defensives* are proved (HBD-1-6). The epithelium cells of many organs, including skin, lungs, kidney, uterus and eye, produce them. In the epithelium cells of oral mucosa (ceratinocites), saliva glands (epithelium cells of the channel) the presence of three beta-defensives is proved (HBD-1, HBD-2, HBD-3). In contrast to HBD-1, for the expression of HBD-2 and HBD-3 in epithelium cells, induction factors are needed, like pro inflammatory cytokines (IL-1*beta*, *tumour necrosis –factor (TNF-alfa), gama-interferon (IFN-gama)* and micro organism.

Defensives show antimicrobial activity, because they are able to "kill" all kinds of Gram-positive and Gram-negative bacteria, the fungus (*Candida albicans*), as well as some viruses (*Herpes simplex*).[9,13]

The mechanism of antimicrobial effect of the defensives can be divided into couple of phases:

1. Electrostatic connection between defensives as a cations and the surface of bacteria cell membrane, which has anion characteristics.
2. The increasing of permeability of bacteria membrane is achieved in two ways: the first one is to form of the ionic channels, which dimensions depend on the type of the cell; the second is called *"carpet model"* which means aggregation of those peptides with positive electrified parts of membrane and in this way formation of a transit path for their pass.
3. Disturbance in a protein synthesis in bacteria cell.[9]

Because of its great potential in "killing" bacteria, defensives are popularly called "natural antibiotics". In some studies is suggested the possibility of their use in oral disease therapy. However, their eventual application would be very limited, because, the high concentration of beta-defensive shows toxic effect on many eucaryotic cells. [9]

Laktoferin

Laktoferin je po svojoj strukturi glikoprotein. Pripada familiji transferina, odnosno, ″ne-hemski″- gvožđe vezu-

Lactoferrin

Lactoferrin is, according to its structure, glycoprotein. It belongs to the family of transferrins, that is, un-haemic –

jućim proteinima. Leukociti i epitelne ćelije mukoze proizvode laktoferin, tako da se njegovo prisustvo dokazuje i u sekretima: pljuvačka, suze, seminalna i vaginalna tečnost.

Laktoferin je značajna komponenta nespecifične antimikrobne zaštite mukoza, jer ispoljava bakteriostatičko i baktericidno dejstvo prema Gram-pozitivnim i Gram-negativnim bakterijama. Ima izraziti afinitet za vezivanje gvozđa, tako da ga čini nedostupnim za bakterije, čime su one lišene ovog, i za njih neophodnog, bioelementa. Ovaj fenomen se naziva*"nutritivni imunitet"* i na ovaj način laktoferin sprečava rast i razmnožavanje bakterija. [14,15]

Baktericidno dejstvo laktoferina podrazumeva direktnu interakciju sa ćelijskim zidom Gram negativnih vrsta. Ovaj antimikrobni peptid, kao katjonski molekul, stupa u interakciju sa lipopolisaharidima (LPS) u membranama bakterija, što dovodi do narušavanja njenog integriteta. [16]

Laktoferin ispoljava i antivirusnu aktivnost, jer u „*in vitro*" uslovima može da i inhibira replikaciju virusa. Međutim, istraživanja pokazuju da laktoferin prvenstveno sprečava infekciju ćelija domaćina virusom, a u manjoj meri inhibira replikaciju virusa.

Sprečavanje infekcije ćelija domaćina virusom laktoferin ostvaruje na dva načina.

1. direktnim vezivanjem laktoferina za virus (*virus hepatitisa C (HCV), poliovirus, rotavirus, hepes simplex virus (HSV), virus humane imunodeficijencije (HIV)*
2. vezivanjem laktoferina za ćelije domaćina, i to za one biomolekule u sastavu plazma membrana koji služe virusima kao receptori ili ko-receptori (heparin-sulfat-proteoglikane - HSPGs). [17,18]

Laktoferin ima slično dejstvo na vrstu *Candida albicans*, kao i na bakterije. To se pre svega odnosi na vezivanje gvožđa, kao i direktna interakcija laktoferina i njegovih peptida sa ovom gljivicom, što izaziva poremećaj propustljivosti njene membrane. [19]

Istraživanja su pokazala da je koncentracija laktoferina promenljiva u pljuvački i gingivalnoj tečnosti u toku inflamatornih procesa u usnoj duplji, posebno kod parodontopatije. Koncentracija laktoferina u pljuvački bila je povećana u pljuvački obolelih u odnosu zdrave ispitanike. Kod pacijenata sa progresivnom parodontopatijom, gde je jedan od uzročnika *Actinobacilus actinomyctemcomitans,* ustanovljena je negativna korelacija između broja ovih patogena i koncentracije laktoferina u pljuvački. Nakon odgovarajuće terapije parodontopatije, nivo laktoferina u gingivalnoj tecnosti i pljuvački se značajno smanjio. Ovo ukazuje da laktoferin može biti osetljiv biomarker stepena težine parodontopatije i efikasnosti primenjene terapije.[20,21]

Katelicidini

Katelicidini su peptidi, katjonske elektroforetske pokretljivosti koji ispoljavaju antimikrobno dejstvo. Pri-

ferric connected proteins. Leukocytes and epithelium cells of mucosa make lactoferrin, so that its presence is proved in secretes: saliva, tears, and seminal and vaginal fluid.

Lactoferrin is an important component for unspecific antimicrobial mucosa protection, because it demonstrates bacteriostatic and bactercidal effect towards Gram-positive and Gram-negative bacteria. It has an outstanding affinity to bond with ferritin, so it made it inaccessible for bacteria, and so they are deprived of this bio element necessary for them. This phenomenon is called "nutritive immunity" and this is the way that lactoferrin prevents the growth and reproduction of bacteria. [14,15]

Bactericidal effect of lactoferrin implied direct interaction with the cell wall of Gram-negative species. This anti microbes' peptide, as a cationic molecule, interacts with lipolysacharides (LPS) in bacteria membranes, which leads to disturbance of its integrity. [16]

Lactoferrin demonstrates antiviral activity, too, because in "in vitro" conditions it could inhibit replication of viruses. However, researches show that lactoferrin primarily stops virus infection of the host cells, and to the smaller extent, it inhibits the replication of viruses. Lactoferrin achieves the prevention of the infection of the host cells in two ways:

1. by direct bonding of lactoferrin for the virus (*hepatitis C virus (HCV)*, *polyvirus, rotavirus, herpes simplex virus (HSV), human immunodeficiency virus (HIV)*
2. by bonding of lactoferrin for the host cells, especially for those biomolecules in the structure of plasma membranes which serve the viruses as a receptors or co-receptors (HSPGs). [17,18]

Lactoferrin has the same effect on a kind of *Candida albicans*, as on bacteria. It refers to ferrous bonding, as a direct interaction of lactoferin and its peptides with this fungus, which provoke disturbance in porousness of its membrane.[19]

The researches show that the concentration of lactoferrin is changeable in saliva and gingival fluid during inflammatory processes in oral cavity, especially in the case of periodontal disease. The concentration of lactoferrin in saliva was increased in saliva of the sick in relation to the healthy tested patients. The patients with progressive periodontal disease, where one of the causes is *Actinobacillus actinomicetemcomitans*, negative correlation is found between the number of those pathogens and concentration of lactoferrin in saliva. After the appropriate periodontal disease therapy, the level of lactoferrin in saliva and gingival fluid is significantly decreased. This shows that lactoferrin can be sensitive biomarker for the parodontopathy level and the efficiency of the therapy applied.[20,21]

Cathelicidins

Cathelicidins are peptides of acationic electrophoresis mobility and they show antimicrobial effects. They

sutni su u koži i mukozi, a takođe su detektovani u pljuvački. U oralnim tkivima je dokazano prisustvo katelicidina u jeziku, bukalnoj mukozi, gingivi, pljuvačnim žlezdama.

Antimikrobna aktivnost (protiv mnogih Gram-pozitivnih i Gram-negativnih bakterija, gljivica, virusa, parazita) je primarna uloga ovog peptida. Neutralisanje bakterija je veoma brzo i ostvaruje se formiranjem jonskih kanala ili pora u membrani ovih mikroorganizama. Dokazano je da katelicidini imaju i sposobnost vezivanja lipopolisaharida u bakterijskoj membrani, što rezultira poremećajem njenog intergriteta. Naime, katelicidini, kao i neki drugi katjonski peptidi sa antimikrobnom aktivnošću, interreaguju sa vezujućim mestima dvovalentnih katjona na lipopolisaharidima bakterijske membrane. Ovi peptidi ispoljavaju veći afinitet za lipopolisaharide u odnosu na neke dvovalentne katjone, kao što su Ca^{2+} i Mg^{2+}. Na ovaj način, katelicidini zamenjuju pomenute dvovalentne jone u vezivanju za lipopolisaharide bakterijske membrane. [22,23]

Opisana je povezanost oralnih oboljenja i katelicidina kod pacijenata sa Kostmanovim sindromom, koji je posledica kongenitalne neutropenije. Smanjenje koncentracije katelicidina u pljuvački kod ovih pacijenata je u korelaciji sa stepenom težine parodontalnog oboljenja. [24,25]

Mucini

Mucini ili mukusni glikoproteini (MG) su glavni činioci nespecifične zaštite mukoza respiratornog, gastrointestinalnog i reproduktivnog trakta organizma. Njihovo prisustvo dokazano je i u pljuvački. Sintetišu se od strane acinusnih ćelija pljuvačnih žlezda. U ovom sekretu su prisutna dva tipa mucina: visokomolekularni (MG1) i niskomolekularni (MG2). [26]

Glavna uloga mucina je u mehaničkoj zaštiti oralne mukoze, ali su istraživanja ukazala i na njihovu antimikrobnu aktivnost. Niskomolekularni mucini pljuvačke u *"in vitro"* uslovima ispoljavaju dejstvo protiv raznih gljivica (*Candida albicans, Cryptococcus neoformans*), Gram-pozitivnih bakterija (*Streptococcus mutans),* kao i protiv Gram-negativnih bakterija uzročnika parodontopatije (*Porphyromonas gingivalis)*. [27,28]

Zbog toga što su u stanju da agregiraju bakterije oralne flore, mucini predstavljaju značajan faktor i u prevenciji zubnog karijesa. Podaci iz literature ukazuju da su niskomolekularni mucini efikasniji u ovom dejstvu od visokomolekularnih. Pokazatelj toga je što su visokomolekularni mucini predominantni u pljuvački karijes-osetljivih osoba, dok je u pljuvački karijes-rezistentnih osoba ustanovljena povećana koncentracija niskomolekularnih mucina. [29]

Kalprotektin

Kalprotektin je peptid, poznat u literaturi pod različitim imenima kao što su: kompleks S100A8 i S110A9

are present in skin and mucosa, and they are detected in saliva, too. In oral tissues, the presence of cathelicidins is confirmed on the tongue, buccal mucosa, gum, and saliva glands.

Antimicrobial effect (against many Gram-positive and Gram-negative bacteria, fungus, viruses, parasites) is the primary role of this peptide. Neutralisation of the bacteria is very quick and it is achieved by forming the ionic channels or pores in membranes of this microorganisms. It is proved that cathelicidins have the ability of bonding lipolysaccharides in bacteria membranes, which has its results in disturbance of its integrity. Namely, cathelicidins, as some other cationic peptides with antimicrobial effects, inter react with bonding spots of two valences cations on lipolysaccharides on bacteria membranes. Those peptides show much bigger affinity towards lipolysaccharides than some bi-valent cations like CA^{2+} and Mg^{2+}. In this way, cathelicidins replaced previously mentioned two valences ions in bonding with lipolysaccharides of bacteria membrane.[22,23]

There is, also, described the connection of oral diseases and cathelicidins at the patients with Costman's syndrome, which is caused by congenital neutropenia. Decrease of cathelicidins concentration at those patients saliva is correlated with the parodontopathy disease level.

Mucins

Mucins or mucus glykoproteins (MG) are the main factors of the unspecific mucosa protection of respiratory, gastrointestinal and reproductive tract in organism. Acinous cells of the saliva glands synthesize them. In this secret two types of mucins are present: high molecular (MG1) and low molecular (MG2).[26]

The main role of mucins is in mechanical protection of mucosa, but the researches showed their antimicrobial activity. Low molecular mucins of saliva in "in vitro" conditions show effect against different kinds of fungus *(Candida albicans, Crytococcus neformans*), Gram-positive (*Streptococcus mutans*), and Gram-negative bacteria that cause periodontal disease (*Porphyromonas gingivalis*).[27,28]

Because, they are able to aggregate oral flora bacteria, mucins represent the important factor in dental caries prevention. Literature data show that low molecular mucins are more effective in this kind of effect then high molecular ones. The indicator for this is that, high molecular mucins are predominant in saliva of caries sensitive persons, while in saliva of caries resistant persons higher concentration of low molecular mucins is established.[29]

Calprotectin

Calprotectin is a peptide, in literature known under different names like: complex S100A8 and S110A9 pro-

proteina, 27E10 antigen, makrofagni inhibirajući protein MRP8/14, LIL i LIH protein, kalgranulin A/B.

Kalprotektin ima antimikrobno delovanje, koje ostvaruje vezivanjem cinka. Naime, mikroorganizmi za svoj metabolizam i razmnožavanje „zahtevaju“ prisustvo ovog bioelementa. Pošto kalprotektin ima veliki kapacitet za vezivanje cinka, mikroorganizmi ostaju lišeni ovog esencijalnog elementa, pa se tako sprečava njihov opstanak i dalje razmnožavanje.[30,31]

Prisustvo kalprotektina dokazano je i u pljuvački. Glavni izvori ovog proteina pljuvačke su gingivalna tečnost, mukozni transudat i gingivalni keratinociti. Ovaj protein je uključen u nespecifičnu antimikrobnu zaštitu oralne sredine, jer ispoljava antibakterijsko i antifungicidno dejstvo. Povećana koncentracija kalprotektina u pljuvački dokazana je u nekim oboljenjima oralne sredine, pa se zbog toga može smatrati validnim biohemijskim markerom ovih oboljenja. [32]

Pored direktnog antibakterijskog dejstva kalprotektina, istraživanja su pokazala da ovaj odbrambeni protein ima ulogu i u zaštiti oralne mukoze od kolonizacije bakterijama. Naime, u *"in vitro"* uslovima je dokazano da ovaj multifunkcionalni protein smanjuje mogućnost vezivanja bakterija za epitelne ćelije oralne mukoze. [33]

Lizozim

Lizozim (*muramidaza ili N-acetilmuramid-glukanhidrolaza*) je po strukturi protein, a po funkciji enzim. Po svojim hemijskim osobinama, lizozim pripada baznim proteina (pozitivno je naelektrisan, katodne elektroforetske pokretljivosti).

Prisustvo lizozima je dokazano u mnogim ćelijama, kao što su: neutrofilni leukociti, retikuloendotelne ćelije, monociti/makrofagi, Kupferove ćelije jetre. Takođe je prisutan u telesnim tečnostima i sekretima, kao što su: suze, znoj, krvni serum, urin, mleko, želudačni sok, pljuvačka.

Lizozim ima značajnu ulogu u nespecifičnoj antimikrobnoj zaštiti oralne sredine. Bakteriolitičko delovanje lizozima zasniva se na sposobnosti ovog enzima da raskida β(1,4)-glikozidnu vezu između C1 N-acetilmuraminske kiseline (NAM) i C4 N-acetilglukozamina (NAG) u sastavu peptidoglikana ćelijskog zida bakterija. [34]

Pored bakteriolitičkog delovanja lizozima baziranog na hidrolizi peptidoglikanskih lanaca u ćelijskom zidu bakterija, u literaturi se pominje i njegovo ne-enzimsko antibakterijsko delovanje. S obzirom na katjonsku prirodu lizozima, pretpostavlja se da se ovaj protein vezuje za tajhoinsku i lipotajhoinsku kiselinu u ćelijskom zidu bakterija, a ovi polianjonski molekuli mogu direktno da aktiviraju bakterijske autolizine, koji dovode do lize ćelije. Drugi predloženi mehanizam je da lizozim utiče na oslobađanje dvovalentnih katjona (Ca^{2+}, Mg^{2+}) iz membrana bakterija, što dovodi do njene destabilizacije. [35]

teins, 27E10 antigen, and microphages inhibited protein MRP8/14, LIL and LIH protein, calgranulin A/B.

Calprotectin has antimicrobial effect, which is achieved by zinc bonding. Namely, microorganisms for their metabolism and breed “demand” the presence of this bioelement. Calprotectin has the great capacity for zinc bonding, and that is why microorganisms are deprived of this essential element, and their survival and further breed is prevented.[30,31]

The presence of calprotectin is proved in saliva. The main sources of this saliva protein are gum fluid, mucosa transudate and gum keratonicites. This protein is included in unspecific anti microbial protection of oral environment, because of its antibacterial and antifungal effect. The increased concentration of calprotectin in saliva is proved in some of oral the environment diseases, so it could be considered as a valid marker for those diseases.[32]

In addition to direct antibacterial effect of calprotectin, the researches showed that this defensive protein has a role in the protection of oral mucosa from bacteria colonisation. Namely, in *“in vitro”* conditions it is proved that this multi functional protein lessens the possibility of bacteria bonding for the epithelia cells of the oral mucosa.[33]

Lysozime

Lysozime (*muramidasis or N-acetilmuramid-glykanhydrolases*) is, according to its structure, protein, and according to its function, it is an enzyme. According to its chemical characteristics, lysozime belongs to the base proteins (it is positively electrified, cathode electrophoresis mobility).

The presence of lysozime is proved in number of cells, like: neutrophil leukocytes, reticuloendothelial cells, monocyte/macrophage, and Kupfer’s liver cells. It is also present in body fluids and secretes, like tears, sweat, blood serum, urine, milk, gastric juice, saliva.

Lysozime has an important role in unspecific antimicrobial protection of oral environment. Bacteriolytic effect of lysozime is based on its ability to break beta (1.4)-glycosidic bond between C1 N-acetyl muramic acid (NAM) and C4 N-acetylglykozamin(NAG) in the structure of peptidoglykans of bacteria cell walls.[34]

Besides barteriolitic effect of lysozime, based on hydrolysis of peptidoglykanic chains, in literature is mentioned its non-enzymatic antibacteria effect. According to cationic nature of lysozime, it is assumed that this protein is bonding with tihoinic and lipotihoinic acid in bacteria cell wall, and this polianionic molecules can directly activate bacteria autolisines, that leads to lisa of the cell. The second suggested mechanism is that lisosim influences deliberation of bi-valent cations (Ca^{2+}, Mg^{2+}) from bacteria membranes, that leads to their destabilisation. [35]

Pored antibakterijskog delovanja lizozima, dokazano je i njegovo antifungicidno dejstvo, posebno protiv gljivice *Candida albicans.* Mogući mehanizam delovanja je destabilizacija membrane ove uslovno patogene gljivice.

Lizozim pljuvačke predstavlja značajan faktor nespecifične antimikrobne zaštite oralne sredine. Nekoliko studija je pokazalo da je koncentracija lizozima pljuvačke u negativnoj korelaciji sa akumulacijom dentalnog plaka i pojavom gingivitisa u dece i mladih osoba. Druge studije su ukazale na povišen sadržaj lizozima u pljuvački obolelih od oralne kandidoze.

Pored toga što je značajan u odbrani oralne sredine od raznih bakterija i gljivica, lizozim inhibira adherenciju bakterija *Streptococcus mutans* i *Streptococcus sanguis* za stečenu zubnu peliklu, čime se umanjuje akumulacija dentalnog plaka. [36,37]

Besides antibacteria effects of lisosim, its antifungal effect is proved, especially against fungus *Candida albicans*. The possible mechanism of acting is destabilisation of membrane of this possibly pathogenic fungus.

Lysozime of saliva presents an important factor of unspecific antimicrobial protection of oral environment. A couple of studies showed that the concentration of saliva lysozime is in negative correlation with accumulation of dental plaque and an appearance of gingivitis at children and young. The other studies pointed out on increased content of lysozime in the saliva of the patients with oral candida.

In addition to its importance in defence of oral environment against bacteria and fungi, lysozime inhibits the adherence of bacteria *Streptococcus mutans* and *Streptococcus sanguis* for the acquired dental pelicle, which lessens accumulation of dental plaque.[36,37]

Oralna peroksidaza

Oralna peroksidaza je enzim pljuvačke koji se sastoji iz dva peroksidazna enzima: *salivarne peroksidaze* (80%) i *mijeloperoksidaze* (20%).

Salivarnu peroksidazu sekretuju glavne pljuvačne žlezde, uglavnom parotidna žlezda. To je enzim koji u svom aktivnom centru sadrži selen. Uloga peroksidaze je da katalizuje reakciju između H_2O_2 (produkt metabolizma oralnih bakterij) i jona tiocijanata. Proizvodi ove reakcije su hipotiocijanatna kiselina (HOSCN) i hipotiocijanati (OSCN-) koji blokiraju sulfhidrilne grupe bakterijskih enzima glikolize *heksokinazu, aldolazu i piruvat kinazu.* Ovaj enzim ispoljava antibakterijsko dejstvo protiv mnogih Gram-pozitivnih (Str. Mutans) i Gram-negativinih bakterija (F. nucleatum, P. gingivalis, Prevotela, A. actinomycetemcomitans). Pored uloge u nespecifičnoj antibakterijskoj zaštiti oralne sredine, ovaj enzim doprinosi i efikasnom uklanjaju H_2O_2 iz oralne sredine.

Mijeloperoksidaza je HEM-zavisan enzim koga sadrže leukociti (neutrofili i monociti). U prisustvu H_2O_2 formira se sa mijeloperoksidazom kompleks enzim-supstrat, koji ima sposobnost da oksidiše jodide i hloride, stvarajući toksične proizvode. S obzirom da je jon hlora veoma rasprostranjen u biološkim sistemima, njegovom oksidacijom ovim kompleksom dobija se hipohlorna kiselina (HOCl). Ova kiselina ima izražena oksidativna svojstva i pri tome se dobijaju proizvodi (aktivne forme kiseonika) koji imaju, ne samo baktericidna svojstva, nego učestvuju i u razgradnji neinfektivnih materija (toksini, inflamatorni medijatori). [38,39,40]

Antimikrobni peptidi pljuvačke kao nosioci nespecifične antimikrobne zaštite oralne sredine sinergistički deluju sa salivarnim imunoglobulinima u sprečavanju kolonizacije oralne mukoze patogenim mikroorganizmima i održavanju ravnoteže normalne oralne flore. Pored

Oral peroxidase

Oral peroxidase is saliva enzyme which consists of two peroxidase enzymes: *saliva peroxidase* (80%) and *mieloperoxidase*(20%) .

Saliva peroxidase is secreted from the main saliva glands, mostly parotid gland. It is an enzyme which, in its active centre contains selenium. The role of peroxidase is to catalyze reaction between H_2O_2 (product of oral bacteria metabolism) and tiociyant ions. The product of this reaction is hipotiocianat acid (HOSCN) and hipotiocianates (OSCN-) which blocade sulfurhydric groups of bacteria enzymes glykolises, *hexocinasis*, *aldolasis* and *pruvat cinasis*. This enzyme shows antibacterial effect against a number of Gram-positive (Streptococcus mutans), and Gram-negative bacteria (F. nucleatum,P. gingivalis, Prevoteles, Actinobacillus actinomycetem comitans). Besides the role of unspecific antimicrobial protection of oral environment, this enzyme also contributes effective elimination of H_2O_2 from oral environment.

Mieloperoxidase is HEM-dependent enzyme which is part of leukocytes (neutrophiles and monocites). In the presence of H_2O_2 with mieloperoxidase ,a complex enzyme-supstrat is formed, and it has the ability to oxidise iodides and chlorides, making toxic products. Because of the great diffusion of chlor ions in biological systems, its oxidation gives the hipochloric acid (HOCL). This acid has expressive oxidative capacity and during the reaction it makes products (active forms of oxygen), which have, not only bactericide capacity, but they also participate in demolishment of uninfective substances (toxins and inflammatory mediators).[38,39,40]

Antimicrobial peptides of saliva as carriers of the unspecific antimicrobial protection of oral environment act sinergically with saliva immunoglobulines in the prevention of oral mucosa colonisation with pathogen microorganisms and keep the balance of normal oral flora. In addition to that, they represent an important factors for

toga, predstavljaju značajne činioce zaštite opšteg zdravlja organizma jer sprečavaju transmisiju patogena u unutrašnju sredinu organizma. U poslednje vreme se vrše eksperimentalna i klinička istraživanja vezana za mogućnost upotrebe ovih peptida u prevenciji i terapiji oralnih infektivnih oboljenja, kao dopuna antibiotskoj terapiji.

the organism health protection, because they prevent the transmission of pathogens in the inner parts of organism. In the last couple of years there were experiment and clinical researches connected to the possibility of those peptide usage in prevention an therapy of oral infective disease as a supplement to antibiotic therapy.

Literatura / References

1. *Miles T S, Nauntofte B, Svensson P. Clinical Oral Physiology.* Quintessence Publishing Co.Ltd; Copenhagen, 2004, str. 17-51.
2. *Tenovuo J.* Antimicrobial function of human saliva – how important is it for oral health?. *Acta Odontol Scand*, 1998; 56:250-256.
3. *Tenovuo J.* Antimicrobial agents in saliva – protection for the whole body. *J Dent Res,* 2002; 81(12):807-809.
4. *Devine DA.* Antimicrobial peptides in defence of the oral and respiratory tracts. *Molecular Immunology*, 2003; 40:431-443.
5. *Edgerton M, Koshlukova SE, Lo TE, Chrzan BG, Straubinger RM, Raj PA.* Candidacidal activity of salivary histatins. *J Biol Chem*, 1998; 273(32):20438-20447.
6. *Tsai H, Bobek LA.* Human salivary histatins: promising antifungal therapeutic agents. *Crit Rev Oral Biol Med,* 1998; 9:480-497.
7. *Helmerhorst JE, Troxsler FR, Oppenheim FG.* The human salivary peptide histatin 5 exerts its antifungal activity through the formation of reactive oxygen species. *PNAS*, 2001; 98(25): 14637-14642.
8. *Dale BA, Krisanaprakornkit S.* Defensin antimicrobial peptides in the oral cavity. *J Oral Pathol Med*, 2001; 30:321-327.
9. *Abiko Y, Nishimura M, Kaku T.* Defensins in saliva and the salivary glands. *Med Electron Microsc,* 2003; 36: 247-252.
10. *Dunsche A, Acil Y, Siebert R, Harder J, Schroder JM, Jepsen S.* Expression profile of human defensins and antimicrobial proteins in oral tissues. *J Oral Pathol Med*, 2001; 30:154-158.
11. *Bissell J, Joly S, Johnson GK, Organ CC, Dawson D, McCray PB, Guthmiller JM.* Expression of β-defensins in gingival health and in periodontal disease. *J Oral Pathol Med*, 2004; 33:278-285.
12. *Abiko JY, Mitamura J, Nishimura M, Muramatsu T, Inoue T, Shimono M, Kaku T.* Pattern of expression of beta-defensins in oral squamous cell carcinoma. *Cancer Letters*, 1999; 143:37-43.
13. *Tanida T, Okamoto T, Okamoto A, Wang H, Hamada T, Ueta E, Osaki T.* Decreased excretion of antimicrobial proteins and peptides in saliva of patients with oral candidiasis. *J Oral Pathol Med*, 2003; 32:586-594.
14. *Ward PP, Conneely OM.* Lactoferrin: Role in iron homeostasis and host defence against microbial infection. *BioMetals*, 2004; 17:203-208.
15. *Farnaud S, Evans RW.* Lactoferrin – a multifunctional protein with antimicrobial properties. *Molecular Immunology*, 2003; 40: 395-405.
16. *Orsi N.* The antimicrobial activity of lactoferin: Current status and perspectives. *BioMetals*, 2004; 17:189-196.
17. *Strate BVA, Beljaars L, Molema G, Harnsen MC, Meier DKF.* Antiviral activities of lactoferrin. *Antiviral Research,* 2001; 52:225-239.
18. *Conneely OM.* Antiinflammatory activities of lactoferrin. Journal of the American College of Nutrition, 2001; 20(5): 389S-395S.
19. *Lupetti A, Annema A, Welling MM, Senesi S, Dissel JT, Nibbering PH.* Candidacidal activities of human lactoferrin peptides derived from the N-terminus. *Antimicrobial Agents and Chemotherapy*, 2000; 44: 3257-3263.
20. *Jentsch H, Sievert Y, Gocke R.* Lactoferrin and other markers from gingival crevicular fluid and saliva before and after periodontal treatment. *J Clin Periodontol*, 2004; 31:511-514.
21. *Groenink J, Wallgreen VE, Nazmi K, Bolscher JGM, Veerman ECI, van Winkelhoff AJ, Nieuww AV.* Salivary lactoferrin and low – Mr mucin MG2 in Actinobacillus Actinomicetemcomitans – associated periodontitis. *J Clin Periodontol,* 1999; 26: 269-275.
22. *Murakami M, Ohtake T, Dorschner RA, Gallo RL.* Cathelicidin antimicrobial peptides are expressed in salivary glands and saliva. *J Dent Res.* 2002; 81(12):845-850.
23. *Ramanathan B, Davis EG, Ross CR, Blecha F.* Cathelicidins: microbicidal activity, mechanisms of action, and roles in innate immunity. *Microbea and Infectio*n, 2002; 4:361-372.
24. *Tanaka D, Miyasaki KT, Lehrer RI.* Sensitivity of Actinobacillus Actinomicetemcomitans and Capnocytophaga spp. To the bacteridical action of LL-37: a cathelicidin found in human leukocytes and epithelium. *Oral Microbial Immunol,* 2000; 15:226-231.
25. *Guthmiller JM, Vargas KG, Srikantha R, Schomberg LL, Weistroffer PL, Mc Cray PB, Tack BF.* Susceptibilities of oral bacteria and yeast to mammalian cathelicidins. *Antimicrobial Agents and Chemotherapy*, 2001; 45:3216-3219.
26. *Slomiany BL, Murty VLN, Piotrowski J, Slomiany A.* Salivary mucins in oral mucosal defence. *Gen Pharmac*, 1996; 27:761-771.
27. *Baughan LW, Robertello FJ, Sarrett DC, Denny PA, Denny PC*. Salivary mucins as related to oral Streptococcus mutans in elderly people. *Oral Microbiol Immunol*, 2000; 15:10-14.
28. *Rayment SA, Liu B, Offner GD, Oppenheim FG, Troxler RF*. Immunoquantification of human salivary mucins MG1 and MG2 in stimulated whole saliva: factors influencing mucin levels. *J Dent res*, 2000; 79(10): 1765-1772.
29. *Liu B, Rayment SA, Soares RV, Oppenheim FG, Offner GD, Taylor PF, Troxler RF.* Interaction of human salivary mucin MG2, its recombinant N-terminal region and a sinthetic peptide with Actinobacillus actinomicetemcomitans. *J Periodont Res*, 2002; 37:416-424.
30. *Sohnle PG, Hunter MJ, Hahn B, Chazin WJ.* Zinc-reversible antimicrobial activity of recombinant calprotectin (migration inhibitory factor-related proteins 8 and 14). *J Infect Dis,* 2000; 182:1272-1275.

31. *Strižˇ I, Trebichavsky I*. Calprotectin – a playotropic molecule in acute and chronic inflammation. *Physiol Res*, 2004; 53:245-253.
32. *Kleinegger CL, Stoeckel DC, Kurago ZB*. A comparison of salivary calprotectin levels in subjects with and without oral candidiasis. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*, 2001; 92:62-67.
33. *Nisapakultorn K, Ross KF, Herzberg MC.* Calprotectin expression inhibits bacterial binding to mucosal epithelial cells. *Infection and Immunity*, 2001; 69:3692-3696.
34. *Masschalck B, Michiels CW.* Antimicrobial properies of lysozyme in relation to foodborne vegetative bacteria. *Crit Rev Microb*, 2003; 29(3):191-214.
35. *During K, Porsch P, Mahn A, Brinkmann O, Gieffers W.* The non-enzymatic microbicidal activity of lysozymes. *FEBS Letters*, 1999; 93-100.
36. *Yeh C, Dodds MWJ, Zuou P, Jonhson AD.* A population – base study of salivary lysozyme concentrations and candidal counts. *Arch Oral Biol*, 1997;42:25-31.
37. *Senpuku H, Kato H, Todoroki M, Hanada N, Nisizawa T.* Interaction of lysozyme with a surface protein antigen of Streptococcus mutans. *FEMS Microbiology Letters*, 1996; 139:195-201.
38. *Ihalin R, Loimaranta V, Tenovuo J.* Origin, structure and biological activities of peroxidases in human saliva. *Arch Biochem Biophys*, 2005; article in press.
39. *Nishioka T, Maki K, Kimura M, Takahama U.* Determination of salivary peroxidase activity in human mixed whole saliva. *Arch Oral Biol,* 2003; 48:397-400.
40. *Nagler RM, Klein I, Zarzhevsky N, Drigues N, Reznick ZA.* Characterization of the differentiated antioxidant profile of human saliva. *Free Radic Biol Med*, 2002; 32:268-277.

Autor odgovoran za korespondenciju

Asst dr Ivan Dožić
Ul. Dr Subotića br 1/IV
Mob. Tel. 064-1111237

Address for correspondence

Asst dr Ivan Dožić
Ul. Dr Subotića br 1/IV
Mob. Tel. 064-1111237