Archives of Oral Biology 82 (2017) 256–262

Contents lists available at ScienceDirect

Archives of Oral Biology

journal homepage: www.elsevier.com/locate/archoralbio

Effect of arginine on the growth and biofilm formation of oral bacteria

Xuelian Huang[a,b,c,1], Keke Zhang[b,d,1], Meng Deng[b,e], Robertus A.M. Exterkate[a], Chengcheng Liu[b], Xuedong Zhou[b], Lei Cheng[b], Jacob M. ten Cate[a,*]

[a] *Department of Preventive Dentistry, Academic Centre for Dentistry Amsterdam (ACTA), University of Amsterdam and Free University Amsterdam, Amsterdam, The Netherlands*
[b] *State Key Laboratory of Oral Diseases, West China College of Stomatology, Sichuan University, Chengdu, Sichuan, China*
[c] *Division of General Dentistry, Eastman Institute for Oral Health, University of Rochester, Rochester, NY, USA*
[d] *School and Hospital of Stomatology, Wenzhou Medical University, Wenzhou, Zhejiang, China*
[e] *Oral Biology Program, School of Dentistry, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA*

A R T I C L E I N F O

Keyword:
Dental caries
Alkali
Biofilms
Microbial viability
Exopolysaccharide
Streptococcus
gtf

A B S T R A C T

Background: Alkali production *via* arginine deiminase system (ADS) of oral bacteria plays a significant role in oral ecology, pH homeostasis and inhibition of dental caries. ADS activity in dental plaque varies greatly between individuals, which may profoundly affect their susceptibility to caries.
Objective: To investigate the effect of arginine on the growth and biofilm formation of oral bacteria.
Methods and results: Polymicrobial dental biofilms derived from saliva were formed in a high-throughput active attachment biofilm model and L-arginine (Arg) was shown to reduce the colony forming units (CFU) counts of such biofilms grown for various periods or biofilms derived from saliva of subjects with different caries status. Arg hardly disturbed bacterial growth of *Streptococcus mutans, Streptococcus sobrinus, Streptococcus sanguinis* and *Streptococcus gordonii* in BHI medium, but only inhibited biofilm formation of *S. mutans*. Scanning electron microscope (SEM) showed *S. mutans* biofilms harboured fewer cells grown with Arg than that without Arg, even in the initial 2 h and 8 h phase. Confocal laser scanning microscope (CLSM) images of poly-microbial dental and *S. mutans* biofilms revealed the biofilms grown with Arg had lower exopolysaccharide (EPS)/bacteria ratios than those without Arg ($P = 0.004$, 0.002, respectively). Arg could significantly reduce the production of water-insoluble EPS in *S. mutans* biofilms ($P < 0.001$); however, quantitative real-time PCR (qRT-PCR) did not show significantly influence in gene expression of gtfB, gtfC or gtfD ($P = 0.32$, 0.06, 0.44 respectively).
Conclusions: Arg could reduce the biomass of poly-microbial dental biofilms and *S. mutans* biofilms, which may be due to the impact of Arg on water-insoluble EPS. Considering the contribution to pH homeostasis in dental biofilms, Arg may serve as an important agent keeping oral biofilms healthy thus prevent dental caries.

1. Introduction

Dental caries, one of the most prevalent chronic diseases worldwide, results from an ecological imbalance in the physiological equilibrium between tooth minerals and oral microbial biofilms. Frequent intake of sugars results in the outgrowth of aciduric and acidogenic bacteria, which may start a vicious cycle, leading to increased acid formation, further outgrowth of aciduric species, and finally development of dental caries (Marsh, 2003; Takahashi & Nyvad, 2011; Ten Cate & Cummins, 2013). Numerous clinical and laboratory studies conducted in the past decades have demonstrated the anti-caries effect of fluoride, however, there are still some different opinions about the employment of fluoride. Fluoride is far from a complete cure. Fluoride acts on the hard tissues rather than on the oral bacteria and is often not able to cope with the resultant massive cariogenic challenge (Ten Cate & Cummins, 2013). Further, fluoride has the potential to cause fluorosis through overexposure. Nature provides many options/mechanisms to potentially interfere in this complex interplay, such as antimicrobial peptides in saliva, a wide range of plant products (Huang, Cheng et al., 2012; Huang, Liu, Li, Zhou, & ten Cate, 2012; Jeon, Rosalen, Falsetta, & Koo, 2011) possessing antimicrobial properties, and probiotic bacterial species (Huang et al., 2016). Evidence is now accumulating that alkali generation, particularly through ammonia production from L-arginine (Arg) and urea, plays a major role in pH homeostasis which may moderate initiation and progression of dental caries (Burne & Marquis, 2000; Margolis et al., 1988). Therefore, Kleinberg suggested it is the

* Corresponding author at: ACTA 13N-69, Gustav Mahlerlaan 3004, 1081 LA, Amsterdam, The Netherlands.
E-mail address: j.t.cate@acta.nl (J.M. ten Cate).
[1] Authors contributing equally as first authors.

http://dx.doi.org/10.1016/j.archoralbio.2017.06.026
Received 26 April 2017; Received in revised form 19 June 2017; Accepted 20 June 2017
0003-9969/ © 2017 Elsevier Ltd. All rights reserved.

proportions and numbers of acid-base-producing bacteria that are the core of dental caries activity (Kleinberg, 2002).

Arg in free form is secreted by salivary glands at concentrations averaging about 50 μmol/L. Arg is also abundant in salivary secretions as peptides and proteins from which Arg can be released by proteases and peptidases (Burne & Marquis, 2000). Arg in the oral cavity is catabolized primarily by the arginine deiminase system (ADS) to release ornithine, ammonia and CO_2, with concomitant production of ATP (Liu, Nascimento, & Burne, 2012). The ADS has important physiological functions: it contributes to pH homeostasis, protects cells from lethal acidification, and provides ATP for growth and maintenance (Burne & Marquis, 2000).

Clinical studies have revealed that caries-free (CF) individuals have significantly higher free Arg levels in saliva (Van Wuyckhuyse et al., 1995) and higher ADS activities in their plaque and saliva than caries-active (CA) individuals (Nascimento, Gordan, Garvan, Browngardt, & Burne, 2009). Furthermore, use of a fluoride-free toothpaste containing 1.5% Arg increased ADS activity in plaque of CA individuals and shifted their bacterial composition to a healthier composition (Nascimento et al., 2014). A series of clinical studies has generated convincing clinical evidence that toothpaste containing 1.5% Arg, an insoluble calcium compound, and fluoride had favorable effects on the de-/remineralization balance, as well as superior cavity prevention efficacy compared to toothpaste with fluoride alone (Ten Cate & Cummins, 2013). Several laboratory studies revealed the regulation mechanism of alkali formation from Arg and its potential relationship with dental caries (Burne et al., 2012; Huang, Exterkate, & ten Cate, 2012). Various bacteria in the oral cavity can express ADS, such as *Streptococcus sanguinis, Streptococcus gordonii, Streptococcus parasanguinis, Streptococcus intermedius, Streptococcus cristatus, Streptococcus australis,* certain *Lactobacillus* species and a few spirochetes (Burne & Marquis, 2000; Huang et al., 2016; Huang, Schulte, Burne, & Nascimento, 2015). Studies using laboratory strains of oral streptococci indicate that the expression of ADS genes is substrate inducible, sensitive to carbon catabolite repression (CCR), and requires low pH and anaerobic conditions for optimal expression (Dong, Chen, & Burne, 2004; Dong, Chen, Snyder, & Burne, 2002; Liu, Dong, Chen, & Burne, 2008).

Apart from the effect on pH homeostasis in biofilm, Arg can also influence the co-aggregation between bacteria and the formation and viability of biofilm (Bernier, Ha, Khan, Merritt, & O'Toole, 2011; Borriello, Richards, Ehrlich, & Stewart, 2006; Sato & Nakazawa, 2014; Zhu et al., 2007). The co-aggregation of *Prevotella oris* and *Porphyromonas gingivalis*, which may contribute to periodontitis, was found to be inhibited by Arg and the inhibition of coaggregation by Arg was also found between *P. gingivalis* and *P. intermedia/S. oralis/A. viscosus/T. denticola/T. medium* respectively (Sato & Nakazawa, 2014). Arg can enhance the killing by ciprofloxacin and tobramycin in mature biofilms of *Pseudomonas aeruginosa*, putatively by increasing the metabolic activity of bacteria in the anaerobic zones of mature biofilm (Borriello et al., 2006) and can promote biofilm formation *in vitro* and completely repressed the ability of *Pseudomonas aeruginosa* to swarm over semi-solid surfaces (Bernier et al., 2011). Arg can also affect biofilm growth of *Staphylococcus aureus* USA 300 (Zhu, Weiss, Otto, Fey, & Smeltzer, 2007)

In our series of studies on Arg metabolism in poly-microbial dental biofilms, interesting findings revealed that Arg could also affect the biomass of dental biofilms. Therefore, the present study focuses on those effects in detail and the underlying mechanism.

2. Material and methods

2.1. Bacterial strains, media, and growth conditions

Streptococcus mutans UA159, *Streptococcus gordonii* ATCC 35105, and *Streptococcus sanguinis* SK36 were maintained in brain heart infusion (BHI) agar plates, and *Streptococcus sobrinus* FR 24 was maintained in tryptic soy agar-defibrinated sheep blood (TSA-blood) plates. All the cultures and biofilms were grown in anaerobic jar (MART, Netherlands), except for growth curve assay. L-Arginine used in the study was purchased from Sigma. To measure cell growth in BHI medium, overnight cultures were diluted 1:50 into fresh BHI medium, after growing for 8 h, culture was further diluted 1:50 into fresh BHI medium with different concentration of Arg (0–1.6%) in 96-well plate and the growth was monitored by SpectraMax Plus384 (Molecular Devices, USA) at 37 °C. Clear seal film (Nunc, Thermo Scientific, USA) was used to seal the 96-well plate to minimize the growth-inhibitory effects of oxygen, and the optical density at 600 nm (OD_{600}) was measured every 15 min for 6 h. In mono-species biofilm study, overnight cultures in BHI were diluted 1:50 into fresh ½ BHI + 50 mM PIPES buffer + 0.2% sucrose (BHIPS) with different concentration of Arg (0–1.6%) in Transferable Solid Phases (TSP) 96-well microtiter plate and the TSP lids with 96 built-in pins was placed on the plates. After 8 h incubation, TSP lids were transferred to plates filled with fresh mediums. After another 16 h, the biomass of biofilms on each pin was measured using crystal violet staining and colony forming units (CFU) counts. In crystal violet staining assay, TSP lid was placed in to a 96-well microtiter plate filled with 0.01% crystal violet, incubated for 5 min and then washed by MilliQ water for 3 times. Then TSP lids were transferred into a 96-well microtiter plate filled with 0.2% NaDoc and incubated for 5 min while gently shaking on a labocat. The absorbance (608 nm) of NaDoc solution was measured.

2.2. Saliva collection

Parafilm-stimulated whole saliva was collected from a CF donor for different-biofilm-age study and from three CF and three CA subjects for different-donor study. Informed consent was obtained from all participating subjects under a protocol reviewed and approved by the Medical Ethical Committee of the VU University Medical Center Amsterdam. CF subjects have no evidence of present caries experience [decayed and missing (DMT) = 0] and CA subjects have at least one active, cavitated and unrestored caries lesions (DT ≥ 1, MFT ≥ 0). All of them had natural dentition and did not use antibiotics in the preceding three months. The donors did not brush teeth for 24 h and abstained from food and drink intake for 2 h prior to saliva donation. The saliva was diluted 2-fold with sterile 60% glycerol and was stored at −80 °C (Exterkate, Crielaard, & ten Cate, 2010; Huang, Exterkate et al., 2012)

2.3. Polymicrobial dental biofilms formation and CFU counting

Polymicrobial dental biofilms were formed in a high-throughput active attachment biofilm model as previously described in detail (Exterkate et al., 2010; Huang, Cheng et al., 2012; Huang, Exterkate et al., 2012). In brief, glass disks were attached to a custom-made lid in such a way that each disk fitted into one well of a polystyrene 24-well flat-bottomed microtiter plate. Each well was filled with 1.5 mL of McBain medium (McBain et al., 2005) supplemented with 0.2% (w/v) sucrose and buffered at pH 7.0 with 50 mM of PIPES with or without Arg. A saliva-glycerol stock was added (1:50 final dilution) as inoculum, and the biofilm model was incubated anaerobically at 37 °C for 24, 48, 72, or 96 h to form biofilms with different ages. In all experiments, the medium was refreshed after alternating periods of 8 and 16 h. The biofilms were harvested by sonication (Vibra cell, Sonics & Materials Inc, Newtown, Connecticut, USA) for 2 min with 1 s pulses and 1 s gap. The resulting bacterial suspension was serially diluted in cysteine peptone water (CPW, 0.5% Yeast Extract, 0.1% Peptone, 0.85% Sodiumchloride and 0.05% L-Cysteine-HCl) and plated on TSA-blood agar plates (4% Tryptic Soy Agar, 0.2% glucose, 1:20 defibrinated sheep blood, pH 7.2). CFU values were calculated after the plates were incubated anaerobically at 37 °C for 72 h.

Download English Version:

https://daneshyari.com/en/article/5637895

Download Persian Version:

https://daneshyari.com/article/5637895

Daneshyari.com