Forum Minireview

Roles of Oral Bacteria in Cardiovascular Diseases — From Molecular Mechanisms to Clinical Cases:
Treatment of Periodontal Disease Regarded as Biofilm Infection:
Systemic Administration of Azithromycin

Pao-Li Wang1,*
1Department of Dental Education and Development, Osaka Dental University,
8-1, Kuzuha Hanazono, Hirakata, Osaka 573-1121, Japan

Received November 25, 2009; Accepted January 14, 2010

Abstract. Periodontal disease as a biofilm infectious disease is considered. Periodontal disease–
associated bacteria formed biofilm in periodontal pockets or on the surface of cementum. Planktonic bacteria
from biofilm invade into periodontal tissues and lead to inflammation and destruction
of tissues directly and indirectly by eliciting the host defense mechanism. Supragingival dental
plaques (biofilm) are easily removed by professional mechanical tooth cleaning, while subgingival
dental plaques and bacteria invading into periodontal tissues are difficult to remove. Therefore, the
development of a method for periodontal disease based on the concept that regards periodontal
disease as a biofilm infectious disease is needed. Hereby, I report the effect of antibiotics on an in
vitro biofilm model of periodontal disease and the systemic administration of azithromycin for
early-onset (aggressive) periodontitis like a treatment resistant periodontitis.

Keywords: azithromycin, aggressive periodontitis, biofilm, systemic administration, oral bacteria

1. Expanding studies of biofilm

A meeting held in 1996 at Hotel Hyatt in Monterey, a
small city on the seashore down south of San Francisco,
have become an epoch-making event in the dental field.
The reason is that this was the first international meeting
taking up “biofilm” as the theme. The main symposium
was Dr. Costerton, University of Iowa, USA (1). On the
day of the meeting I foresaw that the era of biofilm would
present itself in the 21st century. Just before it, Dr. Cos
terton published a review article on biofilm in Science, in
which he introduced dental caries and periodontal disease
regarding it as biofilm infection (Table 1) (2). It may
safely be said that the study of biofilm expanded world
wide with this paper as the turning point. Now in our
country the concept that dental caries and periodontal
disease are biofilm infections seems to have been estab-
lished certainly among dental colleges and even in com-
mercial messages in TV programs.

2. Periodontal disease as a biofilm infection

In the periodontal pocket of periodontal disease as a
biofilm infection, there is a condition in which periodontopathic bacteria form a film-like colony by adherence
and aggregation using cementum as a foothold (Fig. 1)
(3). In the periodontal pocket, there are a few hundred
kinds of bacteria, which seem to amount to more than
several hundred millions in number. The situation is as if
it was the inside of a germ culture flask. Within a biofilm
the numbers of bacteria are stratified three dimensionally,
and metabolic products of bacteria, oxygen partial pres-
sure, pH, and nutrients are unevenly distributed (Fig. 2)
(4). In other words, the biofilm offers an environment
inhospitable for bacteria. Nonetheless, bacteria form
biofilms to protect themselves from attacks by immuno-
cytes and antibacterial agents in the external environ-
ment. The biofilm plays a role as a protective barrier for
bacteria.

In the case of periodontal disease, a biofilm is formed
by periodontopathic bacteria in the periodontal pocket
(Fig. 3). Bacteria released from the biofilm float in the
pocket and then attach to and invade into gingival epithe-
Table 1. Biofilm infectious diseases

<table>
<thead>
<tr>
<th>Infection or disease</th>
<th>Common biofilm bacterial species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dental caries</td>
<td>Acidogenic Gram-positive cocci (e.g., <em>Streptococcus</em>)</td>
</tr>
<tr>
<td>Periodontitis</td>
<td>Gram-negative anaerobic oral bacteria</td>
</tr>
<tr>
<td>Otitis media</td>
<td>Nontypable strains of <em>Haemophilus influenzae</em></td>
</tr>
<tr>
<td>Musculoskeletal infections</td>
<td>Gram-positive cocci (e.g., staphylococci)</td>
</tr>
<tr>
<td>Necrotizing fasciitis</td>
<td>Group A streptococci</td>
</tr>
<tr>
<td>Biliary tract infection</td>
<td>Enteric bacteria (e.g., <em>Escherichia coli</em>)</td>
</tr>
<tr>
<td>Osteomyelitis</td>
<td>Various bacterial and fungal species – often mixed</td>
</tr>
<tr>
<td>Bacterial prostatitis</td>
<td><em>E. coli</em> and other Gram-negative bacteria</td>
</tr>
<tr>
<td>Native valve endocarditis</td>
<td>Viridans group streptococci</td>
</tr>
<tr>
<td>Cystic fibrosis pneumonia</td>
<td><em>P. aeruginosa</em> and <em>Burkholderia cepacia</em></td>
</tr>
<tr>
<td>Melioidosis</td>
<td><em>Pseudomonas pseudomallei</em></td>
</tr>
<tr>
<td>Nosocomial infections</td>
<td></td>
</tr>
<tr>
<td>ICU pneumonia</td>
<td>Gram-negative rods</td>
</tr>
<tr>
<td>Sutures</td>
<td><em>Staphylococcus epidermidis</em> and <em>S. aureus</em></td>
</tr>
<tr>
<td>Exit sites</td>
<td><em>S. epidermidis</em> and <em>S. aureus</em></td>
</tr>
<tr>
<td>Arteriovenous shunts</td>
<td><em>S. epidermidis</em> and <em>S. aureus</em></td>
</tr>
<tr>
<td>Schleral buckles</td>
<td>Gram-positive cocci</td>
</tr>
<tr>
<td>Contact lens</td>
<td><em>P. aeruginosa</em> and Gram-positive cocci</td>
</tr>
<tr>
<td>Urinary catheter cystitis</td>
<td><em>E. coli</em> and other Gram-negative rods</td>
</tr>
<tr>
<td>Peritoneal dialysis peritonitis</td>
<td>A variety of bacteria and fungi</td>
</tr>
<tr>
<td>IUDs</td>
<td><em>Actinomyces israelii</em> and many others</td>
</tr>
<tr>
<td>Endotracheal tubes</td>
<td>A variety of bacteria and fungi</td>
</tr>
<tr>
<td>Hickman catheters</td>
<td><em>S. epidermidis</em> and <em>C. albicans</em></td>
</tr>
<tr>
<td>Central venous catheters</td>
<td><em>S. epidermidis</em> and others</td>
</tr>
<tr>
<td>Mechanical heart valves</td>
<td><em>S. aureus</em> and <em>S. epidermidis</em></td>
</tr>
<tr>
<td>Vascular grafts</td>
<td>Gram-positive cocci</td>
</tr>
<tr>
<td>Biliary stent blockage</td>
<td>A variety of enteric bacteria and fungi</td>
</tr>
<tr>
<td>Orthopedic devices</td>
<td><em>S. aureus</em> and <em>S. epidermidis</em></td>
</tr>
<tr>
<td>Penile prostheses</td>
<td><em>S. aureus</em> and <em>S. epidermidis</em></td>
</tr>
</tbody>
</table>

3. Thinking over therapy of biofilm

When a biofilm is formed in the periodontal pocket, effects of mechanical removal and antibiotics are attenuated. The sustained local drug delivery system (LDDS) to inject a tetracycline agent commonly used clinically (minocycline hydrochloride ointment for dental use) into the periodontal pocket is considered to have antibacterial effects. Metabolites, oxygen pressure, pH, nutrients, and saliva play crucial roles in maintaining the biofilm's internal environment. Fig. 1 illustrates the internal environment of biofilm and defense mechanisms.
potency particularly to floating bacteria released from the biofilm. By means of this method, the sustained-release of antibacterial agents acts for a long time in high concentrations. However, tetracycline antibiotics do not invade into the biofilm and cannot destroy it (6).

On the other hand, macrolide agents exert no antibacterial action to *Pseudomonas aeruginosa*, for example, but have a wide-spectrum antibacterial activity, showing such a pharmacological effect as inhibition of biofilm formation and bacterial adherence. In addition, glycocalyx, a constituent of biofilm derived from *Pseudomonas aeruginosa*, is decomposed by macrolide antibiotics.

**Fig. 2.** Oral biofilm. Modified from Ref. 2.

**Fig. 3.** Caries and periodontal disease as biofilm infectious diseases.

**Fig. 4.** Chemical structure of antibiotics.
Treatment of Periodontal Disease

This effect has been suggested to be attributed to their high permeability into the biofilm and the inhibition of polysaccharide production due to inhibition of GMD (guanosine diphosphomannose dehydrogenase) in the glycocalyx-producing system within bacteria (7). On the basis of molecular structure, macrolide antibiotics are broadly classified into 14-, 15-, and 16-membered ring groups according to the number of atoms forming the lactone ring (Fig. 4). Fourteen- and fourteen-membered ring macrolides are used as the basic agents in continuous therapy during the remission period. In the exacerbation period, on the contrary, a temporary combined use of antibacterial agents effective on respective pathogenic bacteria is considered to be a useful therapy at present. In fact, in diffuse panbronchiolitis caused by biofilm-forming bacteria, macrolide agents with a 14-membered ring, clarithromycin, and a 15-membered ring, azithromycin, dissolve the biofilm, and they have already been established as an effective therapy (8).

Since these macrolide agents are capable of dissolving the biofilm, we assumed that they would be effective in treatments of periodontal disease as a biofilm infection (Fig. 5).

4. Development of in vitro model of biofilm (9)

In the basic experiment, first of all, we sought drugs capable of dissolving the biofilm. In the oral cavity, when glycoproteins derived from saliva and exudates in the gingival sulcus are absorbed to the surface of the teeth and the pellicle (acquired pellicle) is formed, various kinds of bacteria adhere to the pellicle. At present, the bacteria binding to the surface of teeth have been identified. Accordingly, we cultivated Streptococcus gordonii, which is involved in initial adherence to the dental surface, under anaerobic condition on a hydroxyapatite disk processed with saliva. In addition, we cocultivated a representative periodontopathic bacterium, Porphyromonas gingivalis, and thus developed an in vitro model of biofilm (Fig. 6). In this model we studied the effects of the tetracycline antibiotic minocycline and the macrolide antibiotics azithromycin, clarithromycin, and josamycin by observing morphological changes in the biofilm with a scanning electron microscope. Our results confirmed that erythromycin and azithromycin have potency to dissolve the biofilm (Fig. 7).

5. Clinical study of combined application of azithromycin in early onset (aggressive) periodontitis (10)

Early onset (aggressive) periodontitis develops before 35 years of age. Because of rapid destruction of the periodontal tissue, it is a morbid condition with a possibility of early loss of teeth. Accordingly, the progress of the periodontal disease must be inhibited as early as pos-
sible. At present, tetracycline, amoxicillin, and metronidazole are administered as a combined drug application therapy, but the reports on the effects of macrolide antibiotics are restricted.

The macrolide antibiotic azithromycin exerts antibacterial action to a wide variety of intraoral bacteria. Furthermore, it was reported that azithromycin was taken up into phagocytes and was released into the inflamed tissue for a long period of time. Accordingly, we performed a clinical study to investigate the effects of azithromycin as an adjunctive therapy in non-operative treatments of early onset (aggressive) periodontitis. Figure 8 illustrates the sequence of treatments using azithromycin. A case report is shown below.

6. Case report (Fig. 9)

Patient: 32 years of age, female (First examination: March, 2002).

Chief complaints: Feeling that her teeth are loosened and separation of the maxillary anterior teeth.

Past history: A few years ago the maxillary anterior teeth began to separate from one another. Since the lacunae gradually became enlarged, she visited an orthodontist and was referred to a specialist of periodontal disease. No particular systemic diseases were noticed in the past history.

Clinical diagnosis: Early onset (aggressive) periodontitis (diffuse type).

Therapeutic strategy: i) Thorough practice of basic treatments of periodontal disease and professional mechanical tooth cleaning (PMTC), ii) administration of antibiotics (azithromycin), and iii) orthodontic treatments.

Course of treatments:

1) First examination: Separation (flare-out) was noticed among the maxillary anterior teeth, and swelling and reddening were found in the gingiva. Periodontal pocket (mean PD): 3.9 mm. bleeding on probing (POB): 78%. Proportion of pockets deeper than 4 mm (pocket rate, PoR): 55.4%.

2) Periodontal treatments: Thorough mouth cleaning and completion of basic periodontal treatments by scaling and root planing. After confirming that the gingival index (GI) was improved to less than 1 and that mouth cleaning was daily carried on, we combined administration of an antibiotic (azithromycin, 500 mg/day for 3 days) during the basic periodontal treatment period. In this case we administered it once 5 months after the first examination. PMTC was carried out every 2 weeks along with an approach under infiltration anesthesia (root planing) as the countermeasure for the vertical bone defect.

3) Reevaluation: The periodontal tissue was improved so much that the condition of stable periodontal tissue endurable for dynamic migration of teeth was prepared for orthodontic treatments in response to her chief complaint. PD: 2.8 mm. BOP: 29%. PoR: 22.6%.

4) Orthodontic treatments: Since there was a possibility for orthodontic appliances to facilitate adherence of biofilm during orthodontic treatments, we tried to stabilize the periodontal tissue by PMTC (orthodontic treatments were performed by Dr. Ikue Watanabe, Omni-
dentistry, Sapporo).

5) Maintenance period: By orthodontic treatments, remission of her chief complaints was gained and stable periodontal tissue was established. Thus, treatments were successfully completed with the patient’s high satisfaction. PD: 2.0 mm, BOP: 8%, PoR: 3%.

7. Results and discussion

We studied the effects of azithromycin as an adjunctive therapy of non-operative treatments of early onset (aggressive) periodontitis. After 2 – 3 months of guidance for oral cleaning, scaling, and root planning as basic treatments, azithromycin was administered (500 mg/day for 3 days). PMTC was made at the pace of once every 2 weeks during the period of treatments of periodontal disease. When GI reached less than 1, we judged that the initial treatments were completed. Clinical parameters PD, BOP, and PoR were improved (Fig. 10). The period of treatments of the azithromycin-administered group (5 cases: 1 male and 4 female subjects, 29.6 years of age on the average) was 4.7 ± 3.2 months, while in the group who received similar basic treatments but did not receive azithromycin, the period of treatments (6 cases, 2 male and 4 female subjects, 26.5 years of age on the average) was 11.2 ± 4.9 months (Table 2). Thus, in the azithromycin-administered group a remarkably improving effect was revealed for the treatment period.

The best feature of azithromycin is its excellent entry into the tissue, especially into the inflamed tissue. Furthermore, since its half-time in the serum and tissue is as long as 60 – 80 h, satisfactory clinical effects can be obtained with its administration once a day for 3 days. According to the basic theory of pharmacodynamics, there are two possibilities in the route of action of antibiotics administered systemically: either they enter the tissue by infiltrating from the capillaries in the periodontal tissue and affect the periodontopathic bacteria or they destroy the biofilm by the effects of the agents exuding into the periodontal pocket (Fig. 11). In future, more novel therapies will be developed by regarding periodontal disease as a biofilm infection.

8. Conclusion: drugs are not the first choice for periodontal treatments

Control of biofilm is a universal treatment as the basis of dental care in general. It consists of active removal and prevention of biofilm by dental professionals with specialized tools on the basis of practice of daily oral cleaning by the patients themselves. It is only for such restricted cases as early onset (aggressive) periodontitis

<table>
<thead>
<tr>
<th>Duration to the end of initial preparation (months)</th>
<th>Group with azithromycin</th>
<th>Group without azithromycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group with azithromycin</td>
<td>4.7 ± 3.2</td>
<td>11.2 ± 4.9</td>
</tr>
<tr>
<td>Group without azithromycin</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The duration of the initial preparation was significantly shortened when combined with administration of azithromycin. Modified from Ref. 9.

Fig. 10. Administration of azithromycin to aggressive periodontitis. Modified from Ref. 9.

Fig. 11. The possible mechanism of biofilm destruction by systemic administration of azithromycin.
that we have established the therapy of systemic administration of antibiotics like a treatment resistant periodontitis. In contrast, there are several reports that azithromycin was used for the treatment of chronic periodontitis. However, regimens of azithromycin administration were different among these reports (Table 3) (10 – 15). There-

<table>
<thead>
<tr>
<th>Authors / Journal, year (reference No.)</th>
<th>Country / sample size / age</th>
<th>Study style / duration</th>
<th>Intervention (method, frequency, duration)</th>
<th>AZM administration regimen</th>
<th>Outcomes</th>
<th>Side effects</th>
<th>Statistical analysis</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fujii et al. / Perio, 2004 (10)</td>
<td>Japan / AZM = 5, Control = 6 / 16 – 34 years</td>
<td>Case-Control study to end initial preparation</td>
<td>plaque control, SRP, 2 months PMTC (each 2 months)</td>
<td>AZM (500 mg × 3 days) after SRP Control: SRP alone</td>
<td>PPD, BOP</td>
<td>n.a.</td>
<td>unpaired t-test</td>
<td>Shortened duration of initial preparation in AZM combination compared to SRP (aggressive periodontitis)</td>
</tr>
<tr>
<td>Smith et al. / J Clin Periodontol, 2002 (11)</td>
<td>England / AZM = 23, Control = 21 / ave. 42.7 years</td>
<td>RCT / 22 weeks</td>
<td>SRP, 3 times, 2 weeks (at 0, 1, 2)</td>
<td>AZM (500 mg × 3 days) after SRP Control: SRP alone</td>
<td>OHI, PPD, BOP</td>
<td>n.a.</td>
<td>ANCOVA</td>
<td>Low PPD in AZM combination compared to SRP alone</td>
</tr>
<tr>
<td>Mascarenhas et al. / J Periodontol, 2005 (12)</td>
<td>USA / AZM = 15, Control = 15 / 45.3 – 47 years</td>
<td>Case-Control study / 6 months</td>
<td>SRP, twice, &lt; 2 weeks</td>
<td>AZM (initial: 500 mg, next 250 mg × 5 days) after SRP Control: SRP alone</td>
<td>PPD, CAL, BOP</td>
<td>n.a.</td>
<td></td>
<td>1) low PPD, and 2) gain of clinical attachment level in AZM combination compared to SRP alone</td>
</tr>
<tr>
<td>Haffajee et al. / J Clin Periodontol, 2007 (13)</td>
<td>USA / AZM = 25, MET = 24, SDD = 20, Control = 23 / 43 – 47 years</td>
<td>Case-Control study / 12 months</td>
<td>SRP, 4 times, 4 weeks</td>
<td>AZM (500 mg × 3 days), MET (250 mg × 14 days), SDD (20 mg × 3 months) after SRP Control: SRP alone</td>
<td>PPD, CAL, BOP</td>
<td>AZM 2 MET 1 SDD 2</td>
<td>ANCOVA</td>
<td>1) low PPD, and 2) gain of clinical attachment level in AZM or MET combination compared to SRP alone</td>
</tr>
<tr>
<td>Gomi et al. / J Periodontol, 2007 (14)</td>
<td>Japan / AZM = 17, Control = 17 / ave. 48.2 years</td>
<td>Case-Control study / 25 weeks</td>
<td>AZM: FMD, once Control: SRP, 4 – 6 times, interval &lt; 7 days</td>
<td>AZM (500 mg × 3 days, before FMD) Control: SRP alone</td>
<td>PPD, CAL, BOP</td>
<td>AZM 1 (diarrhea)</td>
<td>unpaired t-test</td>
<td>1) low PPD, and 2) improvement of clinical attachment level in FMD+AZM combination compared to SRP alone</td>
</tr>
<tr>
<td>Haas et al. / J Clin Periodontol, 2008 (15)</td>
<td>Brazil / AZM = 12, Control = 12 / 13 – 26 years</td>
<td>RCT / 12 months</td>
<td>plaque control, 2 weeks SRP, 2 weeks</td>
<td>AZM (500 mg × 3 days) at SRP start Control: SRP alone</td>
<td>PPD, CAL, BOP</td>
<td>AZM 1 (headache)</td>
<td>ANCOVA</td>
<td>improvement of clinical attachment level in AZM combination compared to SRP alone</td>
</tr>
</tbody>
</table>

fore, the establishment of guidelines for azithromycin administration is needed. The key to successful treatments is the basic treatment of periodontal disease in collaboration with patients, however. Therefore, the first choice of periodontal treatments is not administration of drugs. Even if we combine drugs, we cannot inhibit the progress of periodontal disease if we do not succeed in basic treatments of periodontal disease.

The effect of drugs is always inextricably linked with their side effects. Recently, as seen in “consciousness disorder after taking the antibiotic telithromycin”, “convulsion caused by combined use of new quinolone antibiotics and non-steroid antibiotics”, and in addition, the problem of “MRSA”, there are some antibiotics that are no longer effective due to prevailing resistant bacteria.

In conclusion, at present, we consider that the combination of antibiotics is recommended in chronic (but not acute) periodontitis only when initial preparation is fully performed but inflammation remains.

References

5. Wang PL, Ohura K. Porphyromonas gingivalis lipopolysaccharide signaling in gingival fibroblasts-CD14 and Toll-like recep-