Microbiological Characteristics of *Veillonella spp.* in the Composition of Endodontic Infections

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Abstract: ***A number of studies have confirmed that necrotic dental pulp does not cause periapical inflammation in the absence of microbial infection. The lack of blood circulation (and hence natural protection against invasion and colonization by oral microorganisms) leads to the infection of the microchannel system of the root canal dentin affecting the apical cement, the periodontal membrane and the lamina corticalis of the dental alveolus. Epidemiological studies indicate that up to 400 different microbial strains can be found in infected root canals, alone or more often in combinations. The results obtained from various studies have linked the presence of certain microorganisms with particular symptoms and nosological units, but there is still no definitive evidence for specificity of the bacterial species in different endodontic diseases. About 40 microbial strains are most commonly isolated from infected root canals and associated with apical periodontitis, with 10-12 of them being predominantly anaerobic species (Bacteroides, Prevotella, Porphyromonas, Fusobacterium, Veillonella). In this review, we will attempt to examine in detail the microbiological characteristics of anaerobic Gram-negative, non-spore forming bacteria of the genus Veillonella isolated in endodontic studies.***

Keywords: non-spore-forming bacteria, anaerobic infections, gram-negative bacteria, endodontic infections, periodontitis, Veillonella

1. Introduction

A large body of research has proved that periapical pathology cannot develop without bacterial contamination; the greater the amount of infectants, the more intense the inflammatory process (6). Primary endodontic infections are characterized by a mixed bacterial consortium composed of 10 to 30 bacterial species and 10^3 to 10^8 bacterial cells per root canal (21, 23, 24, 26).Obligate anaerobes (*Bacteroides, Prevotella, Porphyromonas, Fusobacterium, Bilophila, Desulfomonas, Mitsuokella, Tissierella)*predominate in primary endodontic infection.Gram-negative bacteria are some of the most common microorganisms in these infections (25).

Microbiological characteristics of *Veillonella*

The members of the genus ***Veillonella*** are Gram-negative anaerobic cocci and are normal inhabitants of the mucous membranes of the oral cavity, the oropharynx, the respiratory tract and the intestinal tract.They represent less than 1% of all the anaerobes isolated in clinical specimens (1, 2, 18).

V. parvula comprises 5 – 10% of the salivary microbiota. It has been foundon the tongue and in the composition of the dental plaque. In smaller quantities it has also been found on the mucous membrane of the other parts of the oral cavity. Veillonella is also present in endodontic infections.Some researchers have isolated *V. parvula* in 20.6% of the cases of primary endodontic infections. It is followed by *Porphyromonas gingivalis* (14.1%) (20).

Other authors have found a greater presence of*Firmicutes* and *Fusobacteria* in teeth with failed primary endodontic treatment and symptomatic patients than in asymptomatic patients. According to their study, *Proteobacteria* and *Actinobacteria* are the predominant species in patients without pain (10).

The genus *Veillonella* belongs to the family *Veillonellaceae* and includes seven species, some of which are*V. parvula*, *V. alcalescens*, *V. atypic*, *V. dispar* (9, 27). Bacteria of this genus are globular in shape and are arranged in twos (diplococci) or in short chains. They are non-motile and **non**-spore-forming. Their size is 0.3 – 2.5 μm on average. The optimum temperature (t°) for their development is between 30°C and 37°C, but there are exceptions to this finding. They develop at a pH of 6.5 – 8.0 and are very fastidious about their food environments. They grow and reproduce well in media containing lactate, pyruvate, malate and others. On solid media, they form smooth colonies, measuring 1.0 – 3.0 mm. They break down carbohydrates and polyvalent alcohols. They form hydrogen peroxide (H_2O_2), do not dilute gelatin, do not participate in hemolysis and do not form indole. Antigenically, by an agglutination reaction, they are divided into two types: *V. parvula* and*V. alcalescens*, which are subdivided into eight serological groups. The species *V. parvula* does not form peroxidase and includes serological groups IV and VI. The microbes of serological groups IV, V and VI are exclusively isolated from the human body (1, 2, 7).

V. alcalescens breaks down hydrogen peroxide, without containing the catalase enzyme. Antigenically, it comprises groups I, III, IV and VII, with group IV and group VII microorganisms being isolated mainly from the human body (1, 2).

The role of endotoxins in endodontic infections

Pathogenic microorganisms are capable of adhesion, colonization, and survival by overcoming the body's defense mechanisms. They can cause tissue dysfunction through a direct or indirect impact. Their direct impact is achieved by enzymes, endotoxins and metabolites; their indirect impact is achieved by lipopolysaccharides (LPS), peptidoglycans (PG), lipoteichoic acid, fibers, capsule components, etc.

The Veillonella species contain a specific lipopolysaccharide endotoxin (LPS) with pronounced antigenic and pyrogenic properties when released by the microorganisms. Lipopolysaccharides (LPS) belong to the virulent factors of bacteria and are located on the outer membrane of most Gram-negative microorganisms. A bacterial cell can contain approximately $3.5.10^6$ LPS on an area of 4.9 μm^2 (5). Endotoxins are associated with pulpal pain, periapical inflammation and periapical destruction.This association has been confirmed by various studies (22).

LPS may cause:

- Activation of macrophages, monocytes with a subsequent biosynthesis and release of cytokines, prostaglandins;
- These substances are chemical mediators of inflammation and most of them can stimulate bone resorption;
- They can increase vascular permeability;
- They can accelerate the production of bradykinin – an important chemical mediator of inflammation;

The concentration of *LPS* in infected root canals, such as they are in ***acute apical periodontitis,*** increases proportionally to the increase in the number of gram-negative microorganisms. Siqueira's studies, 2001 (22) indicate that the content of endotoxins or LPS in infected root canals is higher in teeth with symptomatic apical periodontitis, teeth with periradicular bone destruction or teeth with persistent exudation (5, 22).

Not all Gram-negative microorganisms produce LPS. The direct pathogenic mechanism involves secretory products of the microorganisms, including enzymes such as proteinase, hyaluronidase, chondroitin sulfatase, acid phosphatase, and metabolic products. Bacterial components stimulate the cells of the body to release chemical mediators of inflammation and pain (5).

Anaerobes function primarily by stabilizing the resident bacterial flora, preventing the colonization by pathogens from exogenous sources, assisting digestion of food by enzymes, and stimulating host immunity. These normal protective microorganisms are involved in causing disease only when they are moved from their endogenous ecological niches to normally sterile tissues or fluids, through injury or disease of the macroorganism (18). *V. parvula* becomes involved in endodontic diseases as part of a secondary infection; it rarely penetrates healthy tissues as a primary causative agent (1, 2, 18).

Handling endodontic infection by medications

In endodontic treatment, the first step to reduce root canal infection and inflammation in periradicular tissues is the removal of the canal contents and the mechanical and chemical treatment of root canal wall dentin. There is enough evidence from clinical practice and the literature that the removal of the infected canal contents, whentimely and properly performed, alleviates the patient's condition. In order to achieve a greater reduction of the microbial count during the mechanical root canal treatment, the use of antiseptic solutions – hypochlorite, chlorhexidine, oxygen water – is of particular importance.(3, 4, 5, 16). Calcium hydroxide is a medication recommended for the treatment of apical periodontitis. Its antimicrobial mechanism of action is influenced by the rate of the chemical dissociation into calcium and hydroxyl ions. As a result, a high pH of the medium is achieved, which in turn inhibits the enzyme activity, which is of particular importance for the metabolism and growth of the bacterial cell (11, 15, 17). Various studies have reported its improved antimicrobial activity when combined with other medications, e.g. paramonochlorophenol, chlorhexidine and the like (12, 13).

The literature data on the use of antibiotics in endodontics is contradictory. Prophylactic endogenous use of antibiotics in endodontics is recommended in patients at risk of infective endocarditis (8, 25). Systemic administration of antibiotics in endodontics is limited in patients who have local symptoms of infection, physical indisposition, and/or elevated body temperature (5, 25). In one of their studies, Ehrmann, E.H. et al. (2003) have reported topical application of a Ledermix paste (containing tetracycline) in root canals in cases where other intracanal medications have failed (14).

Veilonella die at 60°C for 30 min. They are sensitive to antibiotics belonging to the group of ***protein synthesis inhibitors*** – chloramphenicol, chlortetracycline, oxytetracycline, and are resistant to streptomycin (streptomycin: the oldest aminoglycoside synthesized in 1944) and penicillins (β-lactam antibiotics or ***cell wall synthesis inhibitors***).

Tetracyclines inhibit protein synthesis by altering transfer RNA upon its entry, but this is not such a selective action. The selective action is based on the fact that the uptake of the medication by bacterial cells is much greater than the uptake by human cells (7, 18). Disturbance of the normal microflora of the intestines has been reported as one of the side effects of tetracyclines; they also lead to a brown recoloration of tooth structures and inhibit bone growth. Being hepatotoxic and teratogenic, they are not indicated for children under 8 years and pregnant women (7, 18).

Chloramphenicol blocks peptidyl transferase and protein synthesis. Its action is selective. There is a certain inhibitory function on mitochondria in humans, which may in part be a response to the dose-dependent toxicity to bone marrow (7, 18).

2. Conclusion

Knowing endodontic bacterial pathogens in details, and the interactions between them also, is of particular importance for the choice and application of the most effective therapy possible.

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