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Microbiology of endodontic infections and antibacterial chemical agents

Tsvetelina Borisova-Papancheva, Ana-Maria Miteva

Department of Conservative Dentistry and Oral Pathology,

Faculty of Dental Medicine, Medical University of Varna

Abstract

Endodontic therapy's substantial purpose is to create a biologically appropriate environment within the root canal system that is conducive for the healing and maintenance of periradicular tissue health. Bacteria are one of the main factors causing disorders in the pulpal tissues, as they invade the endodontic space through variety of routes, including carious lesions, traumatic pulp exposure, using blood stream or by adjacent tissues. Bacteria found in the pathogenesis of endodontic diseases vary from facultative anaerobes to aerobes to the most resilient species able to survive adverse conditions in the surrounding environment. By using irrigation, mechanical action, and the antibacterial properties of the irrigating solutions, bacteria eradication and postendodontic treatment success are assured.

Keywords: bacteria, microbiota, infection, root canal, disinfection, irrigation

Introduction

Microorganisms play an evident role in infecting the root canal system with moreover than 700 bacterial species that can be found in the oral cavity [1]. Microbiota are found in highly organized and complex entities, known as biofilms, which mediate the infections of root canals. Once the root canal is infected coronally, infection progresses apically until bacterial products or bacteria themselves are in a position to stimulate the periapical tissues [2,3,4,5].

Material and Methods

This review of the literature examines pertinent publications and published research findings in order to provide a summary about the microbiota which mediate the infections of root canals, the battle to eradicate microorganisms with different solutions.

Results

Researched materials on the various bacteria in root canals and the availability of several solutions demonstrate how challenging it is for clinicians to provide effective mechanical and chemical root canal therapy. When compared to their planktonic counterparts, microbes in biofilms can be up to 1000 times more resistant to antimicrobial agents and host defensive systems [2,5]. Among the bacterial species found in the root canal, the most common ones are aerobes, facultative anaerobes and microaerophiles [2,6,7]. The ecology of the root canal is variable which changes with the development of the disease. The factors that play a key role in this process include the release of oxygen tension when root canals are opened up, the effect of root canal irrigating agents, the influence of various materials on the pH levels inside the canal system [8,9].

There are several routes by which microbiota might enter the pulp by dentinal tubules, open cavity, periodontal membrane, blood stream, faulty restorations and extent. Knowing them is critical for treatment strategy [10].

Endodontic infections are classified as either primary or secondary. A primary infection begins with inflammation of the pulp followed by infection of the root canal system and the spread of microbes and/or their by-products in the tooth-supporting tissues, which can get easily inflamed. Multiple microorganisms cause primary endodontic infections, the most common species among them include Peptoniphilaceae, Eubacterium, Prevotella, Veillonellaceae, Camphylobacter, Fusobacterium, Treponema, Prophyromonas, and Bacteroides spp. [7,11,12]. Different forms of secondary infection can be identified, i.e. reinfection, remnant infection or recurrent infection. Multiple microorganisms cause secondary endodontic infections such as facultative anaerobic gram-posiive cocci and rods (Streptococcus, Enterococcus, Peptostreptococcus, Actinomyces spp). Lactobacillus spp and anaerobic gram-negative rods are lower than in primery infection [12].

MICROBIOTA

The human commensal microbiota colonizes the mucosal surface of the oral cavity, the gastrointestinal system, the urogenital tract, and the skin's surface. This commensal microbiota has coevolved with its host and has learned to survive and tolerate host defensive systems [13,14]. Disease can arise when the host is weakened or when invading germs are harmful enough. Pathogenicity is the ability of one organism to induce illness in another. Pathogens are creatures such as bacteria, fungus, viruses, protozoa, and parasites. These pathogens may adhere, colonize, survive, and reproduce while avoiding host defensive systems such as neutrophils, complement, and antibodies. Furthermore, they can directly or indirectly induce tissue damage. Enzymes, exotoxins, and metabolites, which are produced by pathogens can all cause direct tissue injury [13,15]. A host immune response capable of causing tissue destruction that is stimulated by bacterial components such as lipopolysaccharide (LPS), peptidoglycan (PG), lipoteichoic acid (LTA), fimbriae, outer membrane proteins, capsular components, and extracellular vesicles can cause indirect tissue damage. The level of pathogenicity or disease-causing capacity of a microbe is referred to as virulence [13]. Lipopolysaccharide is a component of Gram negative bacteria's cell wall and is know as endotoxin. When LPS is released, it has a variety of physiological impacts, including the activation of inmunosurveillance systems in the pulp [13]. These endotoxins are linked to pulpal discomfort, periapical inflammation, complement activation, and periapical bone degradation [16, 17]. Gram positive cell walls are mostly made up of peptidoglycan [13]. Peptidoglycan is generated after cell lysis and can interact with the innate immune system as well as stimulate T cell overexpression of proinflammatory and anti-inflammatory cytokines [18]. Lipoteichoic acid is a Gram positive bacterium cell wall component made up of echoic acid and lipid [19]. LTA is produced after cell lysis and attaches to target cells, where it interacts with circulating antibodies, activating the complement cascade and causing damage. A capsule is a well-organized layer of polysaccharides and other components found outside the cell wall of bacteria. Capsules protect the bacterial cell from desiccation, phagocytosis, bacterial viruses, and hydrophobic harmful compounds such as detergents. Bacteria and fungi use capsule development to prevent complement activation and resist phagocyte ingestion [13]. Gram negative bacteria create extracellular vesicles, which allow their products to be released into the extracellular environment. Proteins and lipids are found in the contents and are engaged in a variety of activities such as hemagglutination, hemolysis, bacterial adhesion, and proteolytic activities [20].

Because of the extracellular matrix that surrounds the microorganisms in the biofilm form, they are able to withstand challenging growth and environmental circumstances. Bacteria have the ability to produce extracellular secretions, such as extracellular polysaccharide, and cell surface structures, such as capsules. All indigenous bacteria can be protected by the extracellular polysaccharide against a variety of external challenges, including desiccation, osmotic shock, UV radiation, and pH changes. Additionally, it lessens the impact of any dangerous compounds that must first permeate through the extracellular polysaccharide matrix in order to reach the bacteria [2,21,22].

The gram-negative obligate anaerobe Prevotella species is typically connected with human illnesses such as dental caries and periodontitis. Prevotella spp. progress from commensal bacteria to pathobionts as a result of dysbiosis, which alters the immune system, disrupts homeostasis, and increases the production of several virulence factors, allowing them to survive and establish at the infection site regardless of intermittent environment. Prevotella spp. has been shown to have virulence factors such as adhesins, fimbriae, hemolysins, enzymes such as nucleases, proteases, lipopolysaccharide, and exopolysaccharide, which might boost pathogenicity and survival in the host [23].

E.faecalis is a Gram-positive facultative anaerobe that lives in the human gastrointestinal system naturally. E. faecalis's known virulence activities include its capacity to enter dentin tubules to form biofilms and to live for extended periods of time in difficult settings such as low pH, poor nutrition, and low oxygen [24,25,26].

F. nucleatum is an oral Gram-negative strict anaerobe with rigorous growth and survival requirements[24,27]. F. nucleatum is an important 'bridging' bacterium in the production of oral biofilms. The relationship between F. nucleatum and endodontic infection involves virulence factors such as deregulation of inflammasomes in dental pulp cells [24].

IRRIGANTS AND AGENTS USED FOR CHEMICAL ROOT CANAL THERAPY

A challenging task for irrigating solutions in endondontics is reaching hardly accessible areas and successful removal of inflamed and necrotic tissue within the dental biofilm. The root canal irrigant utilized during the chemical cleaning phase is classified as either antibacterial or decalcifying [28].

Root canal irrigants must meet the following primary requirements:
- Strong antibacterial effect against a wide range of microorganisms, including planktonic and biofilm-forming.
- Endotoxins and lipoteichoic acids, both bacterial virulence factors, are rendered inactive.
- Elimination or disruption of the biofilm
- Pulp tissue remnants dissolve
- No negative effects, local on dentine and periapical tissues and systemic toxicity and allergic responses
- Removal of hardtissue debris and the smear layer, as well as avoidance of their creation
- Low cost and widespread availability [28].

Sodium hypochlorite (NaOCl)

Sodium hypochlorite (NaOCl) is considered the strongest disinfectant in endodontics because of its superior antibacterial activity and capacity to destroy necrotic and vital tissues [2,29, 30]. Increasing the volume of the irrigant [29, 30], decreasing the pH of the irrigant solution, using activation techniques, and warming the solution can all increase the efficiency of NaOCl [29].
Sodium hypochlorite is utilized in endodontic treatment at concentrations that range from 1% to 6%, all of which have antibacterial properties [29, 30,31].
The crucial features of NaOCl are its odor and toxicity, as well as the difficulties of eliminating inorganic components accumulated over anatomical areas such as isthmi and anastomosis since they are difficult to reach with endodontic instruments [29].

Chlorhexidine (CHX)

Chlorhexidine is used as an irrigant for periodontal treatment and infected root canals as well as an oral antiseptic mouthwash to manage plaque. CHX is bactericidal and kills Gram-positive and Gram-negative bacteria, as well as facultative and strict anaerobes [32,33,34], yeasts and fungi, particularly Candida albicans [35,36]. It is effective against some viruses such as espiratory viruses, herpes, cytomegalovirus, HIV. At room temperature, it is inert against bacterial spores. The ability of CHX to absorb to negatively charged surfaces in the mouth (such as tooth, mucosa, pellicle, restorative materials) allows it to be slowly released from these retention sites and so sustain extended antibacterial action for several hours, proccess known as substantivity. Because of its broad spectrum antibacterial effect, substantivity, and low toxicity, chlorhexidine gluconate has been recommended as a root canal irrigant. However, the inability of CHX to dissolve tissue has been identified as a major disadvantage. As a result, when using CHX as an irrigant, complete canal instrumentation should be employed to remove all pulp tissue remains, as CHX does not produce superficial necrosis. Dentin and organic tissues that come into touch with CHX during irrigation retain antimicrobial action for a long time because CHX is slowly released from these retention sites [37]. When compared to sodium hypochlorite, it is less toxic and has lower sustained action. Patients do not experience discomfort when CHX is extruded through the apex. It is advised to use a 2% concentration as a root canal irrigant.

Calcium hydroxide ($Ca(OH)_2$)

Many research have discovered various benefits of calcium hydroxide as a preferred medication, including its high alkalinity, tissue dissolving capacity, ability to neutralize endotoxins, and antibacterial qualities. The pH of calcium hydroxide ($Ca(OH)_2$) is around 12. Hydroxyl ions generate free radicals, which breakdown bacterial cell membrane components. Calcium hydroxide's alkaline pH also changes enzyme function, affecting cellular metabolism and structural proteins. Hydroxyl ions can permeate over dentine, raising the pH to 9.0, a process known as transdentinal treatment. This impact might be useful in regulating bacterial reservoirs in dentinal tubules. When sodium hypochlorite is used as an irrigant, $Ca(OH)_2$ can dissolve necrotic tissue on its own or can be utilized to pretreat tissues to improve their disintegration rate [38]. The tissue dissolving effectiveness of 0.5% sodium hypochlorite with ultrasonic irrigation was improved to the level attained with full strength sodium hypochlorite after pretreatment with calcium hydroxide [39]. Calcium hydroxide does not work equally well against all bacteria and cannot be used in place of thorough debridement [38]. Bacteria like Enterococcus and Streptococcus, for example, can withstand pH values in the 9-11 range [40].

During mechanical instrumentation, a smear layer is created, known to be an amorphous structure made of inorganic and organic elements that is 1-2 μm thick on average, but can cover dentinal tubules up to 40 μm. It has been claimed that the smear layer contains bacteria or bacterial products and may function as a reservoir for irritants, indicating that it should be removed. Endodontic therapy employs a variety of chemical chemicals capable of eliminating the smear layer such as ethylenediaminetetraacetic acid and citric acid [41, 42].

Ethylenediaminetetraacetic acid (EDTA)

In root canal treatment, chelating agents such as ethylenediaminetetraacetic acid (EDTA) are suggested as adjuvants. Numerous writers have demonstrated how effective it is in eliminating the inorganic part of the smear layer and has negligible to no antibacterial action.
EDTA helps with root canal preparation, lubricates root canal instruments, reduces corrosion when used with stainless steel instruments, can be used to detect calcified canal orifices, reduces mercury oscillation in amalgamate restoration, and increases the bonding strength of adhesive materials to root canal dentin walls [42].
In order to remove organic and inorganic debris and disturb microbial biofilms, it seems promising to alternately utilize NaOCl and EDTA during root canal therapy [41,42].

Citric acid

Chelating agent citric acid interacts with metals to generate a non-ionic soluble chelate. It has been used on periodontal disease-affected root surfaces. It has also been considered as a conditioning agent for dental hard tissues. It has high chemical stability and antimicrobial activity against facultative and obligatory anaerobes. Citric acid is recommended as a root canal irrigating solution because to its features such as eliminating the inorganic component of the smear layer and decalcifying dentin. It is used in different concentrations ranged from 1% to 50% [42].

FUTURE ANTIMICROBIAL AGENTS

Antibacterial peptides

Antibacterial peptides are potential intracanal alternatives with strong antibacterial effectiveness, good biocompatibility, and little bacterial resistance. Antibacterial peptides are generally cationic oligopeptides produced from natural sources (for example, bacteria, fungus, plants, and animals) or created computationally. Enterococcus faecalis is the most commonly discovered organism in secondary endodontic infections, the antimicrobial effectiveness of antibacterial peptides as intracanal medicines has been basically researched against it [38]. When compared to the standard calcium hydroxide treatment, antibacterial peptides shown much higher activity in removing Enterococcus faecalis and eradicating biofilms [43].

Nanoparticles

Because of their wide range antibacterial action, nanoparticles have become more and more popular for use in root canal disinfection in recent years. The antibacterial action of chitosan (CS-np), zinc oxide (ZnO-np), and silver (Ag-np) nanoparticles is widespread and is attributed to changes in cell wall permeability that lead to cell death.
These nanoparticles are often used as additions in calcium hydroxide paste to increase the antibacterial efficacy of calcium hydroxide [44]. In comparison to several standard intracanal medicines, nanoparticle therapy showed no significant effect on dentine mechanical qualities [38]. Silver nanoparticle (AgNP) is one of the most researched metal nanoparticles against E. faecalis due to its broad range antibacterial action and inexpensive production processes [45].

The combined use of antimicrobial medicines with varied delivery systems may represent the future of intracanal medicaments [38]. Example the use of antibacterial peptides in conjunction with intracanal irrigant [46], chitosan with chlorhexidine gluconate [47]. The incidence of bacterial resistance would be much lower with multiantimicrobial medications than with single antimicrobial agents [38].

Conclusion

The multi-species nature of biofilms combined with complexity and variability of the root canal system, make disinfection of this system extremely challenging. Microbial persistence appears to be the leading factor in root canal treatment failure, which may have an impact on pain. Specific instruments, disinfecting agents such as irrigants and intracanal medicaments are required to inhibit and remove the biofilm, an uneasy task that combines chemical and mechanical processing of the pathologically altered structures.

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Corresponding author:

Dr. Ana-Maria Miteva
anamariya@abv.bg
0883602700
Faculty of Dental Medicine, Medical University - Varna, Bulgaria