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Investigating the Antimicrobial, Anti-inflammatory and Cytotoxic properties of a Biguanide Derivative: A Comprehensive Analysis

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ABSTRACT: Biguanide represents a versatile class of compounds with significant biological and pharmacological relevance. They constitute an important class of therapeutic agents suitable for the treatment of wide spread of diseases. Therapeutic indications of biguanide include antidiabetic, antimalarial, antiviral, antiplaque, and bactericidal applications. Chlorhexidine is widely used skin antiseptic preparation and is an ingredient in toothpaste and mouthwash. Its antimicrobial effects are long lasting due to its ability to bind strongly to proteins present in the skin and mucosa. This property ensures prolonged antiseptic activity, making it ideal for application where sustained bacterial control is critical as it include handwashing, skin preparation for surgery and placements of intravascular access. This review discusses the pharmacology, clinical applications and adverse effects.

KEY WORDS: Biguanide, therapeutic, chlorhexidine, antiseptic, adverse effects, pharmacology.

I.INTRODUCTION

Biguanides are the compounds in which the amidine part is directly bonded to the guanidine part at N2 position to constitute -C=N-C=N- conjugated system.C2N5H7 structure is the simplest component of biguanides [1] .Biguanides have been used in various chemistry fields such as the synthesis of heterocycles, organocathosis, metal complexation, and crystal engineering. Metformin, a biguanide derivative with hypoglycemic activity, was discovered by Slotta and Tschiesche [2]. As a result of this discovery biguanides attracted notice. Jean Sterne pioneered the first medical application of metformin. Since then, biguanide have been utilized as antimalarial, antidiabetic, antiviral, and antiseptic drugs. Beyond these clinical applications, biguanide derivatives are explored for numerous biological purposes, including anticancer, antibacterial, antitubercular, antiviral, anti-Alzheimer's, and agonist activities [3].

Chlorhexidine, developed as an antiseptic agent by Imperial Chemical Industries (Manchester,UK) in the 1950s, is widely utilized in medical practice as an antiseptic. It is also a common ingredient in personal hygiene products, including mouthwash and toothpaste. This review aims to provide an overview of the pharmacology, clinical applications, and potential adverse effects of chlorhexidine.

Chlorhexidine (1:6-di[4-chlorophenyldiguanido]-hexane) is a bisbiguanide that consist of two chloroguanide chains linked by a hexamethylene chain. It is a strong base and is a di-cation at physiological pH. Chlorhexidine, being insoluble in water, is combined with gluconic or acetic acid to create water-soluble digluconate or diacetate salts. These solutions are colourless, odourless, and possess an intense bitter taste [4].

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When applied topically, the N -chlorinated derivative of chlorhexidine forms covalent bonds with proteins in the skin and mucosa, providing a lasting antimicrobial effect while exhibiting minimal systematic absorption, even if ingested orally [5][6][7]. Chlorhexidine is a synthetic chemical disinfectant known for its broad-spectrum antiseptic properties. It is effective against Gram-positive and Gram-negative bacteria as well as fungi [8][9][10][11]. Its bactericidal action significantly enhances the permeability of bacterial cell membranes and alter protein structures. This process leads to the precipitation of macromolecules within cytoplasm, ultimately resulting in cell death due to lysis of bacterial or fungal cells. Historically it was thought to have a bacteriostatic effect, inhibiting ATPase activity and thereby preventing the replication of prokaryotic cells.

In dental practice, Chlorhexidine is commonly used as a mouthwash to reduce oral bacterial flora. It is also utilized for the prevention and treatment of plaque-related diseases, including gingivitis and periodontitis. By addressing this condition, it helps reduce gingival inflammation and bleeding. Chlorhexidine, when combines with xylitol, exhibits a synergistic interaction that enhances its effectiveness. The available evidence presents conflicting conclusions regarding the impact of Chlorhexidine, particularly in its mouthwash formulation, on caries prevention. Current findings suggest that prevention of dental caries is not directly attributable to the use of chlorhexidine, including its gel and antiseptic solution variants.

Dental caries is multifactorial condition, and its effective management necessities adherence to established protocols such as fluoride application, maintaining a balanced diet, and practicing proper oral hygiene. Recent scientific investigations have reported that chlorhexidine contributes to the reduction of Streptococcus mutans levels; however, such reductions, along with plaque minimization, appear to lack a significant correlation with the prevention of caries. Additionally, the usage the chlorhexidine is associated with dental staining, particularly on resin-based restorative materials.[12][13].

II. SIGNIFICANCE OF THE SYSTEM

The paper mainly focuses on the initial criticism that has emerged against chlorhexidine due to its potential to cause pigmentation on dental enamel. This occurs as chlorhexidine disrupts the enamel film, thereby increasing the enamel's susceptibility to pigments present in food and beverages. The objective of this review is to analyse randomised studies concerning the advantages and disadvantages of chlorhexidine usage, and specifically, to assess the various contexts in which it is applied. Beyond clarifying he benefits and drawbacks of this topical antiseptic of chlorhexidine usage, the article aims to provide insights into its diverse application across multiple domains. The study of literature survey is presented in section III, Methodology is explained in section IV, section V covers the experimental results of the study, and section VI discusses the future study and Conclusion.

III. LITERATURE SURVEY

Chlorhexidine is a cationic bisbiguadine with broad-spectrum antimicrobial activity. It exhibits bacteriostatic, bactericidal, fungicidal, and antiviral properties. Its minimum inhibitory concentrations are lower for Gram-positive bacteria compared to Gram- negative bacteria owing to Gram-positive organisms [14][15]. Prolonged exposure to chlorhexidine amplifies its bactericidal effect across most bacterial strains. However, its activity acid-fast bacilli and heat-resistant bacterial spores remains minimal. Unlike povidone iodine, chlorhexidine's antimicrobial effectiveness is not compromised by the presence of body fluids like blood [16].

Clinical applications of Chlorhexidine:
1.) Oral hygiene: Chlorhexidine is a gold standard in dental care, used in mouthwashes to reduce plaque, gingivitis, and periodontal diseases. Chlorhexidine in mouthwash solutions and binds to oral mucosal surfaces via electrostatic forces, inhibits dental plaque formation and exerts a bacteriostatic action that persists for several hours [17][18].

2.) Hand antisepsis: Alcohol-based hand rubs are the most effective agents for minimizing bacterial presence on hands of healthcare personnel. Following them in effectiveness are antiseptic soaps and detergents, while non-antimicrobial

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soaps are least efficient in reducing bacterial counts. Transient hand flora (*Staphylococcus aureus* and Gram-negative bacilli) present in superficial skin layers are associated with nosocomial infection. Resident flora (coagulase negative *Staphylococcus epidermis*) present in the deeper skin layers is less likely to be pathogenic [16]. Solutions containing chlorhexidine are widely used for surgical hand antisepsis (commonly referred to scrubbing). The effectiveness of skin antisepsis is evaluated based on the reduction in bacterial counts, where a 1-log reduction signifies a 10-fold decrease (eliminating 90% of bacteria). However, total bacterial counts do not account for the type or pathogenicity of residual bacteria. Pathogenic bacteria, such as *Staphylococcus aureus*, residing in the skin are more effectively removed through handwashing.
According to the US Food and Drug Agency standards for antimicrobial effectiveness suggest a 1- log reduction of bacterial count at one minute, a 2-log reduction at five minutes and 3-log reduction at five minutes and 3-log reduction at 10 minutes.[16]

3.) Therapeutic purposes on veins and arteries: Alcohol-based chlorhexidine has proven to be a superior antiseptic for skin preparation before venepuncture and vascular access. A multicentre randomized trial found that 0.5% alcoholic chlorhexidine was more effective than 10% povidone, significantly reducing blood culture contamination rates (1.4% vs 3.3%, odds ratio 0.40, 95% Cl 0.21 to 0.75, P=0.004). Additional advantages of alcoholic chlorhexidine include its faster onset of action and sustained antimicrobial activity, even in the presence of substances like blood [19].

Chlorhexidine, chemically classified as a bis-biguanide, is typically available as a counter -cation paired with one of three counter-anionic species: acetate, chloride, or gluconate. Among these, the gluconate form is most commonly used in dentistry. The organic molecule of chlorhexidine is neutral, apolar, and moderately large, characterised by strongly apolar benzene groups at its ends. This structural composition, which often adopts a linear or unfolded form, accounts for its limited solubility in water [20].

IV. METHODOLOGY

By applying the search parameters, the results were refined. These studies were meticulously reviewed and individually analysed by the author to highlight their key features. The selected articles exhibited diverse characteristics. The biochemical activity of chlorhexidine is centred on its ability to penetrate bacterial cell membranes:

- Inhibition of enzymatic functions: The compound interferes with ATPase activity, disrupting bacterial energy metabolism.
- Alteration of membrane permeability: Chlorhexidine binds to bacterial cell surface, increasing its permeability and destabilizing structural proteins.
- Protein precipitation: Chlorhexidine causes cytoplasmic proteins to precipitate, leading to bacterial agent.

Chlorhexidine functions in two distinct ways:

- Powerful Bactericide: It disrupts the protein structure of bacterial cell membrane by excessively increasing its permeability. This leads to the precipitation of cytoplasmic proteins and ultimately causes bacterial cell death through lysis.
- Bacteriostatic Agent: Previously thought to merely inhibit bacterial replication, it is now understood that chlorhexidine is also capable of killing bacteria.

Bactericidal and bacteriostatic concepts can be applied to fungi as fungicidal and fungistatic respectively [19].

The mechanism of chlorhexidine action involves several key steps:

1. A cationic chlorhexidine molecule rapidly binds to the negatively charged surface of a bacterial cell.
2. It strongly absorbs to phosphate-containing components on bacterial cell surface.
3. The molecule penetrates the cell wall, likely via passive diffusion.
4. It targets the cytoplasmic membrane, compromising its structural integrity.
5. Low molecular weight cytoplasmic components, such as potassium ions, are released, and some enzyme associated with membrane lose functionality [21].

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6. Chlorhexidine forms complexes with phosphorylated compounds like ATP and nucleic acid, leading to cytoplasmic precipitation.

V. EXPERIMENTAL RESULTS

The results have been enforced on the basis of review of various search parameters. The table contains data on organisms their classification and their corresponding Minimum Inhibitory Concentration (MIC). The MIC values represent the lowest concentration of an antimicrobial agent required to inhibit the growth of these organisms.

Table 1.Bacteriostatic activity of chlorhexidine on some bacteria[19]

ORGANISM	Mean MIC (mg/l)	Range (mg/l)
Gram-positive cocci		
Staphylococcus aureus	1.6	1-4
Staphylococcus epidermidis	1.8	0.25-8
Gram-negative bacteria		
Acinetobacter anitratus	32	16-64
Acinetobacter lwoffi	0.5	-

Table 2.Bactericidal activity of chlorhexidine on some bacteria[19]

ORGANISM	Mean log reduction in 0.05 chlorhexidine after 20 seconds	Mean log reduction in 0.05% chlorhexidine after 1 minute	Mean log reduction in 0.05% chlorhexidine after 10 minutes
Gram-positive cocci			
Staphylococcus aureus	0.4	0.7	2.5
Staphylococcus epidermidis	2.2	3.4	>5.1
Gram-negative bacteria			
Acinetobacter anitratus	1.4	2.6	>5.3
Bacteroides distastonis	0.9	2.7	>4.9

Table 3. Fungistatic activity of chlorhexidine[19]

ORGANISM	Mean MIC (mg/l)
Mould/Fungi	
Aspergillus fumigatus	32
Aspergillus niger	16
Yeast	
Candida albicans	9

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Table 4.Fungicidal activity of chlorhexidine[19]

ORGANISM	Mean log reduction in 0.05 chlorhexidine after 20 seconds	Mean log reduction in 0.05% chlorhexidine after 1 minute	Mean log reduction in 0.05% chlorhexidine after 10 minutes
Mould/Fungi			
Aspergillus fumigatus	0.7	1.2	2.4
Aspergillus niger	0.7	1.2	3.0
Yeast			
Candida albicans	2.8	>4.1	>4.2

VI. CONCLUSION AND FUTURE WORK

Chlorhexidine is a highly effective antiseptic agent that maintains its antimicrobial properties even when exposed to blood and body fluids. It is extensively utilized across various disciplines in dentistry. It has a strong and rapid antimicrobial and antifungal activity. The biocidal activity of chlorhexidine is mainly against the vegetative forms of Gram-positive bacteria, while it is less active against Gram-negative bacteria-therefore much higher concentrations are required to fight these microorganisms. In this paper, we showed that chlorhexidine is employed in conservative dental therapies and endodontic treatments. Future work involves to implement a new evaluation for its effects on oral microbiome and identifying approaches to maintain a healthy balance without compromising its antiseptic action. Also, studying its potential in non-medical fields, such as water purification or sanitation systems, to capitalize on its broad-spectrum antimicrobial properties.

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