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Photodynamic therapy (PDT) for disinfection of oral wounds. In vitro study

Research article

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Abstract: Objective: The aim of this in vitro study was to compare the antimicrobial effects of photodynamic antimicrobial chemotherapy (PACT), an ordinary antiseptic (chlorhexidini digluconas), and an antibiotic therapy (bacitracinum zincicum and neomycini sulfas) in vitro. Background: Photodynamic therapy (PDT) is an area of great interest for its potential use as an antimicrobial therapy. It is currently a popular topic in modern medical literature. PDT is, according to recent publications, advantageous over other types of therapies because it acts nonspecifically and it is impossible to develop resistance to the therapy. Materials and Methods: We investigated the antibacterial effect of these three forms of antiseptics on the selection of G+, G-, aerobic, and anaerobic bacteria that exist in the oral cavity and are involved in the formation of periodontal diseases. Results & Conclusion: We found that the PACT device did not have a sufficient antimicrobial effect in vitro. In contrast, the disinfection agents containing chlorhexidini digluconas were effective and may be a safe, non-specific alternative to antibiotic treatments. Promising results from some clinical studies can have different mechanism of action as disinfection.

Keywords: *Laser • Chlorhexidin • Photodynamic therapy • Antibiotics • Disinfection • Ozonization • Oral cavity*

1. Introduction

Photodynamic therapy (PDT) is currently an area of great interest regarding oral disinfection. PDT was discovered more than 100 years ago when it was observed that the combination of harmless dyes and visible light could kill microorganisms in vitro. Since then, PDT has primarily been developed as a treatment for cancer, ophthalmologic disorders, and the dermatological affections. However, an interest in the antimicrobial effects of PDT has recently been revived, and it has been proposed as a therapy for a large variety of localized infections [1].

Although PDT has been used for a variety of applications over the years, the concept of PDT was first described in 1900 when Raab explored the antimicrobial action of acridine and light on a Paramecium species [2]. Photodynamic antimicrobial chemotherapy (PACT), which uses the principles of PDT, has the potential to be a very useful technology, especially in the treatment of local infections. PDT is non-specific and does not induce resistance to the therapy, which are key advantages over other types of therapies. Therefore, PDT can be used as a bactericidal treatment of resistant bacterial strains, such as methicillin-resistant Staphylococcus Aureus (MRSA). PDT utilizes a combination of light, oxygen, and a chemical known as a photosensitizer, which is a substance that is capable of being activated by light, in order to achieve cytotoxic effects. Over the past 30 years, PDT has been used clinically in the treatment of many different localized cancerous and precancerous

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conditions. The use of PDT for the treatment of skin diseases of non-neoplastic origins has also been explored, including psoriasis, scleroderma, and acne [1].

Another type of antimicrobial treatment in surgical fields is chemical disinfection. Antiseptic products containing the active compound chlorhexidini digluconas are currently the most frequently used antibacterial therapies in the oral cavity. These antiseptics are produced with different concentrations of the active ingredient and in different application forms (mainly rinses and gels) [3]. In addition, O_3 disinfectants in the form of ozonized water are also used as disinfectants. Studies on the use of ozonized water for the disinfection of dry socket alveolus have been conducted in our hospital and have shown reasonable clinical results [4], however additional comparative data are needed.

The aim of this study was to compare the antimicrobial effects of PACT, commonly used antiseptics containing chlorhexidini digluconas, and antibiotic therapy (including bacitracinum zincicum and neomycini sulfas) in vitro to avoid other factors (increased local immunity, placebo).

2. The antimicrobial mechanism of action of PDT

The microbial selectivity that has been observed with PDT appears to be due to pharmacokinetic differences between mammalian and bacterial cells [5]. The specificity of PDT is aided by the fact that singlet oxygen, the main bactericidal species, has a short life span and a limited diffusion distance of 100 nm [6].

The mechanism of PDT involves the use of light with an appropriate wavelength to modify the photosensitizer molecule to the excited singlet state, which subsequently crosses to a more stable but lower energy triplet state. The interactions of the photosensitizer excited states with endogenous oxygen in the target cells or surrounding target tissue subsequently induces cytotoxic effects, which can lead into two pathways [7]. The Type I pathway involves electron-transfer reactions from the photosensitizer triplet state that result in the formation of toxic oxygen species, such as superoxide, hydroxyl radicals, and hydrogen peroxide. The Type II process involves energy transfer from the photosensitizer triplet state to the ground state molecular oxygen, which produces the excited singlet oxygen. In the case of bacteria, the lethal damage associated with PDT has been reported to occur at the level of nucleic acids8, the cytoplasmic membrane [9,10], or both [1,11].

3. Materials and Methods

3.1. Devices and Chemicals

A pact device (Cumdente, Tübingen, Germany) was used that had the following parameters:
diode laser with a wavelength of 635 nm,
device class IIa (93 / 42 / EEC),
laser class 2M (EN 60825-1:1993 / A2:2001),
diode power of 150 mW, and output power of 80-100 mW. The pact gel contained the active ingredient tolonium chloride. Two antimicrobial reagents were used in this study: chlorhexidini digluconas (Asklepion, CZ) and pamycon (Biotika, Stara Lupča, Slovakia). The chlorhexidini digluconas was used at a 1% concentration (10 mg in 10 ml of solvent), which was chosen based on the concentration of Corsodyl gel (GSK, UK) commonly used for the treatment of infectious complications in the oral cavity. The pamycon contained 33000 IU of neomycini sulfas and 25000 IU of bacitracinum in one solution vial, which were the same active ingredients and concentrations as framykoin ointment.

3.2. Bacterial strains

Bacterial strains found in the oral cavity and involved in the formation of periodontal disease were used, including the G+ strains

Staphylococcus aureus,
S. aureus MRSA,
S. epidermidis,
Streptococcus pyogenes,
S. viridians,
S. agalactiae,
and *Enterococcus faecalis*, the
enterobacteriaceae
Klebsiella pneumonia, extended spectrum beta-lactamases (*K. pneumoniae ESBL*+),
E. coli, and
E. coli ESBL+, the G- strain
Pseudomonas aeruginosa, the
Eukaryotic microorganisms
Candida albicans, and the
anaerobes
Propionibacterium acnes and
Peptostreptococcus sp.

3.3. The PDT device (PACT)

Bacterial strains were cultivated under laboratory conditions on solid agar media using standard culture media (Columbia agar, Schädler agar, or VL agar; UKLDB - Antibiotic center, Prague). Inoculated plates were cultured under constant laboratory conditions of 36 ± 0.5°C for 24 hours.

Well-grown bacterial colonies were directly coated with photosensitizer (F+), exposed for 60 s, and then irradiated with the diode laser (635 nm, 100mW) for 60 s. As a control, bacterial colonies located on another section of the same plate were exposed to only the diode laser (635 nm, 100 mW) without the use of the photosensitizer (F0). This method was designed to determine the effectiveness of the chemical components of the experiment.

Several colonies from both sites (F+ and F0) were collected, transferred to new culture medium, and cultured under standard conditions. The effect of the laser on the viability of bacterial populations after 24 hours was then evaluated. Several colonies were isolated from the original plates at the same sites (i.e. F+ and F0) and transferred to new culture plates to evaluate the long-term effects of laser treatment (i.e. 24 hours after the irradiation).

Subtraction was repeated 24 hours after standard cultivation.

In parallel, the bacterial cultures were inoculated in liquid soil/fleshpepton bouillon soil with a volume of 0.25 ml. An equal volume of photosensitizer reagent was added to the solution and incubated for 60 s. The bouillon culture was then exposed to the diode laser (635 nm, 100 mW) for 60 s. After exposure, the samples were inoculated on an agar plate, cultured for 24 hours, and then evaluated. The procotols used were in accordance with the recommended practices published by Cumdente.

3.4. Use of disinfectant substances

The disk diffusion method for testing the susceptibility of bacteria to antimicrobial drugs was used to evaluate the effectiveness of disinfectants. Test strains were suspended in saline at a concentration of 105 bacteria/ml and then inoculated on Müler-Hinton agar. After inoculation, sterile disks (filter paper) containing chlorhexidine digluconas or pamycon solution were prostrated on the inoculated plates. Five discs were used for each bacterial culture. The plates were then cultured under standard conditions.

The diameters of inhibition zones (mm) for each agent and the bacterial strains were calculated after 24 hours. The arithmetic mean was determined for the measured values and the data were plotted on a graph.

4. Results

In this study, we used a simple assessment of the growth of bacterial cultures on control nutrient media when evaluating the antibacterial effectiveness of the PACT device on the persistence of bacteria in the oral cavity. As shown in Table 1, we used “+” to indicate bacterial strains that grew on the control nutrient media and “-” that did not grow on the media.

Table 1. Results of PACT disinfection

	Results of PACT disinfection			
	2 min after irradiation F +	control group F0	24 hours after irradiation F +	liquid soil F +
S. aureus	+	+	+	+
S. aureus MRSA	+	+	+	+
S. epidermidis	+	+	+	+
S. pyogenes	+	+	+	+
S. viridans	+	+	+	+
S. agalactiae	+	+	+	+
K. pneumoniae	+	+	+	+
K. pneumoniae ESBL+	+	+	+	+
E. coli	+	+	+	+
E. coli ESBL+	+	+	+	+
P. aeruginosa	+	+	+	+
C. albicans	+	+	+	+
Propionibacterium ac.	+	+	+	+
Peptostreptococcus	+	+	+	+
E. faecalis	+	+	+	+

Our results showed that laser irradiation of bacterial cultures on agar plates under in vitro conditions did not affect their viability, even after the use of a photosensitizer. Therefore, we conclude that the PACT device is not sufficient for the disinfection of wound surfaces in the oral cavity.

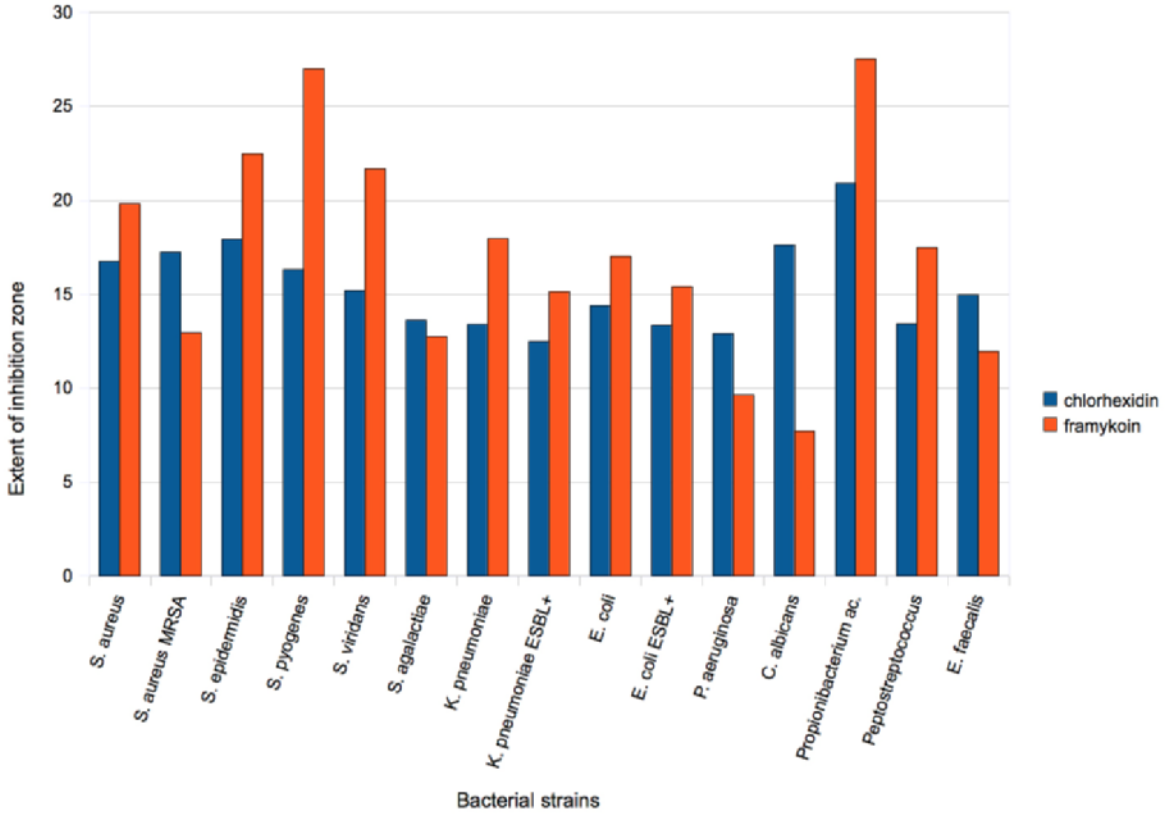
We next evaluated the effectiveness of the disinfection agent chlohexidini digluconas and pamykon on the growth of bacteria by measuring the inhibition zones that formed around the impregnated disks. The diameters (mm) of the inhibition zones are shown in Table 2 and Figure 1.

Both tested substances demonstrated sufficient inhibition of growth (disk diffusion method, inhibition zone > 10mm) of bacterial strains commonly present in the oral cavity. In most cases, the zones of inhibition were more pronounced with the solution containing antibiotics (neomycini sulfas and bacitracin), however the effectiveness of this product was markedly affected by the level of resistance of some of the bacterial species in the region. The *S. aureus MRSA, Pseudomonas aeruginosa, Candida albicanas*, and *Enterococcus faecalis* strains showed strong resistance against these antibiotics.

Table 2. Evaluation of the effectiveness of disinfection agents.

	Chlorhexidini digluconas						Pamykon					
						Arithmetic mean						Arithmetic mean
S. aureus	16.7	16.9	16.6	16.7	17.0	16.77	19.9	20.3	20.1	19.6	19.3	19.83
S. aureus MRSA	17.5	17.5	16.2	17.8	17.3	17.25	12.7	12.3	12.4	13.1	13.0	12.96
S. epidermidis	16.4	18.9	17.6	18.9	17.8	17.94	21.7	22.9	23.0	22.3	22.6	22.49
S. pyogenes	16.6	16.6	17.3	16.5	14.6	16.32	27.2	27.2	27.3	25.9	27.3	26.99
S. viridans	15.3	15.4	14.9	15.1	15.4	15.2	22.7	22.0	21.5	22.1	20.1	21.68
S. agalactiae	13.2	13.5	14.4	12.7	14.4	13.64	13.0	11.7	13.5	12.6	13.1	12.75
K. pneumoniae	13.7	13.4	13.4	13.4	13.3	13.42	17.7	18.4	17.9	17.5	18.3	17.96
K. pneumoniae ESBL+	12.2	12.5	12.7	12.8	12.3	12.5	14.2	15.9	15.3	15.3	15.1	15.15
E. coli	14.1	14.8	14.6	14.5	14.1	14.41	17.0	17.0	17.1	17.0	17.1	17.04
E. coli ESBL+	13.1	13.0	13.8	13.0	13.9	13.35	17.3	16.7	13.7	15.2	14.3	15.43
P. aeruginosa	14.6	13.3	14.2	11.8	10.7	12.91	9.7	9.5	9.6	9.7	9.7	9.64
C. albicans	15.1	18.2	18.3	18.2	18.3	17.63	7.5	6.9	7.6	8.2	8.5	7.73
Propionibacterium ac.	20.9	22.0	20.2	21.0	20.5	20.92	26.5	26.8	28.1	28.5	27.9	27.54
Peptostreptococcus	13.3	14.2	13.7	12.9	13.1	13.43	18.3	16.4	17.6	18.3	16.9	17.48
E. faecalis	15.1	15.2	14.8	14.5	15.2	14.96	12.2	12.0	12.4	11.1	12.0	11.96

Figure 1. Graphical evaluation of the effectiveness of disinfection agents. The diameters (mm) of the inhibition zones are shown (accurate to 1decimal place)



A 1% chlorhexidine digluconas solution has been shown to have a strong, non-specific, and proven effectiveness on bacterial strains of the oral cavity and its surroundings [12,13] The specific effectiveness of this antiseptic observed here against S. aureus MRSA, Pseudomonas aeruginosa, Candida albicanas, Enterococcus faecalis, Propionibacterium acnes, K. pneumoniae ESBL +, and E. coli ESBL + suggests that this disinfectant may be advantageous for the treatment of wound areas that show increased bacterial resistance against antibiotics.

5. Discussion

This study evaluated the antibacterial effectiveness of PACT and commonly used antimicrobial disinfectants against bacterial strains of the oral cavity. Although this study provided a thorough assessment of our aims, some limitations did exist. First, the bacterial spectrum analyzed were only pathogenic strains present in the oral cavity according to the literature. In order to make further clinically relevant assessments, we will need to test the efficacy of PACT on bacterial strains physically present in the oral cavity, examine the effectiveness of this treatment in vivo, or examine the effectiveness of PACT on strains collected directly from patients.

A second limitation of this study (an also main novelty) was that all of the experiments were conducted under in vitro conditions. The efficacy of PACT may be affected by several other factors when used on a live patient. Therefore, the effectiveness of this therapy may depend on other factors (improvement of immunity response, placebo, and photostimulation) in addition to the bactericidal effect of PDT on bacterial strains, despite the fact that other studies have shown a reduction of bacteria in the oral cavity by PACT [14-18]

The therapeutic effect of laser biostimulation has also received recent interest, and therefore it is possible that PACT could induce biostimulation of affected tissue, which could positively affect the healing process.

However, the results of this study have raised several unanswered questions, and additional studies are needed in order to clarify and refine these findings.

6. Conclusion and Summary

Our study has shown that PACT has insufficient antimicrobial effects in laboratory conditions. However, we found that disinfection agents containing chlorhexidini digluconas (golden standard in oral surgery) appear to be safe, non-specific alternatives to antibiotic treatments.

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