ANTIMICROBIAL EFFICACY OF PHOTODYNAMIC THERAPY AS A CO-ADJUVANT IN ENDODONTAL TREATMENT: INTEGRATIVE REVIEW

EFICÁCIA ANTIMICROBIANA DA TERAPIA FOTODINÂMICA COMO COADJUVANTE NO TRATAMENTO ENDODÔNTICO: UMA REVISÃO INTEGRATIVA DA LITERATURA

Sandra Evelyn Moura Silva¹, Marcílio Oliveira Melo², Erick Thiago de Sousa³, Markelane Santana Silva⁴

1 - Associação Brasileira de Odontologia. Endodontic Specialist. E-mail: sandrinha_evelyn@hotmail.com
2 - Associação Brasileira de Odontologia - Secção Piauí (ABO-PI). Master in Endodontics. E-mail: marcelo.melo@hotmail.com
3 - Associação Brasileira de Odontologia - Secção Piauí (ABO-PI). Master in Endodontics. E-mail: thssousa25@gmail.com
4 - Associação Brasileira de Odontologia - Secção Piauí (ABO-PI). Master in Dental Clinic. E-mail: markelanesantanasilva@gmail.com
ABSTRACT

Objective: to carry out an integrative review on the use of PDT as an adjunct to conventional endodontic treatment and its antimicrobial efficacy. Materials and Methods: Articles published between 2006 and 2018, in the national and foreign languages, were selected through the Pubmed, Medline, Scielo and Science Direct databases with the descriptors Photodynamic Therapy, Enterococcus faecalis and Endodontia. Results: Of the 204 articles, eleven were selected. We included articles available in full that related the antimicrobial efficacy of PDT and endodontics. Articles that did not fit the proposed theme and / or the content would not be relevant for the accomplishment of the work and / or articles outside the time limits were excluded. Conclusion: The studies have shown promising results regarding the use of PDT as an adjunct to conventional endodontic treatment and its antimicrobial potential. However, a protocol for the use of PDT in daily clinical practice has not been well defined, suggesting further studies in the area.


RESUMEN

Objetivo: realizar una revisión integrativa sobre la utilización de la PDT como coadyuvante al tratamiento endodóntico convencional y su eficacia antimicrobiana. Materiales y Métodos: Se seleccionaron artículos publicados entre los años 2006 a 2018, en los idiomas nacional y extranjero, a través de las bases de datos Pubmed, Medline, Scielo y Science Direct con los descriptor Terapia Fotodinámica, Enterococcus faecalis y Endodoncia. Resultados: De los 204 artículos, once fueron seleccionados. Se incluyeron artículos disponibles en su totalidad que relacionaran la eficacia antimicrobiana de la PDT y la endodoncia. Se excluyeron artículos que no se adecuaron al tema propuesto y / o el contenido no tendría relevancia para la concreción del trabajo y / o los artículos fuera de los límites temporales. Conclusión: Los estudios demostraron resultados prometedores en cuanto al uso de la PDT como coadyuvante al tratamiento endodóntico convencional y su potencial antimicrobiano. Sin embargo, aún no está bien definido un protocolo de utilización de la PDT en la práctica clínica diaria, sugiriendo más estudios en la área.


RESUMO

Objetivo: realizar uma revisão integrativa sobre a utilização da PDT como coadjuvante ao tratamento endodôntico convencional e sua eficácia antimicrobiana. Materiais e Métodos: Foram selecionados artigos publicados entre os anos 2006 a 2018, nos idiomas nacional e estrangeiro, por meio das bases de dados Pubmed, Medline, Scielo e Science Direct com os descritores Terapia Fotodinâmica, Enterococcus faecalis e Endodontia. Resultados: Dos 204 artigos, onze foram selecionados. Foram incluídos artigos disponíveis na íntegra que relacionassem a eficácia antimicrobiana da PDT e endodontia. Foram excluídos artigos que não se adequassem ao tema proposto e/ou o teor não teria relevância para a concretização do trabalho e/ou artigos fora dos limites temporais. Conclusão: Os estudos demonstraram resultados promissores quanto ao uso da PDT como coadjuvante ao tratamento endodôntico convencional e seu potencial antimicrobiano. Entretanto, ainda não está bem definido um protocolo de utilização da PDT na prática clínica diária, sugerindo mais estudos na área.

Introduction

One of the main objectives of endodontic therapy is the maximum disinfection of the root canal system (SCR), as well as to prevent a possible reinfection.\(^1,2,3\) This is possible thanks to the mechanical action of the endodontic instruments associated to the chemical action of the solutions irrigation, physical actions of irrigation and aspiration,\(^4\) culminating with the obturation of the ducts in a way that promotes a suitable hermetic seal of the same ones.\(^5\)

Microorganisms play an important role in endodontic infections, and their toxic metabolites are responsible for the appearance and persistence of periapopathies.\(^1\) Although endodontics has evolved a great deal in recent decades, the complexity of the internal anatomy of the root canal system still makes it difficult the total elimination of microorganisms by the currently known instrumentation techniques.\(^4,5,6,7\)

While mechanical debridement and the chemical action of irrigators eliminate parts of the microorganisms, residual bacterial contamination persists in approximately a quarter of the root canal system after biomechanical preparation, and this residual contamination is one of the main causes of endodontic treatment failure.\(^5\)

Enterococcus Faecalis is an important persistent pathogen that is associated with failures in endodontic therapy.\(^2,5,6,7,8\) In addition to being organized in biofilms, they have several virulence factors and are frequently found in chronic periapical reactions.\(^5\)

Recently, the limitations of conventional endodontic therapy have given rise to many attempts to introduce Photodynamic Therapy (PDT) as an alternative treatment.\(^8\) In order to optimize bacterial elimination, avoiding the residual bacteria, PDT appears as a new auxiliary method of disinfecting root canal systems with significant microbial reduction.\(^1,5,9\) This article aims to carry out an integrative review of the literature regarding the use of PDT as a coadjuvant to conventional endodontic treatment, as well as its antimicrobial efficacy and potential of reducing the viability of E. Faecalis through the use of different photosensitizers and irradiations.

Materials and Methods

Articles published between 2006 and 2018, in the national and foreign languages, were selected through Pubmed, Medline, Scielo and Science direct, using the descriptors: Photodynamic Therapy, Enterococcus faecalis and Endodontia. We found 204 articles, 11 of which were selected. We included articles available in full with methodology described in detail, possible reproduction, that related to the use of PDT in endodontics with the use of different photosensitizers and irradiations, as well as its antimicrobial efficacy reducing the viability of E. faecalis. The exclusion criteria rejected articles that did not fit the proposed theme and / or the content would not be relevant for the accomplishment of the work and / or articles outside the temporal limits.

The figure below shows the article selection process for this study.
Results

Among the information obtained from the selected studies, the authors' methodology, results and conclusions are highlighted (Table 1).

**Tabela 1. Results and methodologies of the main studies**

<table>
<thead>
<tr>
<th>Study</th>
<th>Methodology / Results (photosensitizers and irradiations used)</th>
<th>Conclusions (microbial reduction potential)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soukos et al.</td>
<td>Strains of microorganisms were sensitized with methylene blue (25 μg / ml) for 5 min, followed by exposure to red light of 665 nm with an energy yield of 30 J / cm². Methylene blue alone completely eliminated all bacterial species, with the exception of E. faecalis. The same concentration of methylene blue in combination with red light (222 J / cm²) was able to remove 97% of bacteria from the E. faecalis biofilm using an optical fiber with multiple cylindrical diffusers.</td>
<td>PDT is an effective adjuvant therapy to endodontic treatment in microbial reduction and that several light and preparation parameters should be studied in order to define the appropriate dosimetry for the elimination of microorganisms from the root canal.</td>
</tr>
<tr>
<td>Fimple et al.</td>
<td>The SCR was incubated with methylene blue (25 μg / mL) for 10 min, followed by exposure to red light at 665 nm with an energy flow rate of 30 J / cm². Light was supplied from a diode laser through a 250m-diameter polymethylmethacrylate optical fiber that evenly distributed the light by 360°. PDT reduced Colony Forming Units (CFUs) by up to 80%.</td>
<td>PDT is an effective auxiliary therapy when associated with endodontic treatment in reducing microorganisms when PDT parameters are optimized.</td>
</tr>
<tr>
<td>Garcez et al.</td>
<td>Biological samples were collected after access to the root canal, after endodontic therapy and after PDT in 32 anterior teeth of 21 patients with periapical lesions and antibiotic use.</td>
<td>The use of PDT associated with conventional endodontic therapy leads to an even greater reduction of microbial activity on the root canal.</td>
</tr>
<tr>
<td>Study</td>
<td>Description</td>
<td></td>
</tr>
<tr>
<td>-------</td>
<td>-------------</td>
<td></td>
</tr>
<tr>
<td>Polyethyleniminachlorine was used as the photosensitizer and the diode laser as the light source ($P = 40$ mW, $t = 4$ min, $E = 9.6$ J). All patients had at least one antibiotic resistant microorganism. Conventional endodontic treatment alone produced a significant reduction in the number of microbial species, but only 3 teeth were free of bacteria, while the association of PDT with endodontic treatment eliminated all drug-resistant species and all teeth were free of bacteria.</td>
<td>load and that PDT is an efficient treatment to eliminate micro-organisms resistant to multiple drugs.</td>
<td></td>
</tr>
<tr>
<td>Twenty-six teeth received mechanical chemical debridement (CMD) with sodium hypochlorite ($NaOCl$) at 6% and 26 teeth received CMD associated with PDT. For PDT, root canal systems were incubated with methylene blue (50 μg / ml) for 5 minutes, followed by exposure to red light (665nm; 30 J / cm2). After CMD + PDT, 45 of the 52 channels (96.5%) had no CFU, compared to 24 of 49 channels (49%) treated with CMD alone. Post-treatment detection levels for all species were markedly lower for CMD + PDT treated channels than for those treated by CMD alone. Bacterial species within the dentin tubules were detected in 17 of 22 (77.3%) and 15 of 29 (51.7%) channels in the DMC and DMC + PDT groups, respectively.</td>
<td>PDT significantly reduces residual bacteria within the SCR and is an efficient method which, if improved by technical improvements, has a substantial promise in complementing endodontic therapy.</td>
<td></td>
</tr>
<tr>
<td>Sixty human teeth were distributed in 5 groups. The control group received no treatment. Group 1 was treated with 5% $NaOCl$ solution. Groups 2, 3 and 4 were treated with 70 μL of the methylene blue photosensitizer and diode laser irradiation (660 nm) for 1, 2 and 4 minutes, respectively. All roots were inoculated with $E.\ faecalis$ suspension and brain-heart infusion broth (BHI) and stored for 21 days. The load of microorganisms in the control group increased. The lowest reduction in microorganism load was observed in the one minute irradiation group (Group 2 = 99.8%), very close to the results of the other experimental groups (99.9%). There were no significant differences between groups.</td>
<td>PDT is as effective as conventional irrigation with 5% $NaOCl$ relative to the antimicrobial efficiency against $E.\ faecalis$ and that irradiation for one minute is sufficient to achieve this efficiency.</td>
<td></td>
</tr>
<tr>
<td>Twenty unirradicular teeth were divided into 2 groups (n = 10): Control Group and PDT Group. The specimens from the control group were untreated and those in the PDT group underwent photodynamic therapy with the methylene blue dye (25 mg / ml) for 10 minutes and a diode laser (910nm; 1W). $E.\ faecalis$ were cultured in Petri dish for 24 h in BHI medium. It was found that the percentage of CFU reduction in the PDT group was 96.7%. The penetration depth of the bacteria in the control group was about 980μm. In the PDT group there were no bacteria up to 890-900μm.</td>
<td>Photodynamic therapy reduces the number of bacteria in infected root canals effectively and can be used as adjuvant antimicrobial procedure after standard endodontic treatment.</td>
<td></td>
</tr>
</tbody>
</table>
| Thirty extracted teeth were divided into three groups. All samples were infected with an artificial biofilm. The first group was treated with Aseptim Plus ® photoactivated disinfection | PDT failed to break the artificial microbial endodontic biofilm and could not inhibit bacterial growth in a clinically
system (LED), the second group by a diode laser (650nm) and blue toluidine as a photosensitizer; and the third group (control) by Passive Ultrasonic Irrigation (PUI) with 17% Ethylenediaminetetraacetic Acid (EDTA) and 2.6% NaOCl solutions. Working time for the three groups was set at 3min. There was no statistically significant difference between the results obtained in the groups treated by Aseptim Plus® and Diode Laser. In cultures of both groups there was maximum bacterial growth. The group that was treated by PUI presented the best results with statistically significant reduction of bacterial load and destruction of the microbial biofilm.

The effects of PDT were evaluated by 30, 60 and 120s on viability of E. faecalis, using methylene blue and malachite green as photosensitizers. The irradiation source was a diode laser (660nm; 40mW), while the energy density and quartz fiber optic diameter were 120 J / cm² and 400μm, respectively. Incubation with saline had no antimicrobial effect, while NaOCl completely eliminated E. faecalis. A significant reduction in the viability of E. faecalis was observed after laser irradiation. When the diode laser was used with both photosensitizers for 30s, there was a small decrease in bacterial viability, with a statistically significant difference between the positive and negative control groups.

One hundred and fifty-six teeth were collected, sterilized and incubated with E. faecalis and C. albicans. The two groups were subdivided into 6 groups: HELBO® Endo Blue photosensitizing dye application followed by HELBO (100mW; 660nm) laser irradiation for 1, 3 and 5min; irrigation with NaOCl 2.5%, PUI at 10s with NaOCl 2.5% and control group. The percentage of dead cells in the treatment groups was significantly higher in comparison to the control group for both organisms. When comparing disinfection methods, PUI was significantly more effective compared to other groups, followed by laser irradiation of 5min, 3min and irrigation with NaOCl, whereas the lowest percentage of dead cells was detected in the laser irradiation group for one minute.

Methylene blue was used in 0.01% (31.2 mol / l) in association with a red laser (660nm) as a source of excitation in the methylene blue group (MBG). The same test was performed with rose bengal (25mol / l), which was associated with a green laser light source (532nm) in the rose bengal (RBG) group. A saline solution (0.9%) was used in the control group. CFUs per milliliter (CFU / ml) were calculated after 24h of incubation at 37 ° C. The results showed a significant reduction in UFC / ml in the RBG group (0.12 • 108)

The best result regarding the reduction of viable E. faecalis cells was obtained with rose bengal as a photosensitizer, which led the authors to conclude that PDT could be improved if rose bengal is used in association with a green laser source for inactivation of E. faecalis.
compared to control (2.82 • 10^8) and MBG groups (2.66 • 10^8). For the concentration and laser intensity used in the experiments, the MBG group repeatedly showed no significant reduction in bacterial counts compared to the control.

A total of 125 extracted human unirradicular teeth were divided into 6 groups (A-F; n = 20) and incubated with E. faecalis. Group A - photosensitizer (indocyanine green [ICG]); B - diode laser; C-PDT; D-CHX 2.0%; E-PDT with photosensitizer modified by CHX 2.0%; and F - control group (no procedure performed). The results showed that none of the groups tested had biofilm eradication or inhibition. On the other hand, ICG-mediated PDT + CHX 2.0%, CHX 2.0% and PDT groups showed significantly less CFU / mL than the ICG and diode laser groups. The group with the lowest CFU / mL count was 2.0% PDT + CHX, being statistically different from all other groups that could decrease expression levels of the efa, gelE and fsRC genes 6.6-, 8.3 and 12.1 times, respectively.

The synergistic effect of ICG-PDT with 2.0% CHX leads to virulence modulation of the E. faecalis biofilm model, suppressing the expression of genes associated with biofilm formation.

Discussion

It is already agreed in the literature that the complexity of the internal anatomy of the SCR makes the total removal of microorganisms by the techniques of instrumentation known today. Epidemiological data suggest that about 30% to 50% of failures in endodontic treatment are related to residual and permanent infections, which require additional strategies for disinfection.

It was decided to investigate in this journal of the literature studies that evaluated the antimicrobial potential of PDT in reducing the viability of E. Faecalis, since the literature describes this microorganism as the most prevalent species in cases of failures in endodontic therapy, because it is resistant the majority of intracanal medicines and because they have the capacity for proliferation and development of infections, with extremely difficult elimination by the conventional method.

PDT has been shown to be a adjuvant therapy to conventional endodontic treatment for the optimization of SCR bacterial reduction in endodontic infections, in order to eliminate persistent microorganisms in the chemical-mechanical preparation. In addition to having easy and rapid clinical application, PDT does not develop bacterial resistance and can be indicated in endodontic treatment in single or multiple sessions.

There are still disputes among the authors about the efficacy of PDT compared to other techniques used in endodontic treatment. The study by Garcez et al.\(^\text{(12)}\) has shown that the use of PDT associated with conventional endodontic therapy is an efficient treatment to kill multiple drug resistant microorganisms. However, Yildirim et al.\(^\text{(14)}\) concluded in their study that PDT is as effective as conventional irrigation with 5% NaOCl in relation to antimicrobial efficiency against E. faecalis. As well as Xhevdet et al.\(^\text{(6)}\) corroborated that PUI was significantly more effective and that PDT did not completely eradicate the contaminating microorganism in the root canals.

Basically, PDT involves the use of a photosensitizing agent (photoactive dye) with affinity for malignant cells, fungi or bacteria; of a light source of a specific
wavelength for excitation; and an oxygen molecule. The molecules of the photosensitizer are activated by the light source and initiate the transfer of energy to the available oxygen, resulting in the formation of toxic oxygen species, singlet oxygen and free radicals, capable of damaging lipids, nucleic acids, and other microbial cellular components.

In endodontics, photosensitizers that have been widely used in PDT research are those derived from phenothiazines. Phenothiazines are tricyclic heteroaromatic compounds, blue dyes such as methylene blue dye and toluidine blue, chlorines and forphirins. In this review, the authors used methylene blue, polyethyleneiminachlorine, blue toluidine, malachite green, rose bengal green echinocyanin. Of these, most chose to use methylene blue.

Another factor of relevance for the success of the PDT, besides the choice of the photosensitizer, is the concentration of the same. The recommended concentration for use in antimicrobial PDT is from 6μg / ml to 15μg / ml, which may range from 0.1μg / ml to 200μg / ml, in order to obtain an effective photosensitization of the microorganisms. However, the concentrations used by the authors ranged from 25 μg / ml, 50 μg / ml, 25 mg / ml, 31.2 mmol / l, 25 mols / l and 70 μl.

The irradiation time is a critical point for the success of PDT. If the photosensitizer is not close to the area of interest, its activation by light will result in the formation of toxic species in an undesired place. The times used in antimicrobial PDT vary from 1 to 10 minutes.

The time used in the selected studies ranged from 3 to 10 minutes. Three studies compared the irradiation time. Yildirim et al. varied the time in 1, 2 and 4min and showed that the irradiation for 1min is sufficient to achieve the efficiency of the therapy. Silva et al. when comparing times 30, 60 and 120s, concluded that the reduction of strains of E. faecalis with PDT for more than 60s was effective. Already Xhevdet et al. altered the times 1, 3 and 5min and showed the best results with 5min and 3min laser irradiation.

Currently, diode lasers are the most commonly used light sources in PDT, emitting light at wavelengths between 630nm and 690nm (red) at low intensity. Most of the authors of this journal used diode laser at an exposure to red light of 660nm and 665nm. Sebrão et al. compared the methylene blue associated with the red laser (660 nm) as a source of excitation with the pink bengal associated with the green laser (532 nm), verified that PDT can be improved if pink bengal is used in association with a green laser source for the inactivation of E. faecalis.

The interesting study by Bolhari et al. analyzed PDT in combination with Chlorhexidine 2.0% (CHX) as conventional therapy in counting colonies and expression patterns of genes associated with E. faecalis biofilm formation. They used the groups with the green photosensitizer indoindocianina; laser diode; PDT; CHX 2.0%; and PDT with 2.0% CHX-modified photosensitizer and concluded that the synergism effect of indocyanine-PDT with 2.0% CHX leads to virulence modulation of the E. faecalis biofilm model, suppressing the expression of genes associated with biofilm formation.

An alternative source of light for the PDT are the LEDs (light emitting diodes), which can be used as a source of activation in PDT. Because they have advantages as a lower cost, are portable, consume little electricity and have a longer life span than laser systems, LEDs have been discussed in recent studies as a source of light in PDT. Muhammad et al. compared the LEDA septim Plus® as a 650nm diode laser and Passive Ultrasonic Irrigation (PUI) and verified that there was no statistically significant difference between the results obtained in the Aseptim Plus®
and diode laser groups. They further added that although PDT may play a role in the destruction of the biofilm and in the decrease of the bacterial load of the intact microbial biofilm, these effects are statistically inferior to those obtained by the PUI.

Conclusion

According to the selection criteria of this literature review, the studies have shown promising results regarding the use of PDT as a coadjuvant in conventional endodontic treatment and its antimicrobial potential against E. faecalis, with the advantages of being selective, easy to apply and not promoting bacterial resistance.

However, a protocol for the use of PDT in daily clinical practice regarding the choice of the best photosensitizer and its concentration, light sources and parameters, wavelength and time of exposure has not yet been well defined. Therefore, more studies are needed to improve the PDT protocol in endodontics.

References