Evaluation the Effects of Led Photo-Activated Disinfection on Periodontal Clinical Parameters in Patients with Chronic Periodontitis

Introduction

Periodontal disease is an inflammatory disease with a complex aetiology, among which the bacteria play an essential role. The breakdown of the periodontal tissue is initiated by the host response to bacterial activity and virulence factors. There are various parameters to quantify the inflammation and the tissue lost, such as: bleeding on probing, clinical attachment loss, probing depth, tooth mobility.

It is well known that specific periodontal therapy includes 3 major steps: etiologic therapy, corrective therapy and maintaining therapy, individualized for each patient. The etiologic therapy combines different methods for bacterial plaque removal (supra-gingival and sub-gingival calculus removal, root planing, periodontal curettage). Adjunctive therapy methods can be added to the standard measures of etiologic therapy, including the antibiotic therapy or the host defence modulators. In the last decade a new method was emphasized: the photodynamic therapy.

The photodynamic therapy involves 3 major components: the visible light, the oxygen and a nontoxic photosensitizer (a photo-actable substance). The photosensitizer binds to the target cells and is activated by the light source, producing singlet oxygen and other reactive agents, highly toxic to bacteria\textsuperscript{1,2}. The excited singlet oxygen can oxidize many biological molecules (proteins, nucleic acids and lipids), leading to its cytotoxicity. Singlet oxygen has a diffusion distance of approximately 100nm and a half-life of <0.04 ls\textsuperscript{3}. The photodynamic activity is influenced by the type, the dose, the incubation time and the localization of the photosensitizer, the wavelength of the light source (nm), the light power density (mW/cm\textsuperscript{2}) and the light energy fluency (J/cm\textsuperscript{2}). In this type of therapy the toxic effect is mainly due to the damage of the cytoplasmic membrane and of the DNA\textsuperscript{4}.
The ideal photosensitizer should present the following properties: a high quantum yield of triplet state to obtain large concentrations of the activated drug; a high single oxygen quantum yield; high binding affinity for microorganisms; a broad spectrum of action; low binding affinity for mammalian cells to avoid the risk of photodestruction of host tissues; a low propensity for selecting resistant bacterial strains; a minimal risk of promoting mutagenic processes; and low chemical toxicity.

Toluidine blue O (tolonium chloride) is a vital dye used for detecting the mucosal abnormalities of the uterine cervix and oral cavity and for demarcating the extent of the lesion before surgery. Toluidine blue O (Fig. 1), which undergoes a pronounced cationic charge, can bind to the outer membrane of G-bacteria, penetrating the bacterial cells. It has been demonstrated in vitro that toluidine blue O interacts with bacterial lipopolysaccharides, with a great photo-bactericidal effect.

**Figure 1. Chemical structure of the phenothiazine photosensitizer toluidine blue O: C15H16N3SCl (tolonium chloride, basic blue 17, blutene chloride and methylene blue T50 or T extra)**

The purpose of our study was to assess the clinical efficiency of the adjunctive photodynamic therapy in the etiologic periodontal treatment, associated to classic mechanical bacterial plaque removal.

**Materials and Method**

The present study was conducted in the Periodontology Clinic of “Gr.T.Popa” University of Medicine and Pharmacy in Iași. The research methodology respected the international standard. The experiments were conducted according to the ethical directives of the Helsinki Declaration and the methods were certified for clinical and para-clinical use. Information and confirmation principles for research purposes were strictly respected; the signed informed consent for study inclusion was obtained from each patient.

We recruited 72 patients with chronic periodontitis, divided in 2 groups: the study group and the control group. The patients with periodontal therapy in the last 12 months or with antibiotic therapy in the last 6 months, patients with inflammatory or infectious systemic diseases, patients taking various types of drugs, which can affect the periodontal status, and smokers were excluded from the study.

Each subject received a rigorous clinical examination; the periodontal clinical indexes (bleeding on probing, periodontal pocket depth, clinical attachment loss) were recorded before the treatment; all the measurements were conducted with periodontal probes (Williams). The bleeding on probing (BOP) was quantified as follows: 0 (no gingival bleeding), 1 (point of bleeding), 2 (linear bleeding), 3 (triangular bleeding) and 4 (drop of blood). The probing depth was registered for all the teeth present in the dental arch, measured in 6 points per tooth (mesial-facial, middle-facial, distal-facial, mesial-oral, middle-oral, distal-oral), from the free gingival margin to the base of the pocket; the measurements higher than 3mm per site were considered as pathological. The clinical attachment loss was measured from the amelo-cemental junction to the base of the periodontal pocket. The periodontal diagnosis was set after the completion of the clinical examination. The method for dividing the groups was randomized.

The study group received etiologic therapy (supra- and sub-gingival scaling, root planing, professional brushing), followed by photo-activated disinfection of the periodontal pockets (LED PAD therapy). The control group received only etiological standard therapy (supra- and sub-gingival scaling, root planing, professional brushing), without photo-activated disinfection.

The LED source used in this study was in the red spectrum (wavelength of 635nm, Denfotex UK) and a viscous solution of tolonium chloride 0.01mg/ml provided by the manufacturer served as a photosensitizer (Denfotex UK). We followed all the steps from the operatory protocol, according to the manufacturer’s recommendations. After the tooth isolation, the photosensitizer was meticulously placed in the periodontal pockets, followed by a LED irradiation for 60 seconds. For the periodontal pockets with a depth higher than 5 mm we used a special Perio-tip for the light source. During photo-activation we used protection goggles for the protection of the patient and of the medical staff.

The PAD therapy was repeated at 7, 14 and 21 days from the first session. The patients were recalled after 2 months for re-assessment. The periodontal clinical parameters were also re-assessed.

The baseline and data registered after 2 months were statistically analysed; for the statistical analysis we used the Microsoft Excel 2010 and PASW 18 Statistics softwares.

**Results and Discussion**

All 72 subjects were divided in the study group (n=35) and the control group (n=37). There were 48 males
and 24 females. The age of the subjects in the study group ranged between 31 and 75 years (with a mean value of 47.3 ± 3.9 years) and in the control group, it was between 36 and 68 years (with a mean value of 49.6 ± 2.8 years).

After 2 months, we observed a significant improvement of the periodontal parameters. Decreased values for the probing depth and for the BOP were noticed for both groups, with higher differences for the study group than the control group. We also remarked a gain of periodontal clinical attachment, more significant for the study group. The clinical statistic results are presented in table 1.

The results of our study support a series of published data reporting positive results of the photo-activation therapy. Improved results were observed in a study using PAD with methylene blue on chronic periodontitis patients8. The same favourable results were obtained in another study with LED PAD and phenothiazine chloride9.

Our re-evaluation results contradict other studies that did not observe a clear advantage of laser PAD in chronic periodontitis patients. A study conducted on patients with chronic periodontitis who received only one session of LED PAD and toluidine blue therapy did not reveal significant changes of the clinical parameters10. Another study demonstrated that PAD therapy reduces a series of clinical parameters but not the BOP, demonstrating a non-significant diminishing of the inflammation degree11. The optimal parameters required for effective antimicrobial photodynamic therapy-induced killing of supra-gingival periodontal pathogens using the combination of different toluidine blue O concentrations and laser-irradiation energies were investigated and reported that diode laser irradiation at 12 J/cm² with 1 mg/ml of toluidine blue O was the most effective option12. The differences between the various studies can be explained by the different study designs, by the different types of activation sources and by the high variety of photo-sensitizers.

It was also demonstrated in vitro that lipopolysaccharide treated by photodynamic therapy did not stimulate the production of pro-inflammatory cytokines by mononuclear cells13; thus, photodynamic therapy may be inactivating endotoxins, such as lipopolysaccharide, by decreasing their biological activity.

### Conclusions

The photo-activated disinfection therapy of the periodontal pockets proves itself as a viable adjunctive method to the classical mechanical plaque removal in chronic periodontitis patients; the LED source is also less aggressive than the usual lasers, providing a safer and more accessible method; the association of toluidine blue O determines significant improvement of the periodontal clinical parameters (BOP, clinical attachment loss and pocket depths).

### Table 1. The changes of clinical parameters of the study and the control group

<table>
<thead>
<tr>
<th>Periodontal parameter</th>
<th>Study group</th>
<th>Control group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BOP decrease</td>
<td>68%</td>
<td>53%</td>
<td>p&lt;0.03</td>
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<tr>
<td>Pocket depth</td>
<td>1.24 mm</td>
<td>0.57 mm</td>
<td>p&lt;0.05</td>
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<tr>
<td>Gain of clinical</td>
<td>0.37 mm</td>
<td>0.14 mm</td>
<td>p&lt;0.04</td>
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<tr>
<td>attachment</td>
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### References


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