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Photodynamic Therapy in the Treatment of Osteomyelitis

João Paulo Tardivo[1] and Mauricio S. Baptista[2]
[1]*Faculdade de Medicina do ABC, São Paulo*
[2]*Departamento de Bioquímica, Instituto de Química da USP, São Paulo*
Brazil

1. Introduction

Osteomyelitis is a bone infection that may initiate due to a local trauma or infection or it may originate due to infections occurring elsewhere in the body (microorganisms are transported by the bloodstream in this case). The bone may be predisposed to infection because of trauma or because of a medical condition like diabetes. The microorganisms responsible for the infection are usually pyogenic bacteria. The most frequent infectious agents are in order of prevalence *Staphylococcus aureus*, *Streptococcus* (groups A and B), *Haemophilus influenzae*, and *Enterobacter species* [1].

Osteomyelitis is highly prevalent in patients with diabetes (extremity bones), patients with fractured and exposed bones and with bone systems that have continuous internal or external trauma/aggression/inflammation (vertebrae in adults). In theory any bone may develop the disease. Extremity bones (arms and legs, especially in children) and spine (vertebral column, especially in adults) are the most affected. In diabetic patients, a large percentage of the patients who have foot ulcers developed osteomyelitis in extremity bones (feet and toes) [1].

Patients with diabetes usually have chronic diseases in the lower ends because they have a higher incidence of vascular failure and reduced sensitivity to pain (diabetic neuropathy). Consequently, they may have skin lesions called skin ulcers that grow in length and in depth, often without the patient realizing it, due to the absence of pain. These ulcers greatly increase the chance of other complications, like soft-tissue infection, tissue infection that may culminate in osteomyelitis and some sort of amputation due to infection that installs both in soft and bone tissues. It is known that diabetic patients have lower immunity responses than the population in general and added with the poor peripheral blood circulation, become more vulnerable to infections, which are more difficult to respond to the treatment. 10% of diabetic patients will develop some sort of foot ulcers followed by amputation. 85% of amputations are preceded by a foot ulcer and 70-100% of the ulcers show signs of peripheral neuropathy associated with various degrees of commitment of the peripheral vasculature [2].

The mortality rate is usually low, unless sepsis occurs. The severity of the disease increases with the dissemination of the infection to surrounding tissues and articular cavity; evolution to chronic osteomyelitis, amputation of the involved extremity, generalized infection or

sepsis. It is a disease that can only be treated with long-term systemic and local antiobiotics treatments [1,3]

Photodynamic therapy (PDT) is a promising clinical modality for the management of various tumors and nonmalignant diseases, due to two main effects that are induced by photo-activation of specific drugs called photosensitizers: cell killing properties [4-12] and its ability to modulate the immune response [13, 14]. PDT is based on an excited-state process called photosensitization (see below), which is a combination of a photosensitizer that is selectively localized in the target tissue and illumination of the lesion with visible light, resulting in photodamage and subsequent cell death (microbial killing). The first time the photodynamic effect shown was in the area of microbiology. Raab conducted in-vitro experiments showing the killing of paramecium parasites by PDT [15]. Several other papers were published showing the photo-induced killing of microorganisns [4]. However, development in this area was bunged for several decades because of the discovery of penicillin. With the growing problem of bacterial antibiotic resistance in the last 15 years, there was a resurge in the research focusing in the anti-microbial application of PDT. Currently, PDT is a potential approach to treat localized infectious diseases mainly due microbial resistance. Numerous worldwide clinical studies have shown that PDT represents an effective and safe modality for various types of diseases [4-9], including osteomyelitis [10-12].

2. Mechanisms and drug development in PDT

The cell killing mechanism of PDT is based on the process called photosensitization (Figure 1), which involves a molecule that absorbs light and gets excited from the ground-state (PS) into singlet excited state ($^1PS^*$), which is short-lived ($\cong 10^{-9}$ seconds) and can be quickly deactivated by radiative and non-radiave processes before it has time to react. A good photosensitizer (PS) will undergo a photophysical process called intersystem crossing, which converts the singlet state to a triplet state ($^3PS^1$). Triplet states cannot relax efficiently having a higher tendency to donate or accept electrons, being therefore both stronger reducing and oxidant agents compared to the ground state. Triplets also have a large tendency to engage in energy transfer reactions especially with oxygen. When triplet states are disabled by electron transfer reactions, it originates radical species, in mechanisms that are called type I. These radicals can initiate radical chain reactions leading to damage in lipids, proteins and nucleic acids. In oxygenated environments the photosensitizers can undergo type II photochemical process that involves energy transfer between the excited triplet state of the photosensitizer and stable triplet oxygen ($^3O_2$), producing short lived and highly reactive singlet oxygen ($^1O_2$) [15-17].

Therefore, one can separate the mechanisms of PDT in two classes. The mechanism TYPE I: light energy passes from triplet excited molecules to biomolecules by electron transfer reactions (radical mechanism) that culminates in direct damage to biomolecules or the formation of reactive oxygen species. TYPE II mechanism in the excitation energy is transferred to molecular oxygen, resulting in the formation of singlet oxygen ($^1O_2$), which is highly electrophilic and capable of causing damage to membranes, proteins and DNA [16-18]. The actual chemical mechanism that takes place in cells will depend on the specific microenvironment of the photosensitizer, and clearly depend on specific interactions with membranes and proteins [18-24]. Although most photosensitizer can bind DNA and can

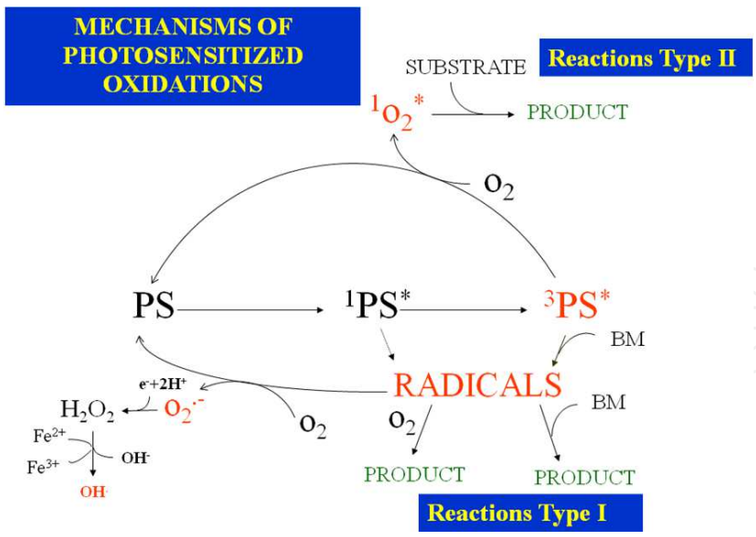


Fig. 1. Photosensitization mechanisms where PS is a photosensitizer that absorbs light going to the first singlet state ($^1PS^*$), converting into a triplet state ($^3PS^*$) by intersystem crossing. The excited species, specially $^3PS^*$ can react by electron transfer forming radical species (Type I mechanism) and starting radical chain reactions or react with oxygen by energy transfer forming Singlet oxygen (Type II mechanism).

photo-damage it in *in-vitro* solution-based experiments, the charged photosensitizers in use do not enter and do not accumulate in cell nuclei, decreasing the possibility of mutagenesis. Adjuvants like hydrogen peroxide can increase the photodynamic efficiency by allowing better uptake of the photosensitizer [25]. Also, nanoparticle platforms have a great potential to control the mechanisms and efficiency of photodynamic action [26-30].

Penetration of light through the skin is dependent on the characteristics of the treated tissue, the absorption by endogenous chromospheres and the scattering of the skin tissues. Wavelengths shorter than 600 nm are absorbed mainly by hemoglobin and other skin choromophores, whereas vibration overtones of water and other molecules induce absorption at wavelengths longer than 950 nm. Tissue scattering, which decreases as the wavelength increases, hinders light penetration of shorter wavelength photons. Forward directed scattering allows for penetration even in high absorbing and scattering tissues. By considering all these processes one can explain light penetration depth (10-20 mm) inside the tissue in the therapeutic window, which is light wavelength from 600 to 950 nm [16]. Therefore, one should choose a photosensitizer that absorbs in the therapeutic window in order to treat internal tissues by PDT. The light dose, which is usually given in joules per square centimeter, is empirical and varies widely. For interstitial applications, radiant exposures between 20 and 300 J/cm2 are needed [5-10, 16].

As mentioned above the first report of PDT action was of a dye (Acridine), which was used to kill a microorganism (paramecium) under illumination [15, 31]. Just few years after the experiment of Raab, the photodynamic concept was applied to dermatology using another dye (eosin) [31]. In 1912 Friedrich Betz Meyer published results of an experiment he did on himself, by injecting himself with a mixture of porphyrin oligomers and showed that he only had erythema reaction in tissues that were illuminated with light, proving that the photodynamic effect depends on light and on photosensitizer [31, 32].

In the 70´s-80´s the protocols developed by Dougherty and his group used a photosensitizer similar to the one Meyer injected himself with, which was called hematoporphyrin derivative (HPD, later called PhotofrinR, Figure 2D). HPD showed great potential to treat tumors, leading to the PDT approval by FDA as a treating modality for head and neck cancers [5, 33]. The protocols developed by Dougherty´s group rely on expensive laser systems, being restricted to only very few research centers in the world. PhotofrinR targets mainly the leakage vasculature of tumor tissues [33, 34]. Intracellular photosensitizers were subsequently tested clinically and have shown to be potentially more efficient. Oseroff and co-workers were the first to propose the strategy of targeting intracellular organelles instead of the tumor vasculature [35].

In the clinical realm, new generation photosensitizers were designed to have intracellular targets. A successful example was Foscan (Tetra-meso hydroxyphenyl chlorin, Figure 2F), which is a powerful intra-cellular photosensitizer. Its high photodynamic efficiency has been explained by the photooxidation of intracellular targets, because the quantum yields of singlet oxygen production is comparable or inferior to other relevant photosensitizers [36, 37]. The intracellular generation of protoporphyrin IX by administration of its metabolic precursors (ALA), was another successful example that helped to expand the applications of PDT (Figure 2E). Today almost any dermatologist around the world knows and uses PDT with ALA to treat a variety of skin diseases.

It is important to mention that although being more efficient than the initial protocols, the available PDT treatments are still very expensive and consequently are nor very useful in terms of public health, especially for developing and under-developed countries**.** In fact, during three decades of clinical PDT, most of the treating protocols were based on the use of hematoporphyrin and its derivatives, irradiated with lasers or very sophisticated non-coherent light sources [5,16]. The combination of inexpensive photosensitizers and light sources have allowed the development of new PDT protocol that are inexpensive, safe and efficient [6-10]. Therefore, we can conclude that there is a great potential for the widespread use of PDT even in underserved populations.

The concept of photodynamic cell killing was soon extended to treat infectious diseases, i.e., its photoantimicrobial action [4, 38]. The effect of the generation of large amounts of radicals and non-radicals oxidizing species on microbials is extremely destructive for two main reasons: lack of effective microbial defenses against these species and multiple sites of attack. Although cells have several natural defenses against reactive species, the level of redox imbalance inflicted by PDT is several orders of magnitude larger than the level or protection allowed by enzymatic and molecular antioxidants within the cell. Besides that, bacteria can defend itself against superoxide radical and hydrogen peroxide, but hydroxyl radical and singlet oxygen cannot be naturally deactivated. Consequently, an important advantage of PDT is the absence of microbial resistance [4, 38].

The development of improved drugs in any pharmaceutical application depends on the knowledge of structure activity-relationships and of the action mechanisms involved. We and others have invested a lot of efforts on solving these issues related with PDT [6,16-25]. New molecular structures, nanoparticles or nanoemulsions are being investigated in order to develop new and more efficient photo-active drugs [18-30]. Several medical conditions were treated by different protocols and depending on the medical condition, specific molecules and delivery systems were designed [5-10]. There are several low-cost options to

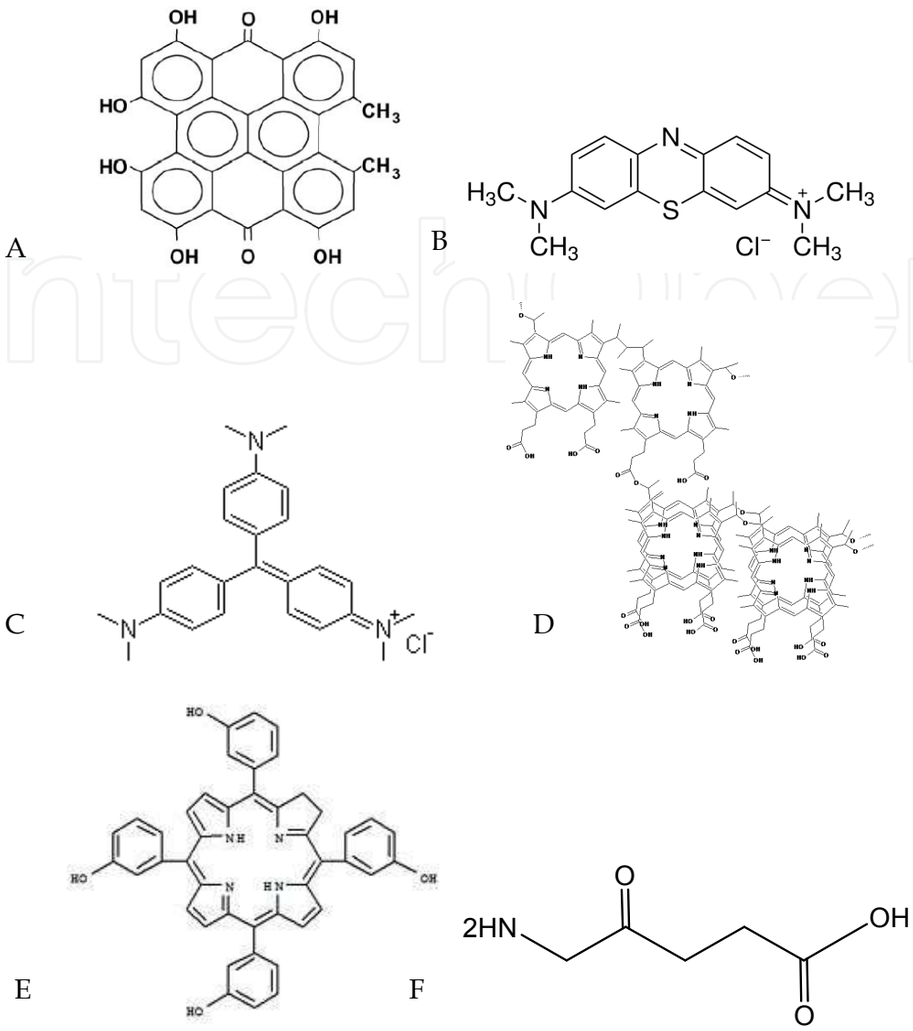


Fig. 2. Examples of photosensitizers used in PDT, from A to E, hypericin, methylene blue, crystal violet, hematoporphyrin derivative and tetra-meso-hydroxyphenyl-chlorin (FoscanR). Compound labeled F is 5-aminolevulinic acid (ALA), which is a pro-drug that is metabolized in the intra-cellular environment to the actual photosensitizer that is Protoporphyrin IX.

be considered as photosensitizers in PDT, which include hypericin, methylene blue and other phenothiazines, crystal violet and other tryarilmethanes (Figure 2A-C).

3. PDT in the case of osteomyelitis

The classical treatment of osteomyelitis requires surgical removal of the diseased tissue and high doses of antibiotics, which are often nephrotoxic, further compromising the health of patients. In-vitro and animal studies in the laboratory have shown the potential of PDT for causing efficient cell death of infecting bacteria as well as treating animals with the disease [11,12]. Low-cost PDT protocols were also tested with success in diabetic patients with osteomyelitis [10].

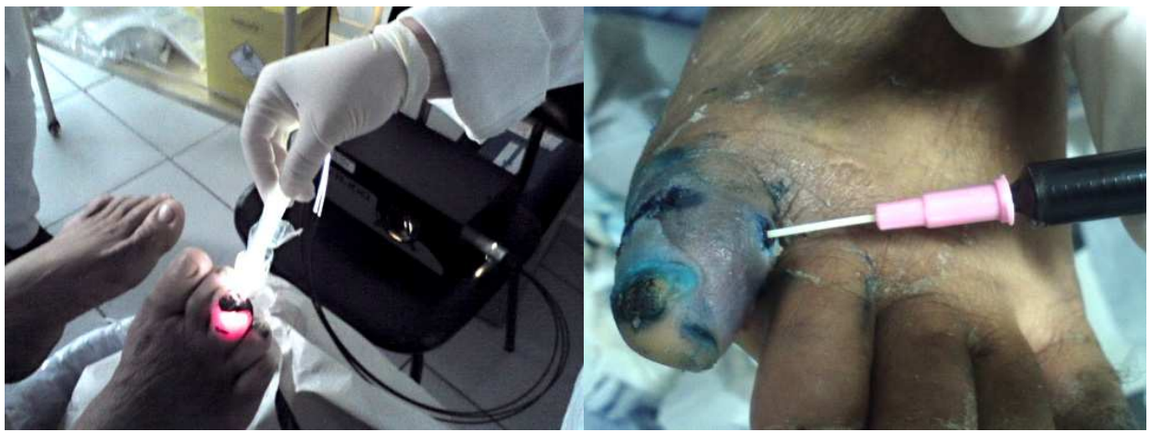


Fig. 3. Two osteomyelitis patients that were selected in the clinical studies conducted by JP Tardivo in the Hospital da Fundação ABC. The picture in the left shows how Methylene Blue solution is injected in the bone through the fistula. In the right the illumination with optical fiber conducted by the continuous white-light source (FASA).

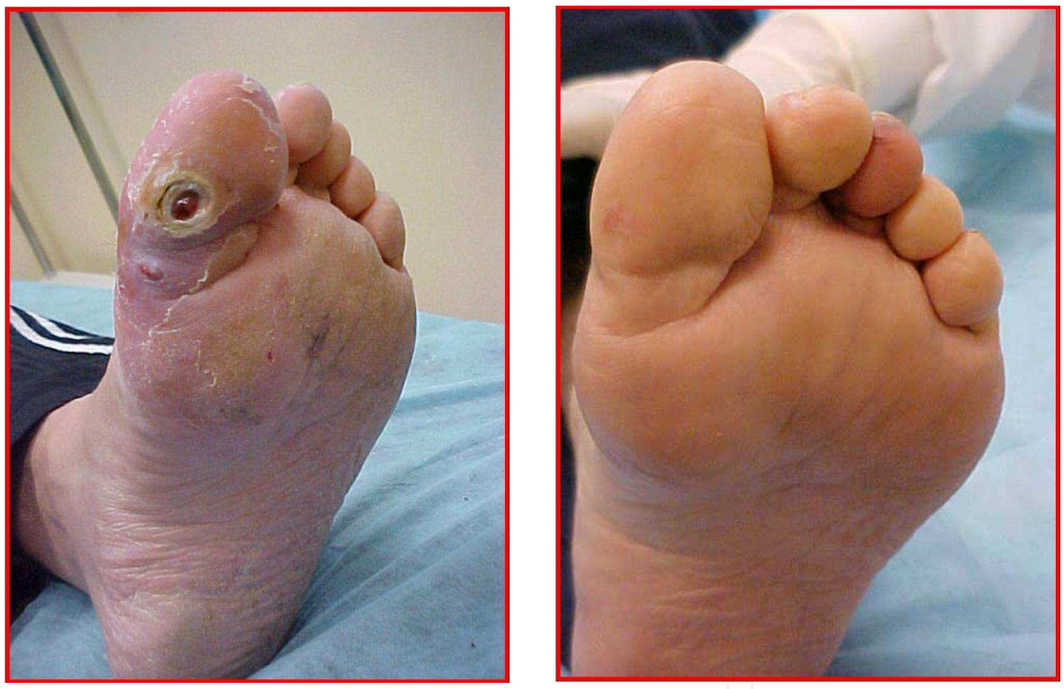


Fig. 4. Plantar region of the thumb of a diabetic patient with osteomyelitis pre and post treatment with PDT. A 1:1 mixture of MB 2% and TB 2% was injected into the patient's great toe. Further details of treatment and conditions are described in **Reference 10**.

PDT in osteomyelitis is intended to eliminate any micro-organism that is installed in bone tissue. In order to combat infection in the bone and soft tissue a photosensitizer solution has to be introduced in the lesion tissue that must be subsequently illuminated (Figures 3, 4). Usually this can be easily realized by administrating the photosensitizer by the drainage of the skin fistulas, which reaches the bone (Figure 3). Illumination should be conducted for around 10 minutes to allow a light dose of around ~20-30 J/cm2, by internal and external

irradiation. Internal irradiation is obtained by introducing optical fibers delivering light directly in the bone and external irradiation with non-coherent polychromatic light sources (Figures 3 and 4). Patients are only accompanied in the ambulatory throughout the treatment, which usually lasts for several weeks. In that period, spontaneously removal of bone fragments was observed by radiographies, fistulas were healed and patient were considered cured (Figures 3 and 4). In the clinical studies conducted so far, 12 patients were treated with success and 8 are being treated. The patients selected in this clinical study had not responded well to usual antibiotic therapy and had had indication of amputation.

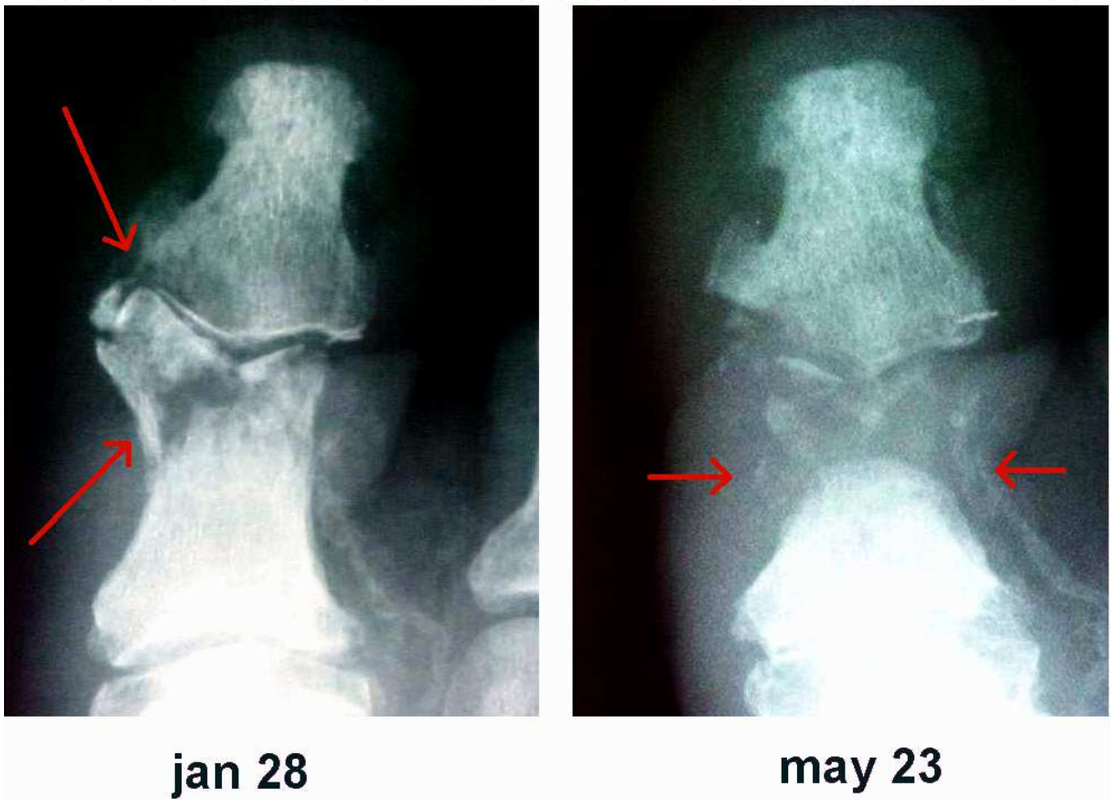


Fig. 5. Radiography of great toe of P1 before (A) and after (B) PACT treatment. Arrows in A: fractures in distal and proximal phalanges. Arrows in B: bone fragments spontaneously removed after treatment. A 1:1 mixture of MB 2% and TB 2% was injected into the patient's great toe. Further details of treatment and conditions are described in **Reference 10**.

4. Conclusions

Photodynamic therapy reduces the risk of amputation, causing cell death to microorganisms that are infecting bone tissues and consequently accelerate healing of osteomyelitis lesions. It also reduces inflammation and pain in the tissue ulcers. PDT provides an efficient and quick treatment that helps avoiding amputations. The physiological/cosmetic outcomes are impressive with small side effects (small pain during treatment is sometimes reported). Besides all these benefits, PDT may be performed in office or clinic and low-cost protocols are available. Consequently, PDT can clearly help to improve the quality of life of diabetic patients.

5. References

[1] Lew, D.P.; Waldvogel, F.A. Osteomyelitis. *N Engl J Med* 1997, 336, 999-1007.

[2] Diabetic Foot, International Consensus, 2003; http://www.iwgdf.org.

[3] Calhoun, J.H.; Mader, J.T. Treatment of Osteomyelitis With a Biodegradable Antibiotic Implant. Clin *Orthop Related Res* 1997, 341, 206-214.

[4] Wainwright, M. Photodynamic antimicrobial chemotherapy (PACT). *J Antimicrob Chemother* 1998, 42, 13-28.

[5] Dougherty T.J. Photochemistry in the treatment of cancer in *Advances in Photochemistry*, Vol. 17, Eds. D.H. Volman, G.S. Hammond and D.C. Neckers, 275-311, Wiley: New York, 1992.

[6] Tardivo, J.P.; Del Giglio, A.; Oliveira, C.S.; Gabrielli, D.S.; Junqueira, H.C.; Tada, D.B.; Severino, D.; Turchiello, R., Baptista, MS. Methylene Blue in Photodynamic Therapy: From Basic Mechanisms to Clinical Applications *Photodiag Photodyn Ther* 2005, 2/3, 175-191.

[7] Tardivo JP; Del Giglio A; Paschoal LH; Baptista MS; A New PDT protocol to treat AIDS-related Kaposi's sarcoma, *Photomed Laser Surg* 2006, 24 (4), 528-531.

[8] Tardivo, J.P., Del Giglio A., Paschoal, L.H.C., Ito A.S., Baptista, M.S., "Treatment of Melanoma Lesions Using Methylene Blue And RL50 Light Source" *Photodiag Photodyn Ther* 2004, 1, 345-346.

[9] Baptista, M.S.; Wainwright, M. Photodynamic antimicrobial chemotherapy (PACT) for the treatment of malaria, leishmaniasis and trypanosomiasis *Braz J Med Biol Res* 2011, 44(1) 1-10.

[10] Tardivo, J.P.; Baptista, M.S. Treatment of Osteomyelitis in the Feet of Diabetic Patients by Photodynamic Antimicrobial Chemotherapy *Photomed Laser Surg* 2009, *27,* 145-150.

[11] Bisland, S.K.; Chien, C.; Wilson, B.C.; Burch, S. Pre-clinical *in vitro* and *in vivo* studies to examine the potential use of photodynamic therapy in the treatment of osteomyelitis *Photochem. Photobiol. Sci.*, 2006, *5*, 31–38.

[12] Goto, B.; Iriuchishima, T.; Horaguchi, T.; Tokuhashi, Y. Nagai, Y.; Harada, T.; Saito, A.; Aizawa, S.Therapeutic Effect of Photodynamic Therapy Using Na-Pheophorbide a on Osteomyelitis Models in Rats. *Photomed Laser Surg* 2011, *29*, 183–189.

[13] Pawel, M.; Huang, Y.-Y.; Hamblin, M.R. "Photodynamic therapy for cancer and activation of immune response." Biophotonics and Immune Responses V. Ed. Wei R. Chen. San Francisco, California, USA: SPIE, 2010. 756503-8.

[14] Ana P. Castano, A.P.; Mroz, P.; Hamblin, M.R. Photodynamic therapy and anti-tumour immunity *Nat Rev Cance*r 2006, 6(7): 535–545.

[15] Raab, O. Action of fluorescent materials on infusorial substances *Zeitschrift fuer Biologie* 1900, 39, 524-546.

[16] Ochsner M. Photophysical and photobiological processes in the photodynamic therapy of tumors. *J Photochem Photobiol B* 1997, 39:1-18.

[17] Castano, A.P.; Demidova, T.N.; Hamblin, M.R. Mechanisms in photodynamic therapy: Part three—Photosensitizer pharmacokinetics, biodistribution, tumor localization and modes of tumor destruction *Photodiag. Photodyn. Ther.* 2005, *2*, 91-106.

[18] Baptista, M.S.; Indig, G.L. Effect of BSA Binding on Photophysical and Photochemical Properties of Triarylmethane Dyes *J Phys Chem B* 1998, *102*, 4678-4688.

[19] Junqueira, H. C.; Severino, D.; Dias, L.G.; Gugliotti, M. ; Baptista, M. S. "Modulation of the Methylene Blue Photochemical Properties Based on the Adsorption at Aqueous Micelle Interfaces" *Phys Chem Chem Phys* 2002, 4, 2320-2328.

[20] Gabrieli, D.; Belisle, E.; Severino, D.; Kowaltowski, A.J.; Baptista, M.S. Binding, aggregation and photochemical properties of methylene blue in mitochondrial suspensions *Photochem. Photobiol*. 2004, 79, 227-232.

[21] Engelmann, F. M.; Mayer I.; Gabrielli, D.; Araki, K.; Toma H. E.; Kowaltowski, A.; Baptista, M. S. Interactions of Cationic *Meso*-Porphyrins with biomembranes *J Bioenerg Biomembr* 2007, 39(2), 175-185.

[22] Engelmann, F.M.; Mayer, I.; Araki, K.; Toma, H.E.; Baptista, M.S.; Maeda, H.; Osuka, A.; Furuta, H. "Photochemistry of Doubly N-confused porphyrin bond to non-conventional high oxidation state Ag(III) and Cu(III) ions" *J. Photochem.Photobiol.A: Chemistry* 2004, 163, 403-411.

[23] Pavani, C.; Uchoa, A.F.; Oliveira, C.S; Iamamoto, Y.; Baptista, M.S. Effect of zinc insertion and hydrophobicity on the membrane interactions and PDT activity of porphyrin photosensitizers *Photochem. Photobiol. Sci.* 2009, *8*, 233–240.

[24] Uchoa, A.F., Oliveira, C.S., Baptista, MS Relationship between structure and photoactivity of porphyrins *J Porph Phthaloc* 2010, 14: 832–845.

[25] Garcez, *A.S;* Núñez, *S.C.* Baptista, *M.s.;* Daghastanli, *N.A.;* Itri, *R;* Hamblin, M.R.; Ribeiro, M.S Antimicrobial mechanisms behind photodynamic effect in the presence of hydrogen peroxide *Photochem. Photobiol. Sci.* 2011, 10, 483–490, 483-490.

[26] Tada, D.B.; Rossi, L.M.; Leite, C.A.P.; Itri, R.; Baptista, M.S.. Nanoparticle platform to modulate reaction mechanism of phenothiazine photosensitizers *J Nanosci Nanotech* 2010, 10, 3100-3108.

[27] Engelmann, F.M.; Rocha, S.V.O.; Toma, E.; Araki, K.; Baptista, M.S. Determination of *n*-octanol/water partition coefficients and membrane binding of cationic porphyrins. *Int J Pharm* 2007, 329, 12-18.

[28] Tada, D.; Vono, L.L.R.; Duarte, E.Itri, R.; Kiyohara, P.K.; Baptista, M.S.; Rossi, L.M Methylene blue-containing silica coated magnetic particles: a potential magnetic carrier for photodynamic therapy, *Langmuir* 2007, *23,* 8194-8199.

[29] Rossi, L.M.; Silva, P.R.; Uchoa, A.F., Tada, D.B.; Baptista, M.S Protoporphyrin IX nanoparticle-carrier: preparation, optical properties and singlet oxygen generation. *Langmui*r 2008, *24*, 12534-12538.

[30] Deda, D.K.; Caritá, E.; Baptista, M.S.; Toma, H.E., Araki, K. A New Polymeric Nanocapsule Formulation with Potential Applications for thePDT Treatment of Skin Cancer *Int J Pharm* 2009, 376, 76-83.

[31] Roberts, D.J., Cairnduff, F. Photodynamic therapy of primary skin cancer: a review. *Brit. J. Plast. Surg.* 1995, *48*, 360-370.

[32] Meyer-Betz F. Untersuchungen uber die bioloische (photodynamische) wirkung des hamatoporphyrins und anderer derivative des blut-und gallenfarbstoffs. *Dtsch Arch Klin Med.* 1913, *112*, 476-503.

[33] Dougherty, T.J.; Kaufman, J.E.;.Goldfarb, A.; Weishaupt, K.R.; Boyle, D. Mittleman, A. Photoradiation Therapy for the Treatment of Malignant Tumors. *Cancer Res* 1978, *38*, 2628.

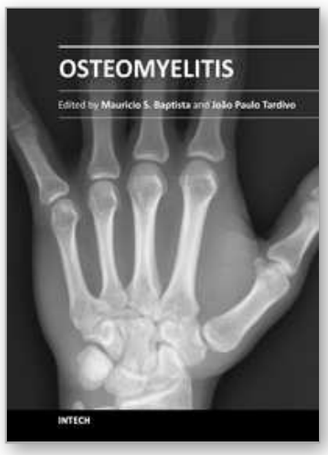
[34] Dougherty, T.J. Photodynamic therapy (PDT) of malignant tumors *Critical Reviews in Oncology/hematology* 1984, *2*(2):83-116.

[35] J.S. Modica-Napolitano, J. L. Joyal, G. Ara, A.R. Oseroff and J.R. Aprille Mitochondrial toxicity of cationic photosensitizers for photochemotherapy *Cancer Res* 1990, 50, 7876-7881.

[36] H. J. Jones, D. I. Vernon, S. B. Brown, Photodynamic therapy effect of *m*-THPC (Foscan®) *in vivo*: correlation with pharmacokinetics *Brit. J. Cancer* 2003, 89, 398–404.

[37] M-H.Teiten, L.Bezdetnaya, P.Morliere, R.Santus, F.Guillemin, Endoplasmic reticulum and Golgi apparatus are the preferential sites of Foscan® localisation in cultured tumour cells *British J. Cancer* 2003, 88, 146-152.

[38] Wainwright, M *Photosensitizers in Biomedicin*e, 1ed. Oxford:Wiley, 2009.



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If you want to learn more about osteomyelitis you should not miss this book. The editors are professionals and scientists working in health sciences and the chapters have been prepared by experts in the field, covering subjects related with the fundamentals of osteomyelitis and new diagnosis and treatment tools. You will have the opportunity to review concepts as well as to learn state-of-the-art alternatives for diagnosis and treatments.

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