Chapter 11

Inactivation of Malaria Parasites in Blood: PDT vs Inhibition of Hemozoin Formation

Régis Vanderesse, Ludovic Colombeau,
Céline Frochot and Samir Acherar

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/65053

Abstract

Malaria causes hundreds of thousands of human deaths every year, and the World Health Assembly has made it a priority. To help eliminate this disease, there is a pressing need for the development and implementation of new strategies to improve the prevention and treatment, due in part to antimalarial drug resistances. This chapter focuses on two strategies to inactivate the malaria parasite in blood, which are photodynamic therapy (PDT) and inhibition of hemozoin formation. The PDT strategy permits either a control of the proliferation of mosquito larvae to develop some photolarvicides for the prevention or a photoinactivation of the malaria parasite in red blood cells (RBCs) to minimize infection transmission by transfusion. The inhibition of hemozoin formation strategy is used for the development of new antimalarial drug by understanding its formation mechanism.

Keywords: hemozoin, photodynamic therapy, blood decontamination, heme-drug interaction, preventive treatment, curative treatment

1. Introduction

Malaria in humans is an infectious disease caused by parasites of the genus *Plasmodium*, and it is spread to humans by the bite of the female anopheles mosquito. Among the species of *Plasmodium*, five are capable of inducing human disease. These are the species: *falciparum*, *vivax*, *malariae*, *ovale*, and *knowlesi*. The first is the most widespread and the most virulent, which is responsible of 80% of infections and about 90% of deaths, especially in Africa.

In 2000, malaria was seen as one of the most critical constraints on global development and considered as a priority challenge of the "Millennium Development Goals" (MDGs). The main objective was to halt and begin to reverse the incidence of malaria by 2015 (Target 6C). The *World Malaria Report 2015* written by the World Health Organization (WHO) summarizes advances that have taken place in each WHO region over the 2000–2015 period [1]. Malaria is endemic in 95 countries mainly in Africa (88%). This report shows that this goal was achieved with an almost 37% and 60% drop in malaria incidence and in death rates, respectively, over this period. In 2015, 214 million cases of malaria were recorded including 438 000 that have led to the death of the patients, reflecting a decline of 18% and 48% in cases and deaths, respectively, over the 2000–2015 period. In May 2015, the *Global Technical Strategy for Malaria 2016–2030* was endorsed by the World Health Assembly. This strategy has its goal to reach a 90% reduction in global malaria incidence and mortality by 2030.

With regard to preventing malaria in countries at risk, the WHO recommends sleeping under an insecticide-treated mosquito net (ITN) and protecting by indoor residual spraying (IRS). Furthermore, the recommended treatment is an artemisinin-based combination therapy (ACT).

Despite a slight decrease, this disease remains a leading cause of death of children in Africa due in part to antimalarial drug resistances. Declines in cases and deaths caused by malaria are due to the development of new strategies such as the use of photodynamic therapy (PDT) for the control of the infection vector or to induce inactivation of *Plasmodium falciparum* and targeting the hemozoin inhibition, with the aim of preventing and treating malaria.

2. Inhibition of hemozoin formation

2.1. Generalities on the hemozoin production by *P. falciparum*

Hemoglobin, the main component of red blood cells (RBCs), represents almost 95% of the protein part of the cytosol (liquid fraction of the cell cytoplasm) up to reach 5 mM concentration in the cytoplasm (>300 mg/mL) [2]. Hemoglobin essential for cellular respiration is composed of a protein portion (globin) and a complex molecular structure centered on an iron atom (heme, ferriprotoporphyrin IX, Fe(II)PPIX which carries oxygen, and carbon dioxide from breathing).

During its life cycle in the red blood cell (RBC), the human malaria parasite (**Figure 1**), *P. falciparum*, gobbles up between 60 and 80% of hemoglobin from the host cell cytoplasm [3] by using a cytostome (cell mouth) for the purpose of transporting it to its acidic digestive food vacuole (pH ≈ 5.0–5.4 [4, 5]). At this acidic pH maintained by means of an ATPase pump enabling activation of a proton gradient, the hemoglobin is degraded into amino acids that are used for the production of parasite proteins, thereby allowing the release of free heme which is toxic to the parasite [6–9]. The hemoglobin degradation mechanism was studied in detail, and it was shown that it implies enzymes (proteases) present in the food vacuole of the parasite such as two aspartic (plasmepsins I and II) and cysteine (falcipain) proteases [10–14].

http://dx.doi.org/10.5772/65053

The heme detoxification is a crucial step for the survival and growth of the parasite [15]. Heme is assumed to generate the formation of reactive oxygen species (ROS), *via* the Fenton reaction catalyzed by its iron atom [16–18], and hydroxyl radicals that may lead to peroxidation of lipid membranes [19–21]. It was postulated also that specific heme-H_2O_2 reaction might produce free radicals [21] which may result in oxidation of lipids, proteins, and DNA [22, 23].

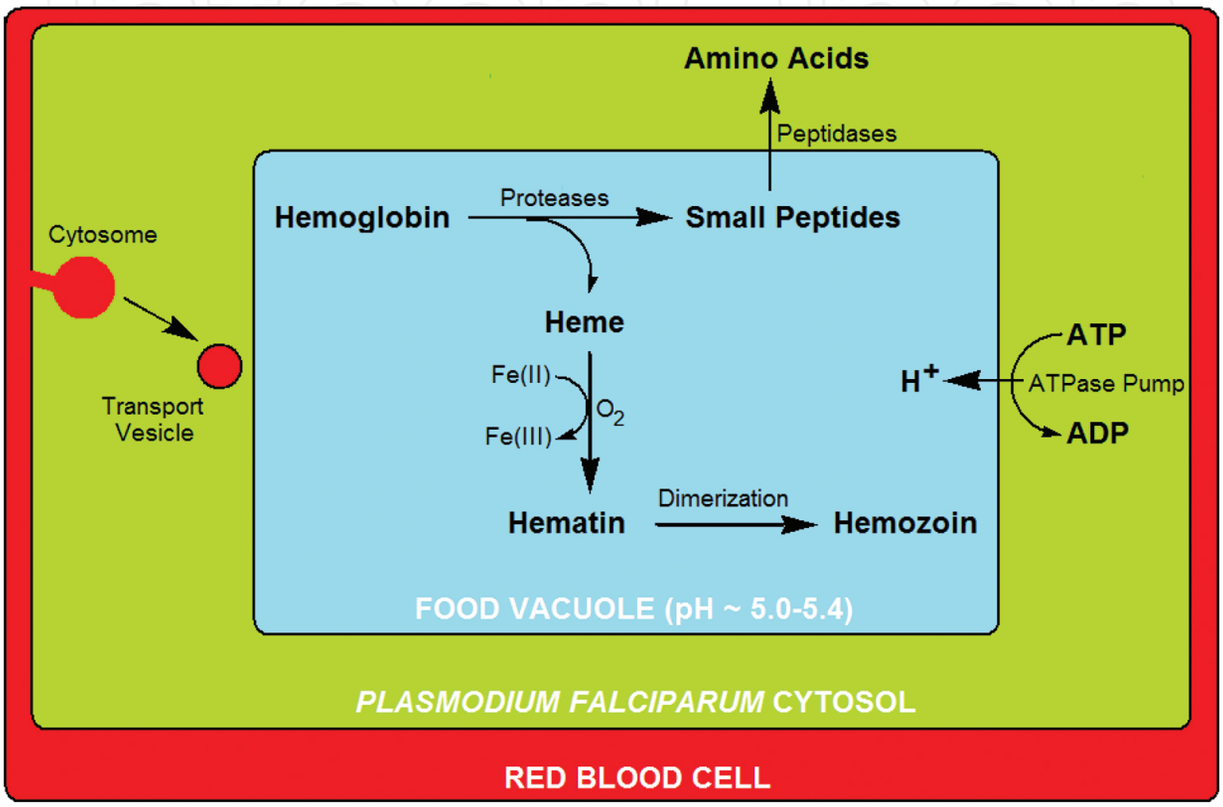

Figure 1. Hemoglobin degradation by *Plasmodium falciparum* in RBC.

The detoxification of heme begins with the self-oxidation of the Fe(II) in heme group into Fe(III) to form potentially toxic hydroxyferriprotoporphyrin IX (hematin, HO-Fe(III)PPIX; **Figure 2**) [8, 24, 25]. This detoxification ends with the formation of highly insoluble brown crystals known as hemozoin (malaria pigment; **Figure 2**) [26, 27] according to biomineralization or biocrystallization processes [28, 29] and not *via* a polymerization as previously believed; the x-ray structure is identical to a synthetic Fe(III)PPIX compound called β-hematin [30]. In 2000, the crystalline structure of β-hematin was determined to be a cyclic dimer of Fe(III)PPIX, involving two coordination bonds between the propionate side chain of one and the Fe(III) atom of the other [28]. These cyclic dimers are self-assembled in the crystal lattice *via* intermolecular hydrogen bonds which link the propionic acid side chains of each Fe(III)PPIX, thereby losing their toxic potential (heme detoxification), and then eliminated from the food vacuole. In 2010, the crystal structure of *P. falciparum* hemozoin has been solved by Klonis et al. after a reanalysis of x-ray crystallographic data for β-hematin [31].

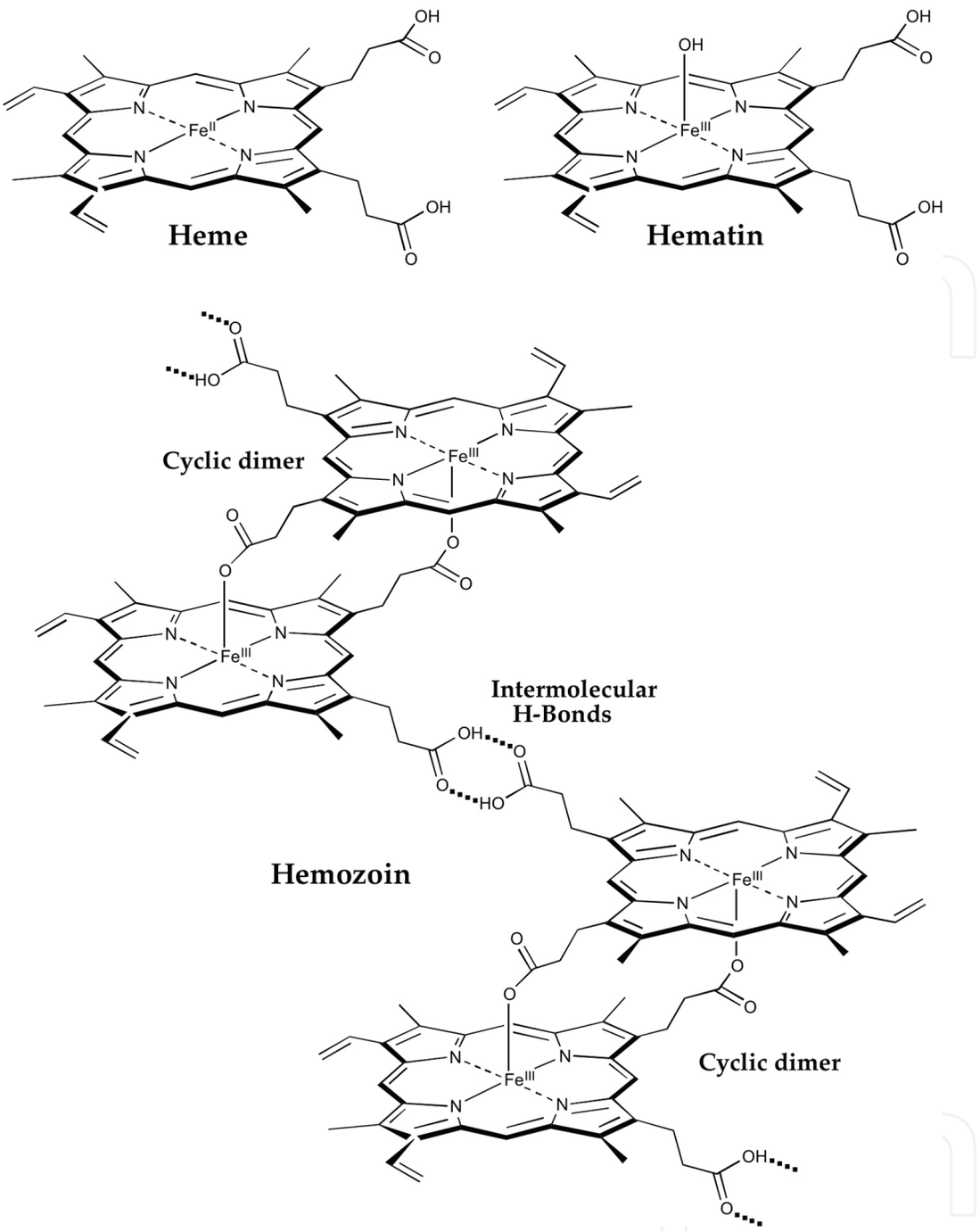

Figure 2. Chemical structure of heme, hematin, and hemozoin.

The mechanism concerning formation of β-hematin (hemozoin) in vivo and in vitro is still ambiguous and will be discussed in the following section.

2.2. Mechanistic assumptions about the hemozoin formation

The heme detoxification by *P. falciparum* results in the formation of hemozoin by template-mediated crystallization ("biocrystallization"), and a number of studies have been conducted

http://dx.doi.org/10.5772/65053

in order to understand the hemozoin formation. Hemozoin or β-hematin (synthetic hemozoin) was used for these studies, and various mechanisms about the β-hematin formation have been postulated for the in vivo process.

Before beginning the discussion about mechanistic assumptions of the hemozoin formation, it is worth noting that when comparing the natural hemozoin and its synthetic version (β-hematin), we see a considerable difference in their size and shape. The natural hemozoin consists of small crystals ranging in size from 50 to 500 nm, whereas for the synthetic β-hematin, these crystals are bigger (50 nm to 20 μm) and depend on solvent used for the recrystallization. This difference in size can lead to diverse immunomodulatory responses [32].

The various studies of this mechanism gave rise to a number of assumptions [11, 33, 34] such as spontaneous [35, 36], autocatalyzed [37, 38], enzyme-catalyzed [39], lipid-catalyzed [40–43], and initiated or catalyzed by histidine-rich proteins (HRPs) [44–48], which can be divided into two main types: non-biological and biological conditions (**Figure 3**).

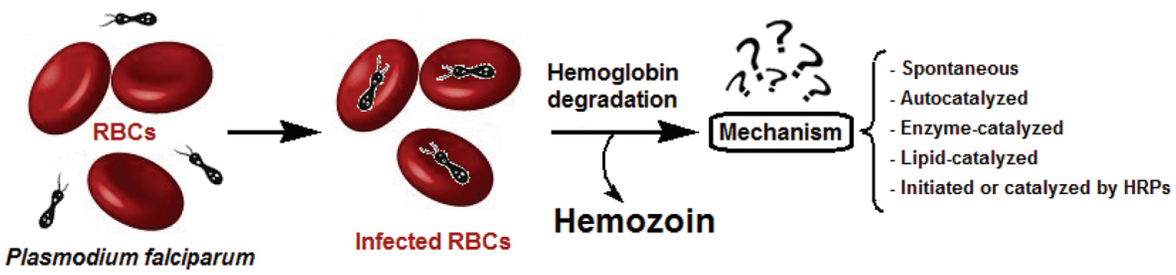

Figure 3. Postulated mechanisms about the hemozoin formation by *Plasmodium falciparum*.

The first category (non-biological conditions) is based on the assumption that β-hematin formation can happen spontaneously without any external help [35]. This observation comes from studies conducted in acetate solution, which shows that the β-hematin can be formed at a moderate low pH compared to the acidic digestive food vacuole [36].

The second category includes of all other mechanisms and provides a presumption that the β-hematin formation can catalyze itself or requires the presence of biological material (biocrystallization). The first idea about an autocatalytic process is, among other things, due to a recent observation of the continued growth of a preexisting hemozoin crystal [37].

As regards the second idea, it began in 1992 with the work of Slater and Cerami [39] which have shown that heme can react with trophozoite lysate extracts at pH 5–6 to generate hemozoin and that chloroquine, an antimalarial drug, can inhibit this formation. The authors concluded that the creation of the two propionate-Fe(III) linkages during the heme detoxification is catalyzed by an enzyme named heme polymerase. The use of extracts from *Plasmodium berghei* (rodent malaria) by Chou and Fitch gave equivalent results [49].

Despite being challenged, this heme polymerase theory attempted to explain hemozoin propagation without clarifying its initiation. This breach paved the way for other hypotheses about the formation of hemozoin involving a protein or enzyme [38]. Firstly, Hempelman in 2007 introduced the concept of "biocrystallization" instead of "polymerization" to describe

the hemozoin formation process [29]. One of the hypotheses suggested that biocrystallization is caused by enzymes, which postulate the presence of proteins such as histidine-rich proteins (HRPs). Sullivan and coworkers [48] showed that HRPs I, II, and III, present in the parasite's digestive vacuole, may be able to promote the formation of hemozoin in vitro. In 2008, Jani et al. [46] identified a novel heme detoxification protein (HDP) from *P. falciparum*, which is considered as one of the most powerful of the hemozoin-producing enzymes. As an example, we could cite the work of Choi and coworkers in 2002 and Nakatani et al. in 2014 concerning the elucidated reaction mechanisms of HRP II and HDP [44, 47]. These authors have shown that some histidine residues are active sites in these proteins and can bind with heme to promote the heme dimerization by bringing two molecules. This dimer would be used as a crystal growth initiator of hemozoin. Recently, Chugh and coworkers have established that HDP and falcipain-2 can work in tandem within the digestive food vacuole of the parasite to transform hemoglobin efficiently into hemozoin [45].

Finally, the last proposed mechanism is the biocrystallization catalyzed by lipids [40–43, 50]. These lipids, produced by the parasite after digesting the transport vesicles and trapped in its food vacuole, have been characterized with spectroscopic studies [7, 41] and known as a neutral lipid blend (NLB) and monopalmitoylglycerol (MPG). In 2007, Pisciotta et al. proved that Fe(III)PPIX can be processed into β-hematin through the action of these lipids with the yield of 80% or more [51] as assumed by Sullivan two years before [27].

The design and development of new antimalarial drugs first begin with the understanding of the mechanism of action of *P. falciparum* after invading RBCs and giving rise to hemozoin formation *via* the heme detoxification. Although this mechanism is not completely elucidated and still requires much work, these assumptions allow researchers to develop new strategies with a view to solving the problem of antimalarial drug resistance concerning chloroquine and artemisinin, the two most antimalarial drugs used to treat malaria.

By way of example, new strategies envisaged include the use of PDT (Section 3) in order to kill mosquito larvae (prevention Section 3.2) or to inactivate malaria parasites in the RBCs (treatment Section 3.3) but also the design of new antimalarial drugs that are able to inhibit the β-hematin formation by heme-drug interaction (treatment Section 4).

3. Photodynamic therapy for preventive and curative treatments

3.1. Generalities

The therapeutic effects of light are known since ancient times and were widely used in combination with natural substances for centuries in Chinese, Egyptian, or Indian civilizations for the treatment of numerous diseases such psoriasis, vitiligo, and rickets [52]. The integration of the concepts of "phototherapy" and then "photosensitivity" in modern medicine is much more recent, since it originated in the work of Niels Finsen, a Danish doctor who demonstrated in the 1890s the positive influence of light on the healing process (Nobel Prize for Medicine in 1903) [53]. However, the concept of exogenous photosensitizer (PS), that is to say, therapeutic

http://dx.doi.org/10.5772/65053

molecule introduced for the specific purpose of interacting with light to generate the desired therapeutic effect, was introduced only a few years later, at the turn of the twentieth century by Raab and von Tappeiner as related by Spikes in a very good historical review [54]. In 1900, Oscar Raab, a student at the Department of Pharmacology of the University of Munich in the group of Hermann von Tappeiner, tried to characterize the influence of acridine on the development of *Paramecium caudatum* and *Plasmodium malariae*, a paramecium responsible for malaria. Very quickly, Raab noted that, according to the hour of the treatment and the weather conditions, the impact of administered acridine on the survival of microorganisms seemed extremely variable. He quickly demonstrated that the mechanism of cell death induced by acridine requires activation by light irradiation. Later, von Tappeiner identified oxygen as the third component (with the PS and light source) involved in photo-induced mechanism. He proposed the term "photodynamic therapy" (photodynamische Wirkung in the original) to define all therapeutic protocols involving these three elements [55]. von Tappeiner's team experimented eosin as PS to treat tumors in six patients, and some promising results were obtained [56]. Unfortunately, in this period, the concept did not arouse reactions on behalf of the scientific world in Western medicine [57].

In summary, PDT is an innovative medical treatment involving the concomitant action of three components that are photoactivatable molecule called PS, light of a suitable wavelength, and oxygen present in the biological medium. After light excitation of the PS and energy transfer from the excited PS to oxygen, reactive oxygen species are produced especially singlet oxygen ($^1O_2$) that can destroy cancer cells in proximity. It is interesting to notice that the PS itself is nontoxic and turns out to be toxic only with light. Light is also nontoxic by itself. The selectivity of action of PDT allows through a localized light radiation to eradicate tumor cells while preserving healthy cells. PS fluorescence properties are also an asset that is utilized to visualize the diseased tissue. The mechanisms are summarized in **Figure 4**.

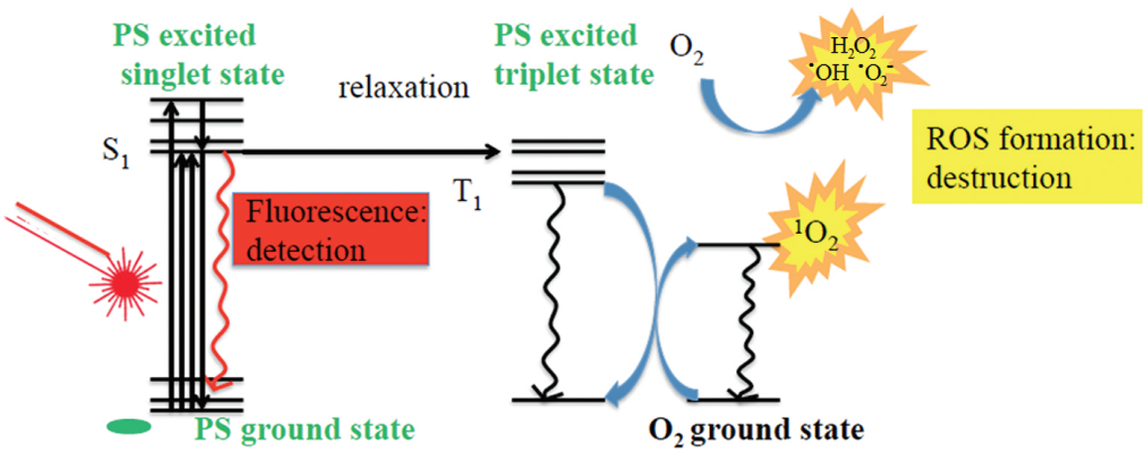

Figure 4. Mechanism of PDT (simplified Perrin-Jablonski diagram).

This technique was used clinically for many years, and in 1993, bladder cancer Photofrin PDT receives government approval in Canada. Since then, PDT has been developed in many countries of the world. PDT is an obvious treatment for dermatology applications, and it is

used daily for skin diseases such as actinic keratoses, acne, and wine stain [57]. PDT has been also widely employed as a treatment for age-related macular degeneration (ARMD). However, since 2006, intravitreal injections of Avastin, humanized monoclonal antibody having anti-angiogenic activity, significantly reduced the use of PDT to treat ARMD. In urology, the French company Steba Biotech has invested heavily to develop a new PS, the TOOKAD® (currently in phase 3) for the treatment of prostate cancer. The first clinical applications demonstrate the technical feasibility [58]. In gastroenterology, PDT demonstrated its effectiveness for the treatment of superficial cancers of the esophagus in patients ineligible for further treatment, with a postradiation recurrence, severe dysplasia in Barrett, and unresectable cholangiocarcinoma [59]. In gynecology, the interest of PDT has been shown in the treatment of cervical dysplasia of low- and high-grade cervical lesions [60]. Our team developed folic acid-targeted photosensitizers that could be very efficient to treat peritoneal carcinosis, and a preclinical evaluation is under progress [61, 62]. In pulmonology, the number of studies on the treatment of lung cancer is still limited, and the role of PDT in the therapeutic arsenal of the practitioner remains to be demonstrated. PDT appears to be a promising treatment for malignant pleural mesothelioma (MPM). Thus, PDT has been tested in phase I and phase II clinical trials to MPM patients in combination with extrapleural pneumonectomy or pleurectomy/decortication and an intravenous chemotherapy. The first work of the team of Professor Friedberg (University of Pennsylvania, Philadelphia, USA) has shown promising results with a median overall survival of 31 months [63]. PDT is not only a powerful technique to destroy human cells but also for viruses [64], yeasts [65], molds [66], bacteria [67], protozoa [68], parasites [69], and insects (Section 3.2). PDT is used in the development of new strategies to treat malaria and more generally to treat tropical diseases, either by controlling the propagation vector of the disease (Section 3.2), by inactivation of microorganisms responsible for these diseases, or by inactivating parasites (Section 3.3).

3.2. Prevention: destruction of mosquito larvae

3.2.1. Generalities

More than 700 million people are affected annually by mosquitoes in Asia, Mexico, Central America, South America, and Africa. A promising strategy to control diseases transmitted by mosquitoes (malaria, filarial, and dengue fever) is the control of these vectors. Mosquitoes are vectors of pathogens: *Aedes* is responsible for dengue fever, yellow fever, and encephalitis; *Anopheles* for malaria and encephalitis; and *Culex* for yellow fever and encephalitis [70]. Pesticides such as DDT (dichlorodiphenyltrichloroethane) have been used in affected area leading to decline of the mosquito population. Nevertheless, the use of these pesticides induces risks for safety reasons, development of resistance in major vectors, environmental and human health problems, etc. There is a need for developing improved insecticide, and the use of light with a PS is a possibility. In this case, the PS is called a photopesticide. One of the first researchers who described the potential of photosensitive molecules as insecticides was probably A. Barbieri in 1928 [71]. In 1979, rose bengal was used to treat Culex larvae [72]. In 1983, a review was written by J. Robinson about the photosensitizing dyes used as insect control agent [73]. The concept is to make a gulp down a small amount of PS to a mosquito larva, and then,

http://dx.doi.org/10.5772/65053

after PS excitation by sunlight, the larva dies. Reviews have been published recently on this topic [70, 74]. The use of porphyrins has been described from the late 1980s [75, 76]. Different porphyrin derivatives have been then tested such as chlorophyllin, pheophorbide, and hematoporphyrin in laboratory conditions but also in semi-field conditions. A synthetic *meso*-substitute developed by Lucantoni et al. in 2012 had a potent photosensitizing activity on *Aedes aegypti* larvae that are responsible for the dengue in laboratory conditions [77].

3.2.2. Anopheles mosquitoes: the primary vector for malaria

Malaria is spread to humans by the bite of the female anopheles mosquito. In 2012, Fabris et al. described the photolarvicidal activity of a new PS called C12-porphyrin (5-(4-*N*-dodecyl-pyridyl)-10,15,20-tri(4-*N*-methylpyridyl)-21*H*,23*H*-porphyrin tetraiodide) [78]. This molecule was first supplied by Frontier Scientific Inc. (US Patent no. 6 573 258), and our team improved the synthesis and performed the photophysical properties study [79]. The structure of molecule is presented in **Figure 5**.

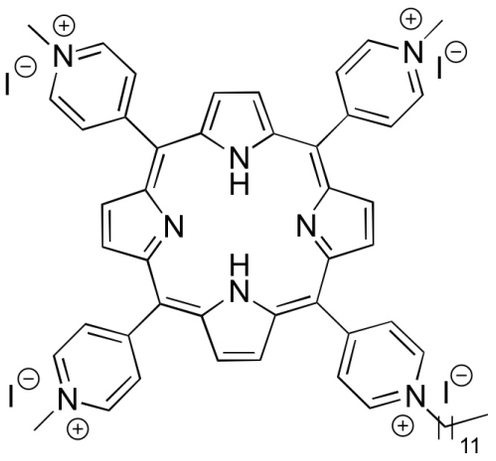

Figure 5. Chemical structure of C12-porphyrin.

In collaboration with the Institut de Recherche en Sciences de la Santé (IRSS) located in Burkina Faso, Fabris et al. studied the potential of C12-porphyrin as a photolarvicide for the control of *Anopheles*. Two different formulations with C12 were prepared: one is composed of Eudragit S100, an anionic methacrylic acid/methyl methacrylate copolymer, and the other is a fraction of cat food pellets. Both of them proved to be very efficient in laboratory conditions. The porphyrin-mediated photoinactivation of anopheles larvae could represent an interesting approach in the achievement of reduction of malaria morbidity and mortality.

3.3. Prevention: photoinactivation of parasites in blood

3.3.1. Generalities

With the emergence of many antibiotics, PDT declined for the treatment of parasite-related diseases, and it is only in recent decades that it knew a regain of interest with the increasing

problem of antibiotic resistance [80]. Antibiotic resistance is a global problem that reduces the power of conventional treatments of many diseases (both nosocomial and community-acquired infections). It concerns all pathogens including bacteria, fungi, and viruses.

To circumvent this bio-resistance, an attractive approach is PDT as non-antibiotic strategy to inactivate microorganisms (bacteria, viruses, parasites, etc.). This process is called antimicrobial photodynamic therapy (aPDT) [81, 82] or antibacterial PDT [83, 84] but is also known as photodynamic inactivation (PDI) [85–87] or photodynamic antimicrobial chemotherapy (PACT) [88–91]. This treatment can be effective in the case of chronic ulcers, infected burns, acne vulgaris, and a variety of local bacterial infections but also in the case of periodontitis [92], dengue [93], tuberculosis [94], viral infection [95], and malaria [96–99]. A very large variety of microorganisms have been studied and are listed by Alves et al. in a recent review [70] in which the insect pest elimination, water disinfection, and elimination of food-borne pathogens are described. A state of the art of PDT (potential) applications in animal models and clinical infectious diseases has been submitted by Dai et al. in 2009 [95], and numerous PSs are described [99, 100].

3.3.2. Inactivation of P. falciparum in human RBCs

Malaria is no more considered as poverty-related disease in Western countries, and attention has been paid to developing blood decontamination methods, vaccines, or new therapies. The spread of malaria disease, particularly with *P. falciparum*, in high-risk countries by blood transfusion is very worrying, especially as global traveling is continuously increasing. Inhabitants of tropical and subtropical regions where malaria is endemic can develop an immune response. However, they can carry a significant amount of parasites that can be transmitted by transfusion even if the blood is frozen (3 weeks' survival) [101, 102]. Decontamination of blood can be carried out according to several protocols including solvent-detergent methods, filtration, deleucocytation, photochemical techniques, etc. PSs such as psoralens, porphyrins, acridines, phenothiazines, porphyrins, and others can be used as additive for blood sterilization, and numerous protocols have been described, some of them could be found in [103, 104].

The life cycle of *Plasmodium* can be characterized by two phases: (a) the asexual proliferative phase in humans (intermediate host), called schizogony. This phase takes place in two different locations in humans and chronologically first within hepatocytes in the liver (exoerythrocytic cycle) and in circulating erythrocytes (RBC cycle). It also stands in the mosquito as a result of the sexual phase, (b) a sexual differentiation phase followed by asexual reproduction, called sporogony, which begins in humans and continues in the mosquito by the maturation of these in male and female gametes. As already mentioned, in erythrocytes, *P. falciparum* ingests 30–80% of hemoglobin, which is then digested in the food vacuole (an acidic organelle) and detoxified into hemozoin. This hemozoin itself could be a PS for killing *P. falciparum*, and Leblanc et al. [105] demonstrated that a simple irradiation of infected cultured RBCs by a near IR laser (800 nm) could induce a ~0.5 log reduction in parasitemia, but this is not enough for decontamination of blood.

http://dx.doi.org/10.5772/65053

Historically, Ehrlich's group was the first to use methylene blue (**Figure 6**) as a PS [106] and Rounds et al. conducted a pioneering work on the photokilling potency of a ruby laser and methylene blue on cells infected by *P. lophurae* [107]. Since then, a wide variety of dyes have been explored. Merocyanine 540 was one of the first PSs used for the decontamination of blood [108] which reduced the concentration of parasites by 3 log when exposed to light. However, the overlapping between hemoglobin and the PS absorption made it not suitable for deparasitization.

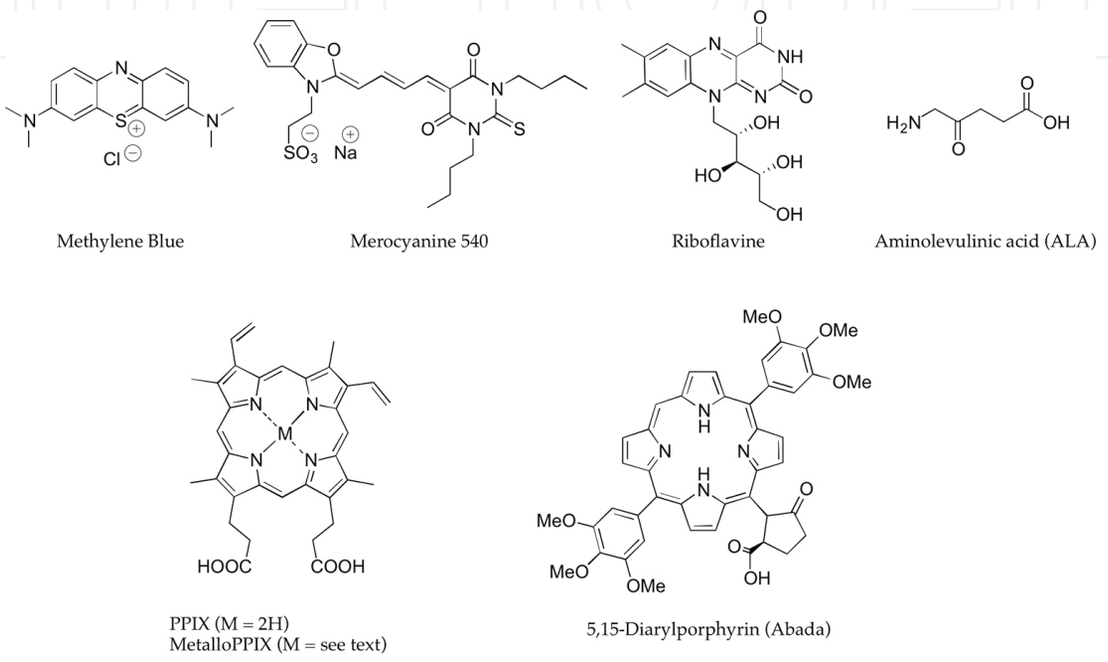

Figure 6. PS used for blood decontamination.

Riboflavin or vitamin B2 (**Figure 6**) deficiency is closely related to malaria [109, 110], and its administration can prevent hemozoin formation in the asexual cycle in the food vacuole of erythrocytes. Akompong et al. observed that addition of riboflavin can induce a 65% decrease of the food vacuole volume and subsequently damage to light-exposed contaminated blood [111]. In 2013, Goodrich's group tested the "Mirasol® pathogen reduction technology" (PRT) system against *P. falciparum* and *P. yoelii* [112]. This PRT system uses riboflavin and UV light for the destruction of a broad range of blood-borne pathogens and receives the European Community mark for both platelet and plasma applications. For *P. falciparum*, the percentage of parasitemia was 0.97 and <0.0005% before and after treatment, respectively. Similar results were obtained in vivo with blood of mice infected by *P. yoelii*. Recently, the "International Society for Medical Laser Applications" ordered a clinical trial named "Antimicrobial photodynamic therapy as a new treatment option for Malaria" in India on a group of 50 patients receiving an antimicrobial photodynamic treatment (riboflavin + 447 nm blue laser) over a period of 5 days plus conventional treatments [113].

In a recent research, Sigala et al. [114] demonstrated that sequencing of *P. falciparum* genome and some gene deletions did not affect the heme formation indicating that the host enzymes

are involved and can be a parallel pathway for the life cycle of *P. falciparum*. They showed the involvement of protoporphyrin IX (PPIX; **Figure 6**) in this parallel pathway and proposed a new treatment based on the chemoluminescence of luminol and aminolevulinic acid (ALA; **Figure 6**), which is the initial building block of PPIX [115] to produce ROS. The combination of ALA, luminol, and stimulating factor (4-iodophenol or dihydroartemisinin) decreased the parasitemia in the range of 75–80% [114]. ALA has also been described by Smith and Kain as a potentiate PS for killing *P. falciparum* in the presence of white light. The culture incubated by 0.2 mM ALA for 8 hours and exposed to light for 30 min exhibited a parasitemia less than 0.002% after 2 days [116].

PS	Conditions	Effects	Reference
Hemozoin	800 nm; 485 mW/cm^2; 60 min	~0.5 log reduction in parasitemia	[105]
Methylene blue	694 nm; 70 J/cm^2	Preferential uptake by infected erythrocytes by imaging	[107]
Merocyanine 540	485 nm; 26 W/m^2; 30 min	1000-fold reduction in parasitemia	[108]
Riboflavin	No irradiation /48h	65% decrease in food vacuole volume	[111]
Riboflavin	UV ; 6.24 J/mL ; 72 h	<0.002% survival	[112]
ALA	White light; 0.57 W/cm^2; 30 min	<0.0005% survival	[116]
ALA	Chemoluminescence by luminol	75–80% death	[114]
SnPPIX	No irradiation	IC_{50} = 6.5 μM (85 μM for chloroquine) on trophozoite lysate	[118]
Zn-PPIX	No irradiation	IC_{50} = 330 nM on RBC	[119]
Diarylporphyrin	No irradiation	IC_{50} = 20 nM on erythrocytes	[120]
Pheophorbide Ph4-OH	660 nm; 7 W/cm^2; 20 min	Total eradication with 2 μM/L	[121]
PC4 phthalocyanine	>600 nm; 60 J/cm^2; 10 min	<0.025% survival with 2 μM/L	[122]

Table 1. Bibliographic data.

In 1996, Martiney et al. [117] described a slight inhibition of hemozoin formation by using Zn-PPIX without light. Using trophozoite lysate of *P. falciparum*, Begum et al. obtained similar results with SnPPIX with an IC_{50} = 6.5 μM (to be compared to 85 μM for chloroquine) [118]. Recently, Garcia's group [119] encapsulated metal-PPIX (2H, Fe, Co, Cu, Mn, Ni, and Zn) in marine atelocollagen using the coacervation technique. They obtained an IC_{50} = 330 nM (for Zn-PPIX) on RBC and found that encapsulated Zn-PPIX was 80-fold more effective than the nonencapsulated Zn-PPIX and similar to chloroquine. In 2013, Abada et al. evaluated a series of 11 diversely substituted porphyrins against *P. falciparum* [120]. Only the 5,15-di-(3,4,5-trimethoxyphenyl)-10-(5-oxopyrrolidine-2(*S*)-carboxylate) (**Figure 6**) porphyrin has an

http://dx.doi.org/10.5772/65053

efficiency comparable to chloroquine with an IC_{50} value of 20 nM with a slight delay of infected mice survival.

The photosensitized inactivation of *P. falciparum* has been investigated by Grellier et al. [121] by using *N*-(4-butanol) pheophorbide derivative (Ph4-OH) as PS. Illumination at 660 nm (7 mW cm^{-2}) of parasitized whole blood induced a total eradication using 2 μM Ph4-OH and 20 min illumination, 4 μM Ph4-OH and 10 min illumination, or 8 μM Ph4-OH and 5 min illumination. The blood remained uncontaminated for at least 2 weeks. These results are better than those obtained with merocyanine 540 [108] and comparable to that obtained with phthalocyanines. In fact, Lustigman and BenHur [122] described the phthalocyanine HOSiPcOSi$(CH_3)_2(CH_2)_3N(CH_3)_2$ (Pc 4) as PS for blood decontamination and obtained an inactivation (≥99.8%) of *P. falciparum* clones 7G8 and HB3 by 10 and 40 min irradiation with a xenon short-arc lamp (>600 nm). The same team evaluated an IC_{50} of 24 nM in the dark [123]. The main results are summarized in **Table 1** (when available).

Besides the decontamination of blood or dialysis, numerous studies have been conducted to understand the physiology of the human malaria parasite *Plasmodium*, and some PSs have been used. For example, 4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-hexadecanoic acid (BODIPY FL C_{16}) has been used as a marker for chloroquine resistance [124] or spatial distribution of oxidative stress in infected erythrocytes [125]. Other examples are 5,6-chloromethyl-2,7-dichlorodihydrofluorescein diacetate (CM-H2DCFDA), 5′,6′-carboxy-10-dimethylamino-3-hydroxy-spiro[7*H*-benzo[c]xanthene-7,1′(3*H*)-isobenzofuran]-3′-one (SNARF), and 2,7-bis-(2-carboxyethyl)-5,6-carboxyfluorescein acetoxymethyl ester (BCECF) for the measurement of the parasite's food vacuolar pH [126].

4. Curative treatment: drugs inhibiting β-hematin formation

Among various strategies, we will focus in the following part only on antimalarial drugs that inhibit the β-hematin formation by heme-drug interaction (purely π-π interactions). This strategy of drug development uses the heme scaffold itself as a hematin crystallization inhibitor (**Figure 2**). We can quote quinine, chloroquine, rufigallol and exifone and artemisinin, which are currently used as antimalarial drugs *via* this strategy (**Figure 7**).

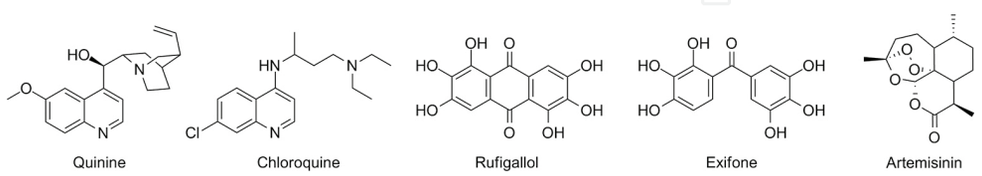

Figure 7. Structures of current antimalarial drugs.

Several studies and reviews [97] reported that porphyrins can inhibit the process of heme crystallization in the acidic food vacuole of the malaria parasite. As current antimalarial drugs,

porphyrins are able to inhibit the β-hematin formation by strong π-π stacking interactions. Several porphyrins have been studied for their use in heme aggregation inhibition.

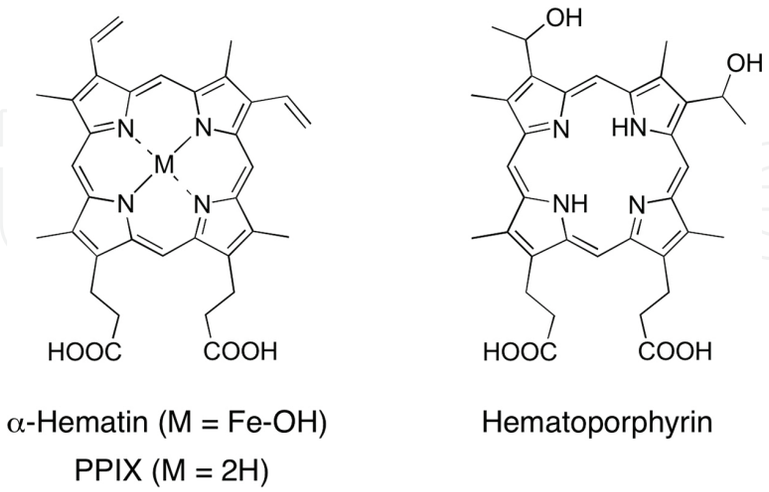

Figure 8. Structures of hematin, PPIX, and hematoporphyrin.

In 1997, Basilico et al. [127] evaluated the effect of two non-iron metalloporphyrins (PPIX and hematoporphyrin) on the crystallization of α-hematin (**Figure 8**) to β-hematin also called synthetic hemozoin (**Figure 2**). Crystallization of hematin may be achieved in 4.5 M sodium acetate buffer at 60°C [35]. Heme and β-hematin may be differentiated by their IR spectroscopic characteristics [128]. IR spectra of β-hematin show two bands at 1662 and 1209 cm^{-1}, which disappear in IR spectra of heme. From this property, Basilico et al. demonstrated that free-base porphyrins inhibit heme crystallization with hematoporphyrin more actively than PPIX. The presence of hydroxyl groups can explain the better inhibitory ability of hematoporphyrin.

In 1999, Tamarelli's team also showed that Fe(III)PPIX is reduced to Fe(II)PPIX as a novel endogenous antimalarial because Fe(II)PPIX molecules inhibit the crystallization process causing the death of the parasite [129].

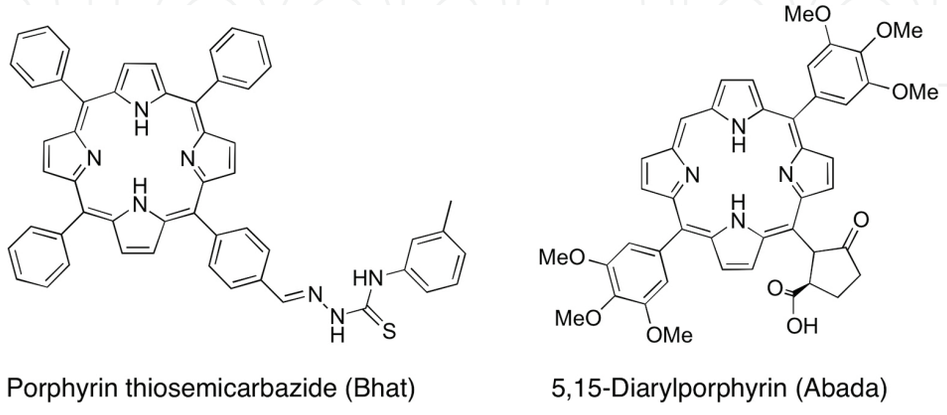

Figure 9. Structure of antimalarial drugs designed by Bhat (left) and Abada (right).

http://dx.doi.org/10.5772/65053

Some researchers are interested in the synthesis of free-base porphyrins. In 2008, Bhat et al. [130] synthesized and evaluated the antimalarial activity of a series of porphyrin thiosemicarbazides. Only one compound (**Figure 9 left**) possesses an ability to inhibit β-hematin formation similar to chloroquine and quinine, the control drugs that are usually used in the malaria treatment. More recently, Abada et al. [120] synthesized a new 5,15-diarylporphyrin (**Figure 9 right**) with a good activity against *Plasmodium* with 20 nM IC_{50} value. The in vivo evaluation on *P. berghei* in mice model showed that this compound allowed delaying the death of the animal on about two days.

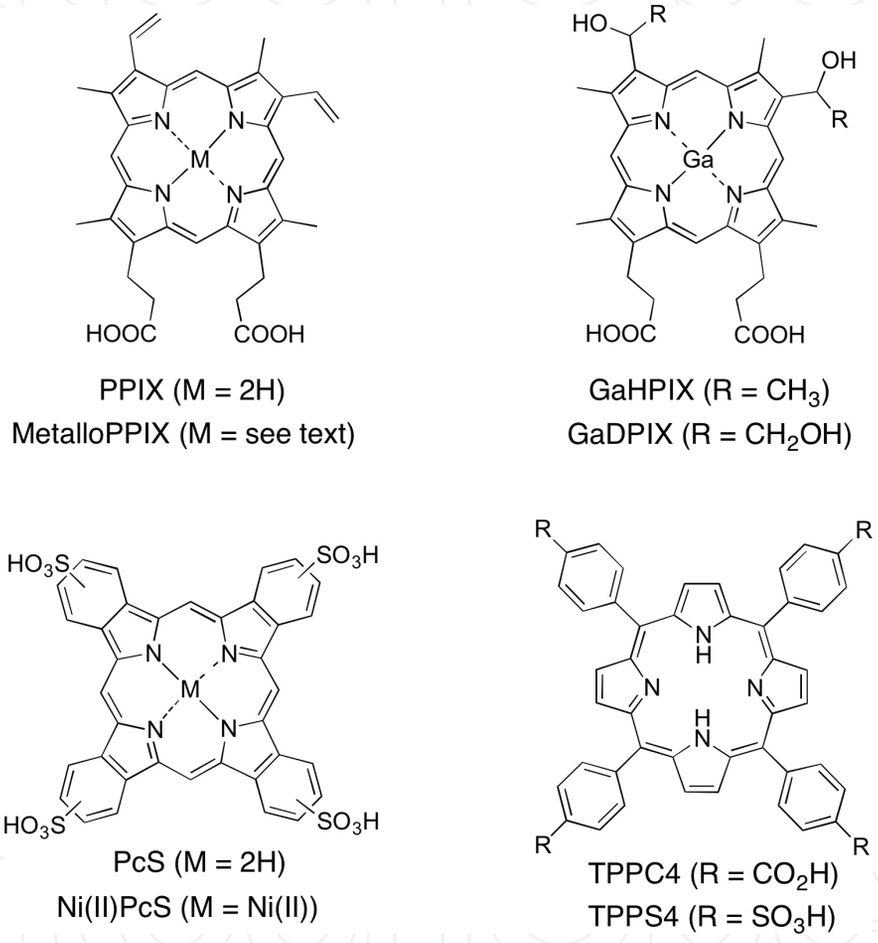

Figure 10. Structure of porphyrins and phthalocyanines developed by Wright and Begum.

In 2000, Wright's team highlighted the presence of other metal ions than Fe(III) can influence the conversion of heme to β-hematin. A number of metallo-PPIX, including Fe(III), Cr(III), Co(III), Cu(II), Mn(III), Mg(II), Zn(II), and Sn(IV) showed in vitro an ability to inhibit the β-hematin formation (**Figure 10**) [131]. In 2003 [132], phthalocyanines, phthalocyanine tetrasulfonate (PcS) and Ni(II)PcS, and anionic porphyrins, *meso*-tetra(4-sulfonatophenyl) porphyrin (TPPS4) and *meso*-tetra(4-carboxyphenyl) porphyrin (TPPC4), came to complete the previous study (**Figure 10**). All of them are inhibitors of heme crystallization. Among them, Mg(II), Zn(II), and Sn(IV) acted six times more efficiently than the free ligand PPIX and were more

efficient than the chloroquine standard as well. These results showed that metalloporphyrins with high oxidation state could form complexes with heme through the Fe-propionate linkages while being efficient crystallization inhibitors.

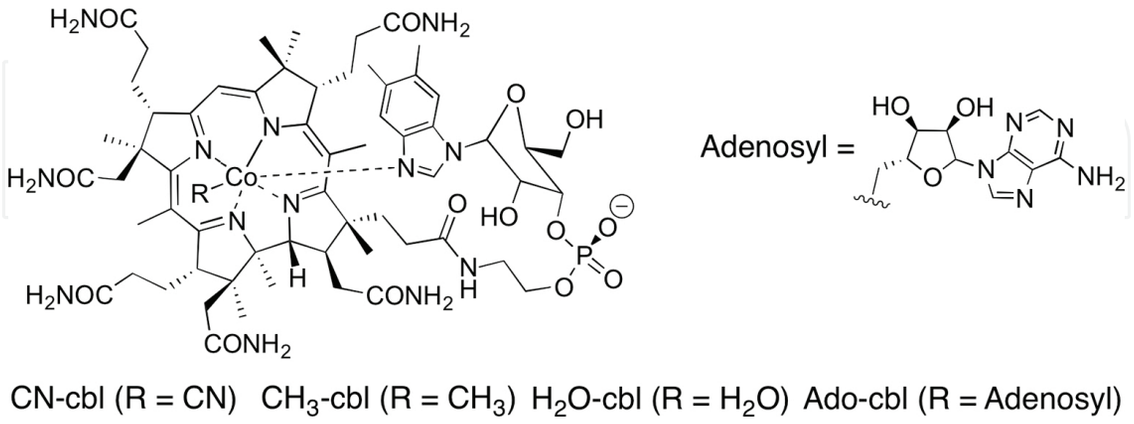

Figure 11. Structure of cobalamin derivatives.

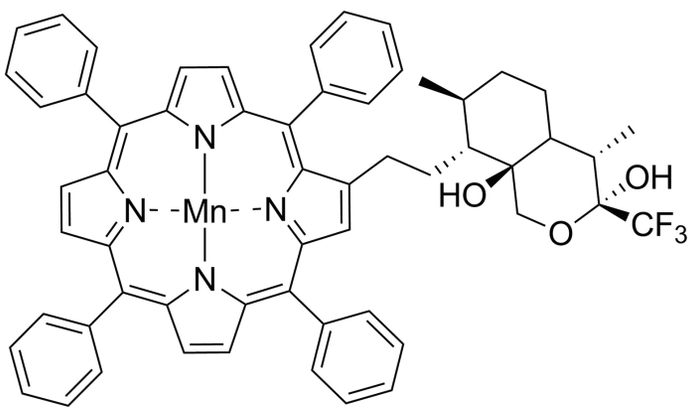

Figure 12. Mn(II) complexes of alkylated tetraphenylporphyrin with a fluorinated artemisinin.

The same behavior was observed by Begum et al. [118] who evaluated the antimalarial activity of free-base PPIX, deuteroporphyrin IX (DPIX), and hematoporphyrin IX (HPIX) and their corresponding complexes with Ga(III), Ag(III), Pd(II), Co(III), Mn(III), Sn(IV), Cr(III), and Fe(III) ions (**Figure 10**). Once again, SnPPIX at 15.5 μM had a better activity than the chloroquine control. Both GaPPIX and GaDPIX showed an antimalarial activity also.

In the same way, Chemaly et al. [133] observed that cobalamins (cbls) also called vitamin B12 (corrin ring with a chemical structure close to the heme but the central iron atom is replaced by an atom of cobalt) possess antimalarial activity. Methylcobalamin (CH_3-cbl), adenosylcobalamin (Ado-cbl), and aquacobalamin (H_2O-cbl) (**Figure 11**) showed increased efficacy over the chloroquine; cyanocobalamin (CN-cbl) was a little more efficient than chloroquine. The in vivo evaluation of vitamin B12 derivatives on the growth of *P. falciparum* (Ado-cbl > CH_3-cbl > H_2O-cbl > CN-cbl) was slightly lower than chloroquine or quinine.

http://dx.doi.org/10.5772/65053

Rodriguez et al. [134] showed that Mn(II) complexes of alkylated tetraphenylporphyrin with a fluorinated artemisinin derivative (**Figure 12**) were effective inhibitors of β-hematin formation with an IC_{50} of 2.6 nM.

Benoit-Vical et al. [135, 136] showed a similar behavior with anionic metalloporphyrins. Alone the *meso*-tetrakis(4-sulfonatophenyl)porphyrin (TPPS) and *meso*-tetrakis(3,5-disulfonatomesityl)porphyrin (TMPS) complexed to manganese (**Figure 13**) inhibited slightly the β-hematin formation. However, the fact of combining them with β-artemether enhanced strongly the in vitro and in vivo antimalarial activity of β-artemether.

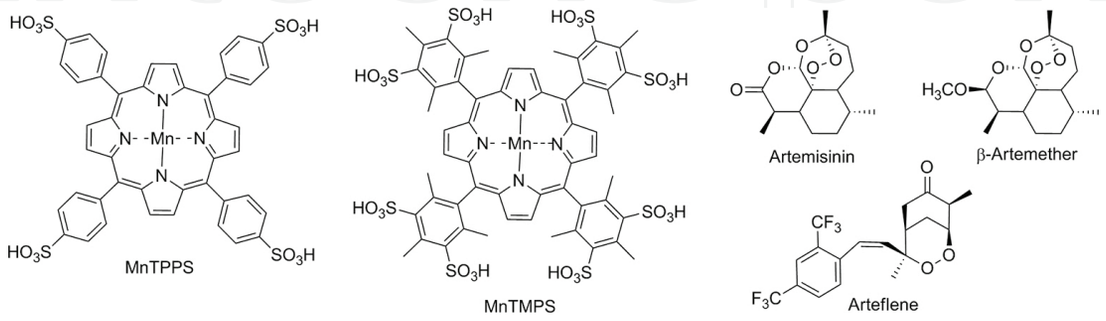

Figure 13. Structure of MnTPPS, MnTMPS, and other antimalarial derivatives.

5. Conclusion and perspectives

Malaria eradication is one of the great issues for humankind in the decades ahead. Based on figures from the *World Malaria Report 2015*, today more than ever, we are on the right track to reach this objective. The decline in cases and deaths caused by malaria stems from the relentless efforts of researchers to understand how the *P. falciparum* affects the RBCs. These different studies generated a wide range of strategies to prevent and treat malaria. Transfusion-transmitted malaria (TTM) must be understood as a high-risk situation, not only in African countries at risk but also around the world due to the increased immigration and travel from malaria-endemic areas. As mentioned in Section 3.3.2, malaria parasites can be transmitted by transfusion even if the blood is frozen (3 weeks' survival). In Europe, for example, all donated bloods are subjected to a large number of safety procedures including nucleic acid testing, blood filtration, or bacterial culture, but these are not done in many developing countries because of limited funds. All blood products are currently available in sterilized forms, except red blood cell (RBC) and platelet concentrates (PCs). The treatment of whole blood with a photosensitizer and light is a promising strategy. Very recent studies showed that this treatment can be achieved by riboflavin plus irradiation [137] and does not alter the quality of the blood [138]. As we already mentioned in Section 3.3.2, a first clinical study worldwide employing antimicrobial PDT is under progress in India using riboflavin as a photosensitizer and 447 nm blue laser. This chapter report focuses on innovative approaches using PDT or the design of new antimalarial drugs that is able to inhibit the β-hematin formation *via* heme-drug interaction.

Author details

Régis Vanderesse[1], Ludovic Colombeau[2], Céline Frochot[2] and Samir Acherar[1*]

*Address all correspondence to: samir.acherar@univ-lorraine.fr

1 Macromolecular Physical Chemistry Laboratory, University of Lorraine, Nancy Cedex, France

2 Reactions and Process Engineering Laboratory, University of Lorraine, Nancy Cedex, France

References

[1] WHO. World Malaria Report. 2015. Available from: http://www.who.int/malaria/publications/world-malaria-report-2015/report/en/

[2] Francis SE, Sullivan DJ, Goldberg DE. Hemoglobin metabolism in the malaria parasite *Plasmodium falciparum*. Annu Rev Microbiol, 1997;51:97–123. DOI: 10.1146/annurev.micro.51.1.97

[3] Krugliak M, Zhang JM, Ginsburg H. Intraerythrocytic *Plasmodium falciparum* utilizes only a fraction of the amino acids derived from the digestion of host cell cytosol for the biosynthesis of its proteins. Mol Biochem Parasitol, 2002;119:249–256. DOI: 10.1016/s0166-6851(01)00427-3

[4] Klonis N, Tan O, Jackson K, Goldberg D, Klemba M, Tilley L. Evaluation of pH during cytostomal endocytosis and vacuolar catabolism of haemoglobin in *Plasmodium falciparum*. Biochem J, 2007;407:343–354. DOI: 10.1042/BJ20070934

[5] Krogstad DJ, Schlesinger PH, Gluzman IY. Antimalarials increase vesicle pH in *Plasmodium falciparum*. J Cell Biol, 1985;101:2302–2309. DOI: 10.1083/jcb.101.6.2302

[6] Chou AC, Fitch CD. Mechanism of hemolysis induced by ferriprotoporphyrin IX. J Clin Invest, 1981;68:672–677. DOI: 10.1172/JCI110302

[7] Fitch CD, Chevli R, Banyal HS, Phillips G, Pfaller MA, Krogstad DJ. Lysis of *Plasmodium falciparum* by ferriprotoporphyrin-IX and a chloroquine-ferriprotoporphyrin-IX complex. Antimicrob Agents Chemother, 1982;21:819–822. DOI: 10.1128/AAC.21.5.819

[8] Har-el R, Marva E, Chevion M, Golenser J. Is hemin responsible for the susceptibility of Plasmodia to oxidant stress? Free Radic Res Commun, 1993;18:279–290. DOI: 10.3109/10715769309147495

[9] Orjih AU, Banyal HS, Chevli R, Fitch CD. Hemin lyses malaria parasites. Science, 1981;214:667–669. DOI: 10.1126/science.7027441

[10] Banerjee R, Liu J, Beatty W, Pelosof L, Klemba M, Goldberg DE. Four plasmepsins are active in the *Plasmodium falciparum* food vacuole, including a protease with an active-site histidine. Proc Natl Acad Sci USA, 2002;99:990–995. DOI: 10.1073/pnas.022630099

[11] Coronado LM, Nadovich CT, Spadafora C. Malarial hemozoin: from target to tool. Biochim Biophys Acta, 2014;1840:2032–2041. DOI: 10.1016/j.bbagen.2014.02.009

[12] Eggleson KK, Duffin KL, Goldberg DE. Identification and characterization of falcilysin, a metallopeptidase involved in hemoglobin catabolism within the malaria parasite *Plasmodium falciparum*. J Biol Chem, 1999;274:32411–32417. DOI: 10.1074/jbc.274.45.32411

[13] Goldberg DE. Hemoglobin degradation. Curr Top Microbiol Immunol, 2005;295:275–291. DOI: 10.1007/3-540-29088-5_11

[14] Goldberg DE, Slater AF, Beavis R, Chait B, Cerami A, Henderson GB. Hemoglobin degradation in the human malaria pathogen *Plasmodium falciparum*: a catabolic pathway initiated by a specific aspartic protease. J Exp Med, 1991;173:961–969. DOI: 10.1084/jem.173.4.961

[15] Meshnick SR. Artemisinin: Mechanisms of action, resistance and toxicity. Int J Parasitol, 2002;32:1655–1660. DOI: 10.1016/S0020-7519(02)00194-7

[16] Kirschner-Zilber I, Rabizadeh E, Shaklai N. The interaction of hemin and bilirubin with the human red-cell membrane. Biochim Biophys Acta, 1982;690:20–30. DOI: 10.1016/0005-2736(82)90234-6

[17] Shinar E, Rachmilewitz EA. Oxidative denaturation of red blood cells in thalassemia. Semin Hematol, 1990;27:70–82. PMID: 2405497

[18] Vincent SH. Oxidative effects of heme and porphyrins on proteins and lipids. Semin Hematol, 1989;26:105–113. PMID: 2658086

[19] Klouche K, Morena M, Canaud B, Descomps B, Beraud JJ, Cristol JP. Mechanism of *in vitro* heme-induced LDL oxidation: effects of antioxidants. Eur J Clin Invest, 2004;34:619–625. DOI: 10.1111/j.1365-2362.2004.01395.x

[20] Sadrzadeh SMH, Anderson DK, Panter SS, Hallaway PE, Eaton JW. Hemoglobin potentiates central-nervous-system damage. J Clin Invest, 1987;79:662–664. DOI: 10.1007/s12029-013-9496-4

[21] Tappel AL. Unsaturated lipide oxidation catalyzed by hematin compounds. J Biol Chem, 1955;217:721–733. DOI: 10.1007/BF02633109

[22] Aft RL, Mueller GC. Hemin-mediated DNA strand scission. J Biol Chem, 1983;258:12069–12072. DOI: 10.1016/j.ejps.2012.04.014

[23] Aft RL, Mueller GC. Hemin-mediated oxidative-degradation of proteins. J Biol Chem, 1984;259:301–305. PMID: 6323403

[24] Atamna H, Ginsburg H. Origin of reactive oxygen species in erythrocytes infected with *Plasmodium falciparum*. Mol Biochem Parasitol, 1993;61:231–241. DOI: 10.1016/0166-6851(93)90069-A

[25] Foley M, Tilley L. Quinoline antimalarials: mechanisms of action and resistance and prospects for new agents. Pharmacol Ther, 1998;79:55–87. DOI: 10.1016/s0163-7258(98)00012-6

[26] Scheibel LW, Sherman IW. Metabolism and organellar function during various stages of the life cycle: proteins, lipids, nucleic acids, and vitamins, in: Malaria: Principles and Practice of Malariology. Churchill-Livingstone, Edinburgh, 1988. p. 219–252.

[27] Sullivan DJ. Hemozoin: a biocrystal synthesized during the degradation of hemoglobin, in: Biopolymers Online, Wiley-VCH Verlag GmbH & Co. KGaA, 2005. p. 129–163. DOI: 10.1002/3527600035.bpol9007

[28] Egan TJ, Mavuso WW, Ncokazi KK. The mechanism of beta-hematin formation in acetate solution, parallels between hemozoin formation and biomineralization processes. Biochemistry (Mosc), 2001;40:204–213. DOI: 10.1021/bi0013501

[29] Hempelmann E. Hemozoin biocrystallization in *Plasmodium falciparum* and the antimalarial activity of crystallization inhibitors. Parasitol Res, 2007;100:671–676. DOI: 10.1007/s00436-006-0313-x

[30] Bohle DS, Dinnebier RE, Madsen SK, Stephens PW. Characterization of the products of the heme detoxification pathway in malarial late trophozoites by X-ray diffraction. J Biol Chem, 1997;272:713–716. DOI: 10.1074/jbc.272.2.713

[31] Klonis N, Dilanian R, Hanssen E, Darmanin C, Streltsov V, Deed S, et al. Hematin-hematin self-association states involved in the formation and reactivity of the malaria parasite pigment, hemozoin. Biochemistry (Mosc), 2010;49:6804–6811. DOI: 10.1021/bi100567j

[32] Coban C, Yagi M, Ohata K, Igari Y, Tsukui T, Horii T, et al. The malarial metabolite hemozoin and its potential use as a vaccine adjuvant. Allergol Int, 2010;59:115–124. DOI: 10.2332/allergolint.10-RAI-0194

[33] Egan TJ. Recent advances in understanding the mechanism of hemozoin (malaria pigment) formation. J Inorg Biochem, 2008;102:1288–1299. DOI: 10.1016/j.jinorgbio.2007.12.004

[34] Egan TJ. Haemozoin formation. Mol Biochem Parasitol, 2008;157:127–136. DOI: 10.1016/j.molbiopara.2007.11.005

[35] Egan TJ, Ross DC, Adams PA. Quinoline antimalarial-drugs inhibit spontaneous formation of beta-hematin (malaria pigment). FEBS Lett, 1994;352:54–57. PMID: 7925942

[36] Orjih AU, Mathew TC, Cherian PT. Erythrocyte membranes convert monomeric ferriprotoporphyrin IX to beta-hematin in acidic environment at malarial fever temperature. Exp Biol Med, 2012;237:884–893. DOI: 10.1258/ebm.2012.012013

[37] Chen MM, Shi L, Sullivan Jr DJ. Haemoproteus and Schistosoma synthesize heme polymers similar to Plasmodium hemozoin and β-hematin. Mol Biochem Parasitol, 2001;113:1–8. DOI: 10.1016/S0166-6851(00)00365-0

[38] Dorn A, Stoffel R, Matile H, Bubendorf A, Ridley RG. Malarial haemozoin/beta-haematin supports haem polymerization in the absence of protein. Nature, 1995;374:269–271. DOI: 10.1038/374269a0

[39] Slater AFG, Cerami A. Inhibition by chloroquine of a novel heme polymerase enzyme-activity in malaria trophozoites. Nature, 1992;355:167–169. DOI: 10.1038/355167a0

[40] Ambele MA, Egan TJ. Neutral lipids associated with haemozoin mediate efficient and rapid beta-haematin formation at physiological pH, temperature and ionic composition. Malar J, 2012;11:Article Number 337. DOI: 10.1186/1475-2875-11-337

[41] Dorn A, Vippagunta SR, Matile H, Bubendorf A, Vennerstrom JL, Ridley RG. A comparison and analysis of several ways to promote haematin (haem) polymerisation and an assessment of its initiation in vitro. Biochem Pharmacol, 1998;55:737–747. DOI: 10.1016/S0006-2952(97)00509-1

[42] Hoang AN, Ncokazi KK, de Villiers KA, Wright DW, Egan TJ. Crystallization of synthetic haemozoin (beta-haematin) nucleated at the surface of lipid particles. Dalton Trans, 2010;39:1235–1244. DOI: 10.1039/b914359a

[43] Hoang AN, Sandlin RD, Omar A, Egan TJ, Wright DW. The neutral lipid composition present in the digestive vacuole of *Plasmodium falciparum* concentrates heme and mediates beta-hematin formation with an unusually low activation energy. Biochemistry (Mosc), 2010;49:10107–10116. DOI: 10.1021/bi101397u

[44] Choi CYH, Schneider EL, Kim JM, Gluzman IY, Goldberg DE, Ellman JA, et al. Interference with heme binding to histidine-rich protein-2 as an antimalarial strategy. Chem Biol, 2002;9:881–889. DOI: 10.1016/S1074-5521(02)00183-7

[45] Chugh M, Sundararaman V, Kumar S, Reddy VS, Siddiqui WA, Stuart KD, et al. Protein complex directs hemoglobin-to-hemozoin formation in *Plasmodium falciparum*. Proc Natl Acad Sci USA, 2013;110:5392–5397. DOI: 10.1073/pnas.1218412110

[46] Jani D, Nagarkatti R, Beatty W, Angel R, Slebodnick C, Andersen J, et al. HDP—a novel heme detoxification protein from the malaria parasite. PLoS Pathog, 2008;4:e1000053. DOI: 10.1371/journal.ppat.1000053

[47] Nakatani K, Ishikawa H, Aono S, Mizutani Y. Identification of essential histidine residues involved in heme binding and hemozoin formation in heme detoxification protein from *Plasmodium falciparum*. Sci Rep, 2014;4:6137. DOI: 10.1038/srep06137

[48] Sullivan DJ, Gluzman IY, Goldberg DE. Plasmodium hemozoin formation mediated by histidine-rich proteins. Science, 1996;271:219–222. PMID: 8539625

[49] Chou AC, Fitch CD. Heme polymerase—modulation by chloroquine treatment of a rodent malaria. Life Sci, 1992;51:2073–2078. PMID: 1474861

[50] Ridley RG, Dorn A, Matile H, Kansy M. Heme polymerization in malaria—reply. Nature, 1995;378:138–139. DOI: 10.1038/378138b0

[51] Pisciotta JM, Coppens I, Tripathi AK, Scholl PF, Shuman J, Bajad S, et al. The role of neutral lipid nanospheres in *Plasmodium falciparum* haem crystallization. Biochem J, 2007;402:197–204. DOI: 10.1042/BJ20060986

[52] Pathak MA, Fitzpatrick TB. The evolution of photochemotherapy with psoralens and Uva (Puva)—2000 Bc to 1992 Ad. J Photochem Photobiol B: Biol, 1992;14:3–22. DOI: 10.1016/1011-1344(92)85080-E

[53] Parsons BJ. Psoralen photochemistry. Photochem Photobiol, 1980;32:813–821. DOI: 10.1111/j.1751-1097.1980.tb04061.x

[54] Spikes JD. Photodynamic action: From paramecium to photochemotherapy. Photochem Photobiol, 1997;65:142S–147S. DOI: 10.1111/j.1751-1097.1997.tb07977.x

[55] Dougherty TJ, Gomer CJ, Henderson BW, Jori G, Kessel D, Korbelik M, et al. Photodynamic therapy. J Natl Cancer Inst, 1998;90:889–905. DOI: 10.1093/jnci/90.12.889

[56] von Tappeiner H, Jesionek A. Therapeutic trials with fluorescent substances. (in German). Munch Med Wochenschr, 1903;50:2042–2044.

[57] Taub AF. Photodynamic therapy in dermatology: history and horizons. J Drugs Dermatol, 2004;3:S8–S25. DOI: 10.1007/s10103-009-0716-x

[58] Azzouzi AR, Lebdai S, Benzaghou F, Stief C. Vascular-targeted photodynamic therapy with TOOKAD(R) Soluble in localized prostate cancer: standardization of the procedure. World J Urol, 2015;33:937–944. DOI: 10.1007/s00345-015-1535-2

[59] Shishkova N, Kuznetsova O, Berezov T. Photodynamic therapy in gastroenterology. J Gastrointest Cancer, 2013;44:251–259. DOI: 10.1007/s12029-013-9496-4

[60] Hillemanns P, Petry KU, Soergel P, Collinet P, Ardaens K, Gallwas J, et al. Efficacy and safety of hexaminolevulinate photodynamic therapy in patients with low-grade cervical intraepithelial neoplasia. Lasers Surg Med, 2014;46:456–461. DOI: 10.1002/lsm.22255

[61] Azaïs H, Schmitt C, Tardivel M, Kerdraon O, Stallivieri A, Frochot C, et al. Assessment of the specificity of a new folate-targeted photosensitizer for peritoneal metastasis of epithelial ovarian cancer to enable intraperitoneal photodynamic therapy. A preclinical study. Photodiagnosis Photodyn Ther, 2016;13:130–138. DOI: 10.1016/j.pdpdt.2015.07.005

[62] Stallivieri A, Baros F, Jetpisbayeva G, Myrzakhmetov B, Frochot C. The interest of folic acid in targeted photodynamic therapy. Curr Med Chem, 2015;22:3185–3207. DOI: 10.2174/0929867322666150729113912

[63] Friedberg JS, Mick R, Culligan M, Stevenson J, Fernandes A, Smith D, et al. Photodynamic therapy and the evolution of a lung-sparing surgical treatment for mesothelioma. Ann Thorac Surg, 2011;91:1738–1746. DOI: 10.1016/j.athoracsur.2011.02.062

[64] Costa L, Faustino MAF, Neves M, Cunha A, Almeida A. Photodynamic inactivation of mammalian viruses and bacteriophages. Viruses-Basel, 2012;4:1034–1074. DOI: 10.3390/v4071034

[65] Calzavara-Pinton P, Rossi MT, Sala R, Venturini M. Photodynamic antifungal chemotherapy. Photochem Photobiol, 2012;88:512–522. DOI: 10.1111/j.1751-1097.2012.01107.x

[66] Aspiroz C, Cebamanos BF, Rezusta A, Paz-Cristobal P, Dominguez-Luzond F, Diaz JG, et al. Photodynamic therapy for onychomycosis. Case report and review of the literature. Rev Iberoam Micol, 2011;28:191–193. DOI: 10.1016/j.riam.2011.03.004

[67] Jiblaoui A, Leroy-Lhez S, Ouk TS, Grenier K, Sol V. Novel polycarboxylate porphyrins: synthesis, characterization, photophysical properties and preliminary antimicrobial study against Gram-positive bacteria. Bioorg Med Chem Lett, 2015;25:355–362. DOI: 10.1016/j.bmcl.2014.11.033

[68] Lyon JP, Moreira LM, de Moraes PCG, dos Santos FV, de Resende MA. Photodynamic therapy for pathogenic fungi. Mycoses, 2011;54:E265–E227. DOI: 10.1111/j.1439-0507.2010.01966.x

[69] Richter PR, Strauch SM, Azizullah A, Häder DP. Chlorophyllin as a possible measure against vectors of human parasites and fish parasites. Front Environ Sci, 2014;2:18. DOI: 10.3389/fenvs.2014.00018

[70] Alves E, Faustino MAF, Neves M, Cunha A, Nadais H, Almeida A. Potential applications of porphyrins in photodynamic inactivation beyond the medical scope. J Photochem Photobiol, B, 2015;22:34–57. DOI: 10.1016/j.jphotochemrev.2014.09.003

[71] Barbieri A. Fluorescent sensitizers as larvicides. Photodynamic action of light. (in Spanish). Riv Malariol, 1928;7:456–463.

[72] Pimprikar GD, Georghiou GP. Mechanisms of resistance to diflubenzuron in the housefly, *Musca domestica (L)*. Pestic Biochem Physiol, 1979;12:10–22. DOI: 10.1016/0048-3575(79)90089-0

[73] Robinson JR. Photodynamic insecticides—a review of studies on photosensitizing dyes as insect control agents, their practical application, hazards, and residues. Residue Rev, 1983;88:69–100. DOI: 10.1007/978-1-4612-5569-7_2

[74] Abdel-Kader MH, Eltayeb TA, Photodynamic control of malaria vector, noxious insects and parasites, in: H.M. Abdel-Kader (Ed.) Photodynamic Therapy: From Theory to

Application, Springer Berlin Heidelberg, Berlin, Heidelberg, 2014. p. 269–291. DOI: 10.1007/978-3-642-39629-8_13

[75] Rebeiz CA, Juvik JA, Rebeiz CC. Porphyric insecticides. 1. Concept and phenomenology. Pestic Biochem Physiol, 1988;30:11–27. DOI: 10.1016/0048-3575(88)90055-7

[76] Rebeiz CA, Juvik JA, Rebeiz CC, Bouton CE, Gut LJ. Porphyric insecticides. 2. 1,10-Phenanthroline, a potent porphyric insecticide modulator. Pestic Biochem Physiol, 1990;36:201–207. DOI: 10.1016/0048-3575(90)90011-P

[77] Lucantoni L, Magaraggia M, Lupidi G, Ouedraogo RK, Coppellotti O, Esposito F, et al. Novel, meso-substituted cationic porphyrin molecule for photo-mediated larval control of the dengue vector *Aedes aegypti*. PLoS Negl Trop Dis, 2011;5:e1434. DOI: 10.1371/journal.pntd.0001434

[78] Fabris C, Ouedraogo RK, Coppellotti O, Dabire RK, Diabate A, Di Martino P, et al. Efficacy of sunlight-activatable porphyrin formulates on larvae of *Anopheles gambiae* M and S molecular forms and *An. arabiensis*: a potential novel biolarvicide for integrated malaria vector control. Acta Trop, 2012;123:239–243. DOI: 10.1016/j.actatropica.2012.05.011

[79] Stallivieri A, Le Guern F, Vanderesse R, Meledje E, Jori G, Frochot C, et al. Synthesis and photophysical properties of the photoactivatable cationic porphyrin 5-(4-N-dodecylpyridyl)-10,15,20-tri(4-N-methylpyridyl)-21H,23H-porphyrin tetraiodide for anti-malaria PDT. Photochem Photobiol Sci, 2015;14:1290–1295. DOI: 10.1039/C5PP00139K

[80] Peterson LR. Squeezing the antibiotic balloon: the impact of antimicrobial classes on emerging resistance. Clin Microbiol Inf, 2005;11:4–16. DOI: 10.1111/j.1469-0691.2005.01238.x

[81] Pereira Rosa L, da Silva FC. Antimicrobial photodynamic therapy: a new therapeutic option to combat infections. J Med Microb Diagn, 2014;3:Art 158. DOI: 10.4172/2161-0703.1000158

[82] Sperandio FF, Huang Y-Y, Hamblin MR. Antimicrobial photodynamic therapy to kill Gram-negative bacteria. Recent Patents Anti-Infect Drug Disc, 2013;8:108–120. DOI: 10.2174/1574891X113089990012

[83] Maisch T, Szeimies R-M, Jori G, Abels C. Antibacterial photodynamic therapy in dermatology. Photochem Photobiol Sci, 2004;3:907–917. DOI: 10.1039/B407622B

[84] Perni S, Prokopovich P, Pratten J, Parkin IP, Wilson M. Nanoparticles: their potential use in antibacterial photodynamic therapy. Photochem Photobiol Sci, 2011;10:712–720. DOI: 10.1039/C0PP00360C

[85] Adolfo Vera DM, Haynes MH, Ball AR, Dai T, Astrakas C, Kelso MJ, et al. Strategies to potentiate antimicrobial photoinactivation by overcoming resistant

phenotypes. Photochem Photobiol, 2012;88:499–511. DOI: 10.1111/j.1751-1097.2012.01087.x

[86] Denis TGS, Dai T, Izikson L, Astrakas C, Anderson RR, Hamblin MR, et al. All you need is light antimicrobial photoinactivation as an evolving and emerging discovery strategy against infectious disease. Virulence, 2011;2:509–520. DOI: 10.4161/viru.2.6.17889

[87] Tim M. Strategies to optimize photosensitizers for photodynamic inactivation of bacteria. J Photochem Photobiol B: Biol, 2015;150:2–10. DOI: 10.1016/j.jphotobiol.2015.05.010

[88] Craig RA, McCoy CP, Gorman SP, Jones DS. Photosensitisers—the progression from photodynamic therapy to anti-infective surfaces. Expert Opin Drug Deliv, 2015;12:85–101. DOI: 10.1517/17425247.2015.962512

[89] Nakonechny F, Nisnevitch M, Nitzan Y, Firer MA, New techniques in antimicrobial photodynamic therapy: scope of application and overcoming drug resistance in nosocomial infections, in: A. Méndez-Vilas (Ed.) Science Against Microbial Pathogens: Communicating Current Research and Technological Advances, Volume 2, Formatex Research Center, 2011. p. 684–691. DOI: 10.1016/j.biomaterials.2007.06.015

[90] Ryskova L, Buchta V, Slezak R. Photodynamic antimicrobial therapy. Cent Eur J Biol, 2010;5:400–406. DOI: 10.2478/s11535-010-0032-2

[91] Wainwright M. Photodynamic antimicrobial chemotherapy (PACT). J Antimicrob Chemother, 1998;42:13–28. DOI: 10.1093/jac/42.1.13

[92] Souza E, Medeiros AC, Gurgel BC, Sarmento C. Antimicrobial photodynamic therapy in the treatment of aggressive periodontitis: a systematic review and meta-analysis. Lasers Med Sci, 2016;31:187–196. DOI: 10.1007/s10103-015-1836-0

[93] Carpenter BL, Situ XC, Scholle F, Bartelmess J, Weare WW, Ghiladi RA. Antiviral, antifungal and antibacterial activities of a BODIPY-based photosensitizer. Molecules, 2015;20:10604–10621. DOI: 10.3390/molecules200610604

[94] Chang J-E, Oak C-H, Sung N, Jheon S. The potential application of photodynamic therapy in drug-resistant tuberculosis. J Photochem Photobiol B: Biol, 2016;150:60–65. DOI: 10.1016/j.jphotobiol.2015.04.001

[95] Dai T, Huang Y-Y, Hamblin MR. Photodynamic therapy for localized infections—state of the art. Photodiagnosis Photodyn Ther, 2009;6:170–188. DOI: 10.1016/j.pdpdt.2009.10.008

[96] Baptista MS, Wainwright M. Photodynamic antimicrobial chemotherapy (PACT) for the treatment of malaria, leishmaniasis and trypanosomiasis. Braz J Med Biol Res, 2011;44:1–10. DOI: 10.1590/S0100-879X2010007500141

[97] Deda DK, Budu A, Cruz LN, Araki K, Garcia CRS. Strategies for development of antimalarials based on encapsulated porphyrin derivatives. Mini-Rev Med Chem, 2015;14:1055–1071. DOI: 10.2174/1389557515666150101094829

[98] Jori G, Coppellotti O. Inactivation of pathogenic microorganisms by photodynamic techniques: mechanistic aspects and perspective applications. Antiinfect Agents Med Chem, 2007;6:119–131. DOI: 10.2174/187152107780361652

[99] Wainwright M. The development of phenothiazinium photosensitisers. Photodiagnosis Photodyn Ther, 2005;2:263–272. DOI: 10.1093/jac/42.1.13

[100] Almeida A, Cunha A, Faustino MAF, Tome AC, Neves MGPMS, Chapter 5 Porphyrins as Antimicrobial Photosensitizing Agents, in: Photodynamic Inactivation of Microbial Pathogens: Medical and Environmental Applications, The Royal Society of Chemistry, 2011. p. 83–160. DOI: 10.1039/9781849733083-00083

[101] Lindholm PF, Annen K, Ramsey G. Approaches to minimize infection risk in blood banking and transfusion practice. Infect Disord Drug, 2011;11:45–56. DOI: 10.2174/187152611794407746

[102] Castro E, Chagas and other protozoan diseases, in: Isbt Science Series, Volume 2, No 1: State of the Art Presentations, 2007. p. 1–5. DOI: 10.1111/j.1751-2824.2007.00062.x

[103] Reddy HL, Doane SK, Keil SD, Marschner S, Goodrich RP. Development of a riboflavin and ultraviolet light-based device to treat whole blood. Transfusion (Malden, MA, U.S.), 2013;53:131S–136S. DOI: 10.1111/trf.12047

[104] Goodrich RP, Platz MS. The design and development of selective, photoactivated drugs for sterilization of blood products. Drug Future, 1997;22:159–171. DOI: 10.1358/dof.1997.022.02.400935

[105] LeBlanc D, Story R, Gross E. Laser-induced inactivation of *Plasmodium falciparum*. Malar J, 2012;11:Art267. DOI: 10.1186/1475-2875-11-267

[106] Guttmann P, Ehrlich P. On the effect of methylene blue in malaria. (in German). Berlin Klin Woch, 1891;28:953–956.

[107] Rounds DE, Opel W, Olson RS, Sherman IW. The potential use of laser energy in the management of malaria. Biochem Biophys Res Commun, 1968;32:616–623. DOI: 10.1016/0006-291X(68)90282-9

[108] Smith OM, Dolan SA, Dvorak JA, Wellems TE, Sieber F. Merocyanine-540-sensitized photoinactivation of human erythrocytes parasitized by *Plasmodium falciparum*. Blood, 1992;80:21–24. PMID: 1611086

[109] Das BS, Das DB, Satpathy RN, Patnaik JK, Bose TK. Riboflavin deficiency and severity of malaria. Eur J Clin Nutr, 1988;42:277–283. PMID: 3293996

[110] Traunmuller F, Ramharter M, Lagler H, Thalhammer F, Kremsner PG, Graninger W, et al. Normal riboflavin status in malaria patients in Gabon. Am J Trop Med Hyg, 2003;68:182–185. PMID: 12641409

[111] Akompong T, Ghori N, Haldar K. In vitro activity of riboflavin against the human malaria parasite *Plasmodium falciparum*. Antimicrob Agents Chemother, 2000;44:88–96. DOI: 10.1128/AAC.44.1.88-96.2000

[112] Keil SD, Kiser P, Sullivan JJ, Kong AS, Reddy HL, Avery A, et al. Inactivation of *Plasmodium* spp. in plasma and platelet concentrates using riboflavin and ultraviolet light. Transfusion, 2013;53:2278–2286. DOI: 10.1111/trf.12079

[113] ISLA. Anti-microbial photodynamic therapy as a new treatment option for malaria. 2015. Available from: http://www.isla-laser.org/research-group/en/current/current-projects/

[114] Sigala PA, Crowley JR, Henderson JP, Goldberg DE. Deconvoluting heme biosynthesis to target blood-stage malaria parasites. Elife, 2015;4:e09143. DOI: 10.7554/eLife.09143

[115] Wachowska M, Muchowicz A, Firczuk M, Gabrysiak M, Winiarska M, Wanczyk M, et al. Aminolevulinic acid (ALA) as a prodrug in photodynamic therapy of cancer. Molecules, 2011;16:4140–4164. DOI: 10.3390/molecules16054140

[116] Smith TG, Kain KC. Inactivation of *Plasmodium falciparum* by photodynamic excitation of heme-cycle intermediates derived from d-aminolevulinic acid. J Infect Dis, 2004;190:184–191. DOI: 10.1086/421503

[117] Martiney JA, Cerami A, Slater AF. Inhibition of hemozoin formation in *Plasmodium falciparum* trophozoite extracts by heme analogs: possible implication in the resistance to malaria conferred by the beta-thalassemia trait. Mol Med, 1996;2:236–246. PMID: 8726466

[118] Begum K, Kim HS, Kumar V, Stojiljkovic I, Wataya Y. In vitro antimalarial activity of metalloporphyrins against *Plasmodium falciparum*. Parasitol Res, 2003;90:221–224. DOI: 10.1007/s00436-003-0830-9

[119] Alves E, Iglesias BA, Deda DK, Budu A, Matias TA, Bueno VB, et al. Encapsulation of metalloporphyrins improves their capacity to block the viability of the human malaria parasite *Plasmodium falciparum*. Nanomed Nanotechnol Biol Med, 2015;11:351–358. DOI: 10.1016/j.nano.2014.09.018

[120] Abada Z, Cojean S, Pomel S, Ferrie L, Akagah B, Lormier AT, et al. Synthesis and antiprotozoal activity of original porphyrin precursors and derivatives. Eur J Med Chem, 2013;67:158–165. DOI:10.1016/j.ejmech.2013.06.002

[121] Grellier P, Santus R, Mouray E, Agmon V, Maziere JC, Rigomier D, et al. Photosensitized inactivation of *Plasmodium falciparum* and Babesia divergens-infected erythrocytes in whole blood by lipophilic pheophorbide derivatives. Vox Sang, 1997;72:211–220. DOI: 10.1046/j.1423-0410.1997.7240211.x

[122] Lustigman S, BenHur E. Photosensitized inactivation of *Plasmodium falciparum* in human red cells by phthalocyanines. Transfusion, 1996;36:543–546. DOI: 10.1046/j.1537-2995.1996.36696269514.x

[123] Zhao XJ, Lustigman S, Kenney ME, BenHur E. Structure-activity and mechanism studies on silicon phthalocyanines with *Plasmodium falciparum* in the dark and under red light. Photochem Photobiol, 1997;66:282–287. DOI: 10.1111/j.1751-1097.1997.tb08656.x

[124] Loh CCY, Suwanarusk R, Lee YQ, Chan KWK, Choy KY, Renia L, et al. Characterization of the commercially-available fluorescent chloroquine-BODIPY conjugate, LynxTag-CQ(GREEN), as a marker for chloroquine resistance and uptake in a 96-well plate assay. Plos One, 2014;9:Art. e110800. DOI: 10.1371/journal.pone.0110800

[125] Fu Y, Klonis N, Suarna C, Maghzal GJ, Stocker R, Tilley L. A phosphatidylcholine-BODIPY 581/591 conjugate allows mapping of oxidative stress in *P. falciparum*-infected erythrocytes. Cytometry A, 2009;75A:390–404. DOI: 10.1002/cyto.a.20704

[126] Wissing F, Sanchez CP, Rohrbach P, Ricken S, Lanzer M. Illumination of the malaria parasite *Plasmodium falciparum* alters intracellular pH—implications for live cell imaging. J Biol Chem, 2002;277:37747–37755. DOI: 10.1074/jbc.M204845200

[127] Basilico N, Monti D, Olliaro P, Taramelli D. Non-iron porphyrins inhibit beta-haematin (malaria pigment) polymerisation. FEBS Lett, 1997;409:297–299. DOI: 10.1016/S0014-5793(1097)00533-00534.

[128] Slater AF, Swiggard WJ, Orton BR, Flitter WD, Goldberg DE, Cerami A, et al. An iron-carboxylate bond links the heme units of malaria pigment. Proc Natl Acad Sci U S A, 1991;88:325–329. PMID: 1988933

[129] Monti D, Vodopivec B, Basilico N, Olliaro P, Taramelli D. A novel endogenous antimalarial: Fe(II)-protoporphyrin IX alpha (heme) inhibits hematin polymerization to beta-hematin (malaria pigment) and kills malaria parasites. Biochemistry (Mosc), 1999;38:8858–8863. DOI: 10.1021/bi990085k.

[130] Bhat AR, Athar F, Van Zyl RL, Chen C-T, Azam A. Synthesis and biological evaluation of novel 4-substituted 1-{[4-(10,15,20-triphenylporphyrin-5-yl)phenyl]methylidene}thiosemicarbazides as new class of potential antiprotozoal agents. Chem Biodivers, 2008;5:764–776. DOI: 10.1002/cbdv.200890073

[131] Cole KA, Ziegler J, Evans CA, Wright DW. Metalloporphyrins inhibit beta-hematin (hemozoin) formation. J Inorg Biochem, 2000;78:109–115. DOI: 10.1016/S0162-0134(1099)00216-00210.

[132] Ziegler J, Pasierb L, Cole KA, Wright DW. Metalloporphyrin probes for antimalarial drug action. J Inorg Biochem, 2003;96:478–486. DOI: 10.1016/S0162-0134(03)00253-8

[133] Chemaly SM, Chen C-T, van Zyl RL. Naturally occurring cobalamins have antimalarial activity. J Inorg Biochem, 2007;101:764–773. DOI: 10.1016/j.jinorgbio.2007.01.006

[134] Rodriguez M, Bonnet-Delpon D, Begue JP, Robert A, Meunier B. Alkylation of manganese(II) tetraphenylporphyrin by antimalarial fluorinated artemisinin derivatives. Bioorg Med Chem Lett, 2003;13:1059–1062. DOI: 10.1016/S0960-894X(03)00076-3

[135] Benoit-Vical F, Robert A, Meunier B. Potentiation of artemisinin activity against chloroquine-resistant *Plasmodium falciparum* strains by using heme models. Antimicrob Agents Chemother, 1999;43:2555–2558. PMID: 10508044

[136] Benoit-Vical F, Robert A, Meunier B. In vitro and in vivo potentiation of artemisinin and synthetic endoperoxide antimalarial drugs by metalloporphyrins. Antimicrob Agents Chemother, 2000;44:2836–2841. DOI: 10.1128/AAC.44.10.2836-2841.2000

[137] El Chaar M, Atwal S, Freimanis GL, Dinko B, Sutherland CJ, Allain JP. Inactivation of *Plasmodium falciparum* in whole blood by riboflavin plus irradiation. Transfusion, 2013;53:3174–3183. DOI: 10.1111/trf.12235

[138] Owusu-Ofori S, Kusi J, Owusu-Ofori A, Freimanis G, Olver C, Martinez CR, et al. Treatment of whole blood with riboflavin and UV light: impact on malaria parasite viability and whole blood storage. Shock, 2014;44:33–38. DOI: 10.1097/SHK.0000000000000280

IntechOpen