Inactivation of *Plasmodium falciparum* by Photodynamic Excitation of Heme-Cycle Intermediates Derived from δ-Aminolevulinic Acid

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Transfusion-transmitted malaria (TTM), especially that caused by *Plasmodium falciparum*, is of great concern in malaria-endemic areas. As a result of increased international travel, migration, and the spread of drug-resistant parasites, TTM is also a growing problem in industrialized nations. An effective and inexpensive means of inactivating malaria parasites in blood products would represent an important advance. In this report, we demonstrate that photoactivation of plasmodial heme-cycle intermediates, derived from supplemental δ-aminolevulinic acid (ALA), by exposure to simple white light in the presence of ALA, reduces *P. falciparum* in culture to levels that are undetectable by light microscopy or lactate dehydrogenase assay. Photodynamic excitation of presumed heme-cycle intermediates, which was revealed by fluorescence microscopy, did not appear to adversely affect the viability of erythrocytes. These data suggest that this pathogen-inactivation strategy, which uses inexpensive reagents and white light, may represent an appropriate means of inactivating malaria parasites in blood products in resource-poor settings.

Human malaria, caused by 4 species of apicomplexan protozoa of the genus *Plasmodium*, accounts for an estimated 300 million cases and 1.5–2.7 million deaths annually [1]. There has been a global resurgence of malaria, largely attributable to the development and spread of drug-resistant parasites; as drug resistance continues to escalate, and in the absence of an effective vaccine, the global malaria situation is likely to worsen [2].

Malaria parasites invade and multiply inside human erythrocytes, thus posing a risk to recipients of blood products from infected donors. Transfusion-transmitted malaria (TTM) is of primary concern in malaria-endemic countries. However, TTM is also a growing challenge in industrialized nations, because of substantive changes in international travel, migration, and transmission of malaria [3]. For example, in a recent review of TTM in the United States, the risk of TTM has been estimated to be similar to the risk of transfusion-transmitted hepatitis C virus and HIV [4]. Of particular concern, in 62% of TTM cases, the donor-screening failed, and the overall case fatality rate was 11% [4]. In addition, many potential blood donors who have travelled to malaria-endemic areas are lost to unit-exclusion criteria, when, in fact, only a minute fraction of donors ever contain viable parasites in their blood. In the United States, an estimated 50,000 donations are excluded each year because of travel to a malarious area [4]. Nucleic acid testing is used to prevent many transfusion-transmitted infections. However, Food and Drug Administration–approved tests for malaria are not currently available, and alternate strategies, including inactivating pathogen, such as malaria parasites, in blood products are needed.
Heme is a component of many proteins, including hemoglobin, myoglobin, and cytochrome c. In humans, the first step in the heme cycle is the condensation of glycine and succinyl CoA, by δ-aminolevulinic acid (ALA) synthase, in the mitochondria of all cells, to form ALA, a 5-carbon amino acid. This precursor is then exported to the cytosol, processed by 2 enzymes (to form protoporphyrin IX [PPIX]). Heme is formed by the addition of an atom of ferrous iron to the ring-shaped PPIX, by ferrochelatase [5]. In humans, the synthesis of heme is tightly regulated; in the presence of sufficient levels of heme, the synthesis of ALA by ALA synthase is inhibited by a complex negative-feedback mechanism [6, 7].

Heme-containing proteins have also been identified in Plasmodium falciparum [8, 9], and parasites must either obtain heme from heme-containing proteins of the host or synthesize their own. P. falciparum can metabolize hemoglobin by hydrolyzing the globin component, which provides most of the amino acids required by this parasite, and by detoxifying the heme moiety released during hemoglobin breakdown [10]. However, despite the apparent abundance of heme present within infected erythrocytes, these parasites cannot salvage the porphyrin ring of hemoglobin for their own biosynthetic purposes [11]. Instead, the parasite converts heme obtained through the metabolism of hemoglobin into hemozoin, an insoluble compound composed of excess protein and iron-containing hematin [12, 13].

The ALA synthase enzyme of P. falciparum catalyses the formation of ALA in its mitochondria [14, 15]. Malaria parasites also import ALA synthesized by human erythrocytes or ALA that is present in the medium of cultures of P. falciparum [15–17]. Thus, in theory, a surplus of ALA taken up by parasites would result in a bypass of the negative-feedback inhibition of ALA synthase by heme. Once this inhibition has been bypassed, heme would be produced in the parasite at a rate limited by its own characteristic enzyme-activity profile. If the new rate-limiting step in the presence of excess ALA is the conversion of PPIX to heme, then parasites would be expected to accumulate large quantities of PPIX [18].

PPIX and other heme-cycle intermediates are photosensitizers; in the presence of a certain wavelength of light, these compounds will increase in energy and produce an excited short-lived electronic state called a singlet. This compound, in its singlet state, usually decays to its ground state, emitting fluorescence in the process. However, in many cases, the singlet molecule will undergo spin inversion to a metatriplet state. This state reacts with oxygen, to generate highly reactive singlet oxygen, which targets membranes, particularly those of the mitochondria [19–21]. These membranes become peroxidized and are lysed, which leads to the death of the cell [22].

Photodynamic therapy using ALA and heme-cycle derivatives has been used in the treatment of early stages of malignant skin tumors [23, 24] and upper gastrointestinal cancers [22]. Neoplastic cells appear to lack the complex feedback-inhibition mechanisms of normal human cells, and, therefore, high concentrations of PPIX and other heme-cycle intermediates accumulate [24], rendering these cells vulnerable to destruction by irradiation of light of appropriate wavelength.

We hypothesize that malaria parasites, because of their unusual heme metabolism, are candidates for inactivation by photodynamic excitation of unstable ALA-derived heme-cycle intermediates. We wished to determine the concentration of ALA and the intensity of incoherent white light necessary to photodynamically inactivate P. falciparum malaria parasites and to ascertain whether this novel in vitro method might eventually be applied as a means to inactivate Plasmodium species in blood products.

**MATERIALS AND METHODS**

**Media and reagents.** Endotoxin-free RPMI 1640 medium and heat-inactivated group AB human serum were obtained from Wisent; Percoll was purchased from Pharmacia; and ALA, sorbitol, HEPES, sodium bicarbonate, and hypoxanthine were obtained from Sigma.

**Treatment of cultures with ALA.** Stock cultures of 2 clones of P. falciparum, including 3D7A [25, 26] and ITG, were grown at 5% hematocrit in RPMI 1640 media supplemented with 10% heat-inactivated human serum (complete medium) in 50-mL flasks, at 37°C, as described elsewhere [27, 28]. Stock cultures were treated for 5 min with 5% sorbitol, to remove trophozoites and schizonts from ring stages. Assays were performed in 1-mL volumes in 12-well culture plates after stock cultures had been diluted to a parasitemia of 0.3% ring stages. Parasites were exposed to various concentrations of ALA (0, 200 μmol/L, and 2 mmol/L), made up fresh in complete medium before every experiment [29], for 8 h before cultures were washed with complete medium. These concentrations of ALA have been used previously to study the incorporation of radioactive ALA (50 μmol/L and 2 mmol/L) into heme, in P. falciparum and P. berghei, in vitro [15]; for inactivation of human lymphoma cells in vitro (1 mmol/L) [30]; and for murine studies (1.5 mmol/L) [18].

**Treatment of cultures with ALA and incoherent white light.** After incubation with ALA and washing with complete medium, parasites in culture plates were exposed to incoherent white light (as opposed to light of 1 wavelength [e.g., lasers]) emanating from the glass surface of an overhead projector (410 W bulb and 718 cm² surface area, for a total energy fluence rate of 0.57 W/cm²), for different durations (0, 10, 20, or 30 min) at 37°C. The glass surface of the overhead projector never exceeded 37°C during the exposure time. The total energy flu-
ence rate over time (total light exposure) is determined by multiplying the total energy fluence rate by the number of seconds of exposure to the light source, to give a value in J/cm². Thus, for the various times given above (2–30 min), the total light exposure varied from 342 to 1026 J/cm². Such incoherent light sources have been used in photodynamic therapy of cancer cells, and the required energy fluence rate over time has been estimated at 30–540 J/cm² [24].

**Culture-viability assays.** After exposure to light, cultures were grown for 12 days, with dilutions of fresh blood and complete medium every 2 days so that parasitemia values were never >5%. Generally, all cultures were diluted 1:4 if parasitemia for any 1 of the cultures was >2% of mature asexual stages (trophozoites and schizonts). Viability of treated parasites was determined by quantifying their growth over this period and by assays at the end of this period that ascertain levels of the parasite protein lactate dehydrogenase, which is produced only by live parasites [31, 32]. To quantify the growth of parasites in culture, smears were taken every 24 h, to determine any changes to parasitemia in each of the culture wells in which concentrations of ALA and duration of exposure to light varied. Assays were repeated for each of 2 clones at least 3 times.

**Autofluorescence assays.** Wet mounts of parasite cultures were made immediately after cultures were incubated for 8 h with 0, 200 μmol/L, and 2 mmol/L ALA. Slides were examined using a Nikon Labophot EFD-3 microscope with UV illumination. If photosensitizable heme intermediates, such as PPIX, which has an absorbance of 632 nm [24], are accumulating in parasites or infected erythrocytes, these cells should fluoresce when viewed through a Nikon filter block G-2E/C, which filters all wavelengths of light except those from 605–655 nm. Nikon filter blocks UV-2E/C and B-2E/C, which transmit only blue (435–485 nm) and green (515–555 nm) light, respectively, were also used.

**Erythrocyte-reinfection studies.** Uninfected erythrocytes were incubated for 8 h with 0, 200 μmol/L, and 2 mmol/L ALA, washed with complete medium, and exposed to light for various durations (0, 10, 20, or 30 min). A total of 12 conditions were thus generated by this method. Sixteen hours later, 2 mL of *P. falciparum* (clone 3D7A) culture containing 5% mature stages was layered on 6 mL of 80% Percoll (made up in 1× PBS) and centrifuged at 1550 g for 30 min at 20°C, to concentrate trophozoites and schizonts to a parasitemia of 80%. After washing with complete medium and centrifuging at 700 g for 10 min at 20°C, a total of 5 × 10⁸ mature stages were added to each of 12 culture conditions (each culture contained 1 mL of culture at 5% hematocrit) in a 12-well plate. Viability of cultures was determined as above, and dilution of cultures was performed when necessary.

**Statistical analysis.** The data are expressed as means and SDs of *n* experiments, with each mean representing the results of at least 3 equivalent and independent experiments performed in duplicate. Statistical significance was determined by use of the Student's *t* test. Error bars on figures represent the SE of the means.

**RESULTS**

**Reduction in viability of *P. falciparum* cultures incubated with ALA.** To determine the effect of ALA alone (independent of light) on the viability of *P. falciparum*, the growth of malaria parasites (clones 3D7A and ITG), preincubated for 8 h with different concentrations of ALA (0, 200 μmol/L, and 2 mmol/L), was recorded for 12 days. For clone 3D7A, control cultures and those exposed to 200 μmol/L ALA remained healthy and required dilution every 2 days for the entire 12-day period; at the end of this period, the mean parasitemia of cultures was still high (2.6%–2.7%) (figure 1A). The parasitemia of cultures incubated with 2 mmol/L ALA decreased after 3 days; at 12 days after treatment, the mean parasitemia of 1.0% was significantly lower than control values (*P* < .05). Results were similar with clone ITG. These data suggest that higher concentrations of ALA, even in the presence of ambient white light alone, are moderately toxic to parasites.

**Viability of *P. falciparum* cultures exposed to incoherent light in the absence of ALA.** To determine the effect of incoherent white light alone (independent of ALA) on the viability of *P. falciparum*, the growth of malaria parasites (clone 3D7A), incubated for 8 h without ALA and exposed to simple white light for 0, 10, 20, or 30 min, was recorded for 12 days. All cultures remained healthy and required dilution every 2 days for the entire 12-day period; at the end of this period, the mean parasitemia of cultures was still high (2.7%–3.1%) (figure 1B). Results were similar with clone ITG. These data suggest that exposure to white light alone (no ALA) does not affect parasite viability.

**Reduction in viability of ALA-treated parasites exposed to incoherent light.** To determine the effect on ALA-treated malaria parasites exposed to various periods of incoherent light, the growth of malaria cultures incubated for 8 h with different concentrations of ALA (0, 200 μmol/L, and 2 mmol/L) and exposed to white light (for 0, 10, 20, or 30 min) was documented for 12 days.

Figure 2 illustrates the dose-response experiments with increasing exposure to white light. Cultures of clone 3D7 incubated with 200 μmol/L ALA but not exposed to light grew as well as did those subjected to neither ALA nor light (figure 1A). However, cultures incubated with 200 μmol/L ALA and exposed to light for different durations showed a significant decrease in parasitemia after 12 days (*P* < .03, for 10 min [figure 2A]; *P* < .01, for 20 min [figure 2B]; and *P* < .01, for 30 min [figure 2C]). Results were similar with clone ITG.
Inactivation of Malaria Parasites in Blood Products

Figure 1. Viability of Plasmodium falciparum cultures incubated with δ-aminolevulinic acid (ALA). A, Malaria parasites (clone 3D7A) were incubated for 8 h with different concentrations of ALA (0, 200 μmol/L, and 2 mmol/L), washed, and then cultured for 12 days, as described in Materials and Methods. At day 12, control cultures and those incubated with 200 μmol/L ALA each had a high mean parasitemia (2.67% ± 0.55% and 2.63% ± 0.81%; P = .96). The parasitemia of cultures incubated with 2 mmol/L ALA decreased after 3 days; at 12 days after treatment, the mean parasitemia of was significantly 1.03%. Cultures were diluted 1:4 on days 3, 5, 7, 9, and 11. B, Malaria parasites (clone 3D7A) were incubated for 8 h without ALA and exposed to incoherent white light for 0, 10, 20, or 30 min, washed, and then cultured for 12 days. At the end of this period, all cultures had a high mean parasitemia (2.7%–3.1%). Cultures were diluted 1:4 on days 3, 5, 7, 9, and 11.

Figure 3 shows the dose-response experiments with increasing concentrations of ALA. Cultures of 3D7 exposed to 30 min of light but not incubated with ALA grew as well as did controls subjected to neither light nor ALA (see figure 1B). However, cultures incubated with 200 μmol/L and 2 mmol/L ALA and exposed to 30 min of light were greatly reduced in viability (P < .002, for both concentrations; figure 3A). After 12 days, parasites could not be detected by either light microscopy or lactate dehydrogenase assay in 1 of 3 trials with 200 μmol/L ALA and in 2 of 3 trials with 2 mmol/L ALA (a parasitemia of 0.001% was obtained in the other trial with 2 mmol/L ALA). Results were similar with clone ITG (figure 3B).

These data indicate that photodynamic activation of derivatives of ALA are capable of inactivating P. falciparum to a high degree in vitro, with the effectiveness of killing increasing significantly with higher doses of ALA in the medium and longer exposure to white light.

Accumulation of photosensitizers in parasitized ALA-treated erythrocytes. To determine whether photosensitizers in the form of heme-cycle intermediates are accumulating in ALA-treated parasitized erythrocytes, wet mounts of cultures treated with 0, 200 μmol/L, and 2 mmol/L ALA were examined by use of a UV microscope equipped with 3 filter blocks that allow only blue, green, or red light, respectively. In cultures treated with ALA, infected erythrocytes fluoresced orange-red, whereas uninfected erythrocytes did not fluoresce, when observed with the red filter block (data not shown). Infected and uninfected erythrocytes in control cultures that were not subjected to ALA did not fluoresce when observed with the red filter block, and infected and uninfected erythrocytes in ALA-treated or control cultures did not fluoresce, when observed with the blue or the green filter blocks. This suggests that parasitized erythrocytes exposed to ALA are accumulating a photosensitizing compound that absorbs a wavelength of light between 605 and 655 nm, a range that includes the absorbance of PPIX at 632 nm [24].

Viability of uninfected erythrocytes exposed to ALA and light. To test the viability of uninfected erythrocytes after treatment with ALA and exposure to light, we layered cultured schizonts of P. falciparum on uninfected erythrocytes that had been incubated with various concentrations of ALA (0, 200 μmol/L, and 2 mmol/L) and exposed to light for various durations (0, 10, 20, or 30 min). The molecular mechanism of merozoite invasion is complex, involving the interaction of a number of merozoite ligands and erythrocyte receptors, and is extremely sensitive to any changes in erythrocyte viability, morphology, and membrane physiology [33, 34]. Therefore, we reasoned that merozoites from burst schizonts would only infect ALA-treated erythrocytes if red blood cell (RBC) morphology and physiology were intact. Maturation of the asexual stage and consequent invasion of the next generation of merozoites into ALA-treated erythrocytes 48 h later was thus used as a surrogate marker for RBC viability. Parasites in all 12 culture conditions grew well, at approximately the same rate (cultures had to be diluted 1:4 on days 5, 7, 9, and 11), as, on day 12, all cultures ranged in mean parasitemia from 1.13% to 1.43%; variation among conditions correlated neither to the log of ALA concentration (r = 0.418; slope = 0.045) nor to the duration of exposure to light (r = −0.433; slope = −0.034). As a comparison, among parasite-infected erythrocytes that were exposed to the same concentrations of ALA and light...
Figure 2. Reduction in viability of δ-aminolevulinic acid (ALA)-treated *Plasmodium falciparum* cultures exposed to different durations of incoherent light. Malaria parasites (clone 3D7A) were incubated for 8 h with different concentrations of ALA (0 or 200 μmol/L), exposed to incoherent white light for 10, 20, or 30 min, and then cultured for 12 days. Cultures incubated with 200 μmol/L ALA showed a significant decrease in parasitemia—that is, correlated to the duration of exposure to light—after 12 days (for 10 min [A]; for 20 min [B]; and for 30 min [C]). Cultures were diluted 1:4 on days 3, 5, 7, 9, and 11.

**DISCUSSION**

In the present study, we have demonstrated that *P. falciparum* ring-stage parasites can be inactivated in vitro after incubation with ALA coupled with exposure to white light. Parasitemia of cultures decreases at higher concentrations of ALA and at longer durations of exposure to light. A sensitive measure of erythrocyte viability, namely the ability of *P. falciparum* to invade and mature within erythrocytes, revealed that concentration of ALA and duration of exposure to light did not negatively affect cultures exposed to either or both of these elements. Erythrocytes containing ALA-treated parasites, when subjected to red light, displayed an orange-red fluorescence, likely indicative of the accumulation of photosensitive heme-cycle intermediates, that was absent in uninfected erythrocytes and parasitized erythrocytes not incubated with ALA. Taken together, these data indicate that the reduction in parasite viability was likely caused by the photodynamic excitation of unstable heme-cycle intermediates formed during enzymatic processing of surplus ALA to heme. Photoactivatable intermediates accumulated only in infected erythrocytes, imparting specificity to this inactivation strategy and decreasing the potential for injury to uninfected erythrocytes. This mechanism is novel for malaria parasites and may have potential implications for pathogen inactivation in blood products.

The hemoglobin-degradation pathway of malaria parasites has been investigated in detail (reviewed in [10]) and is a target for antimalarial drugs, including chloroquine, quinine, and artemisinin (reviewed in [35]). Less is known, however, about the heme-biosynthesis pathway of malaria parasites. *P. falciparum* synthesizes ALA synthase de novo, to catalyze the reaction of glycine and succinyl CoA, to form ALA in the mitochondria [14, 15, 36]. Bonday et al. [15, 37] postulated that *P. falciparum* imports some or all of the remaining 7 heme pathway enzymes from the infected erythrocyte. However, the malaria genome project has revealed orthologues of all except 1 of the genes for heme biosynthetic enzymes in *P. falciparum* [5, 35]. One of these is a functional ALA dehydratase, the second enzyme in the heme pathway [5], which appears to be targeted from the nucleus to the apicoplast of the parasite [38]. On the basis of these observations, a model for the biosynthesis of heme has been proposed [35, 39]. Glycine obtained from hemoglobin degradation and succinyl CoA generated from the tricarboxylic acid cycle of the parasite are catalyzed by ALA synthase, to form ALA in the mitochondrion of the parasite. ALA is then transported to the apicoplast, where ALA dehydratase converts 2 molecules of ALA
Inactivation of Malaria Parasites in Blood Products

Inactivation of Malaria Parasites in Blood Products

• JID 2004:190 (1 July) • 189

Figure 3. Elimination of parasites in Plasmodium falciparum cultures treated with 2 mmol/L δ-aminolevulinic acid (ALA) and exposed to incoherent light for 30 min. A, Malaria parasites (clone 3D7A) were incubated for 8 h with different concentrations of ALA (0, 200 μmol/L, and 2 mmol/L), exposed to incoherent white light for 30 min, and then cultured for 12 days. Control parasites not incubated with ALA had a high mean parasitemia (after 12 days), whereas cultures incubated with ALA were greatly reduced in viability (P<.002, for 200 μmol/L and for 2 mmol/L). Cultures were diluted 1:4 on days 3, 5, 7, 9, and 11. Results were similar for clone ITG (B).

into porphobilinogen. At some as-yet-unknown point later in the heme cycle, 1 of the intermediates is transported back to the mitochondrion, where ferrochelatase catalyses the reaction of PPIX to heme. The strategy of segregating hemoglobin degradation and heme detoxification in the food vacuole, from heme biosynthesis in the mitochondrion and apicoplast, initially appears to be wasteful; however, with this mechanism, malaria parasites are able to minimize the contact between toxic hemozoin and the other organelles of the parasite.

Several reports have described the photoinactivation of malaria parasites in vitro; each of these involved incubating cultures with photosensitizing molecules that, when illuminated by light, produce singlet oxygen to destroy various parasite structures. The photosensitizing dye merocyanine 540, when taken up by cultured P. falciparum and activated by white light, caused a 3-log decrease in viability [43]. However, merocyanine 540 appeared to have little specificity to infected RBCs, as the singlet oxygen produced by this compound was subsequently shown to damage uninfected erythrocytes as well [44–47]. Further development of phorphoride derivatives [48] and silicon phthalocyanines [49, 50], with respect to the photoinactivation of P. falciparum, have not appeared in the literature.

There at least 3 new technologies that are designed to inactivate infectious agents in blood products. Each of these methods uses compounds that interact with parasite DNA and prevent DNA synthesis. Riboflavin, a polycyclic, planar naturally-occurring vitamin, reduces the in vitro parasitemia of asexual and sexual stages of P. falciparum [51, 52]. When activated by white light, riboflavin has been shown to inactivate a range of viruses and bacteria [53]. The second technology involves psoralens, which are heterocyclic compounds that are soluble in water, permeable to cells, and intercalate into nucleic acids, to form interstrand cross-links when activated by UV radiation [54–56]. The psoralen compound amotosalen hydrochloride (S-59), when activated by UV radiation, is highly effective at decontaminating bacteria and viruses from platelet fractions, whereas S-303 is designed to inactivate erythrocytic pathogens in the absence of light [56, 57]. The third technology uses a ethyleneimine compound, PEN 110, that inactivates viruses and bacteria in the absence of light by binding to nucleic acids and generating a stop signal for polymerases [58, 59]. Data regarding the effectiveness of the the 3 inactivation methods, with respect to P. falciparum and other blood-borne protozoan parasites, have, so far, appeared only as abstracts or published meeting symposia.

A criticism of singlet oxygen–based photoinactivation methods has been that the activated compounds may induce damage to the erythrocyte membrane. Oxidative damage to erythrocytes has been observed for merocyanine 540 and protoporphyrin when each of these compounds was introduced into the medium [44]. However, in a study of the effect of the administration of ALA (as opposed to protoporphyrin) to erythrocytes, significant differences in the concentrations of porphyrins or other intermediates in the heme biosynthetic pathway were not detected between control and ALA-treated erythrocytes [60]. This report, coupled with our observation, by use of fluorescence microscopy, that uninfected erythrocytes do not reveal detectable photosensitizer, suggests that potentially damaging levels of protoporphyrins only accumulate in parasitized erythrocytes, and not in uninfected RBCs, in which heme biosynthesis is tightly controlled. In addition, since oxidative damage is often manifested by disruptions to erythrocyte-membrane integrity [61] and since merozoites of Plasmodium species require specific erythrocyte receptors and are extremely selective in their choice of erythrocyte [62], our observation that mer-
ozoites of *P. falciparum* could invade and mature within RBCs treated with ALA and exposed to light suggests that any PPIX activity in uninfected cells probably does not adversely affect erythrocyte viability.

A concern with any pathogen-inactivation method is the toxicity or mutagenicity of the residual active compound that remains in treated blood. Initial studies on the systemic administration of ALA to mice revealed relatively low toxicity. Observations from mice injected with 250 mg of ALA/kg of body weight showed an accumulation of PPIX in some body tissues, particularly the sebaceous glands, that resulted in a mild photosensitivity of the skin for a short period; this accumulation and effect vanished after 24 h, with minimal residual effect [18, 62]. In terms of individual human cells, 85% of normal human lymphocytes were found to be viable after treatment with 1 mmol/L ALA and exposure to white light, whereas there was a 2-log decrease in 3 lines of human leukemic cells subjected to the same treatment [30]. Studies on the genotoxicity of ALA-based photodynamic therapy for skin cancers indicate that the administration of ALA in the absence of activating light is not correlated with secondary skin carcinoma [63]. However, additional study is warranted, since overproduction of ALA has been suggested as a potential carcinogen in cases of acute intermittent porphyria, tyrosinemia, and lead poisoning [64].

We have demonstrated that cultured *P. falciparum* parasites treated with ALA and exposed to white light are inactivated or eliminated from culture. Although in vivo murine malaria models, erythrocyte-viability assays, and genotoxicity assays will be required to test the effectiveness of the method and the residual toxicity, if any, of ALA or its derivatives, these in vitro results suggest a potential inactivation strategy for malaria parasites in blood products. Potential benefits of this strategy include the relatively inexpensive cost of ALA (~$1.60/500 mL blood bag, at 200 μmol/L ALA [167.6 g/mol]) and the convenience of using incoherent white light instead of UV light for inactivation. If effective for malaria, this strategy may represent a more general approach to reduce the risk of other transfusion-transmitted parasitic threats, including *Babesia* species, *Toxoplasma gondii*, *Trypanosoma cruzi*, and *Leishmania* species [65], especially those species (*T. cruzi* and *Leishmania* species) that have defective enzymes for, and thus may poorly regulate, heme biosynthesis [66–68].

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Inactivation of Malaria Parasites in Blood Products • JID 2004;190 (1 July) • 191

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