Photodynamic Inactivation of *Candida albicans* by Hematoporphyrin Monomethyl Ether

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Running title: Photodynamic inactivation of *C. albicans* by HMME

ABSTRACT Aim: To evaluate the capacity of hematoporphyrin monomethyl ether (HMME) in the presence of light to cause photodynamic inactivation (PDI) of *C. albicans*. **Materials & methods:** HMME photoactivity was evaluated against azole-susceptible and resistant *C. albican*s. The mechanisms by which PDI of *C. albicans* occurred were also investigated. **Results:** HMME-mediated PACT caused a dose-dependent inactivation of azole-susceptible and resistant *C. albican*s. Incubation

with 10 μM HMME and irradiation with 72 J cm^{-2} light decreased the viability of *C. albicans* by 7 log_{10}, induced damage of genomic DNA, led to loss of cellular proteins, and damaged the cell wall, membrane, and intracellular targets. **Conclusion:** *C. albicans* can be effectively inactivated by HMME in the presence of light, and HMME-mediated PACT shows potential as an antifungal treatment.

KEYWORDS PACT, *Candida albicans*, hematoporphyrin monomethyl ether, mechanism

Candida albicans, which is present in the normal microbiota of healthy individuals, is an opportunistic commensal pathogen that lives in the oral cavity, gastrointestinal tract, and vagina [1]. It causes a wide range of human diseases ranging from superficial mucosal infections to life-threatening invasive candidiasis in immunocompromised patients [2-4]. Most treatments available for systemic and invasive candidiasis are based on antifungal drugs including azoles, polyenes, pyrimidine, and echinocandins. However, these drugs can be toxic to the host [5] and can damage and interrupt cellular functions [6]. Moreover, the extensive and repetitive use of antifungal drugs has resulted in the development of drug-resistant *C. albicans* strains, and the occurrence of infections refractory to standard antifungal therapy has increased [7, 8]. Thus, development of alternative methods to manage drug resistance is imperative.

One promising therapeutic approach is photodynamic antimicrobial chemotherapy (PACT), which uses a photosensitizer (PS) that is excited from a ground state to a triplet state upon illumination with light of an appropriate wavelength. The triplet state PS reacts with oxygen in and around the cells, thereby forming singlet oxygen ($^1O_2$) or other reactive oxygen species (ROS) [9] that rapidly react with nonspecific microbial targets and irreversibly destroy microbial cells through chemical and phototoxic reactions [10]. PACT has several advantages over traditional therapies, including high target specificity through direct application of PS

and light irradiation to the target sites [11], low risk of chemical and thermal side effects [12], biocompatibility with human cells [13], and low potential for development of drug resistance due to the non-specific action of liberated $^1O_2$ or other ROS [5].

Hematoporphyrin monomethyl ether (HMME) is a second-generation, porphyrin-related PS developed in China [14]. It consists of a mixture of the two positional isomers 3-(1-methyloxyethyl)-8-(1-hydroxyethyl) deuteroporphyrin IX and 8-(1-methyloxyethyl)-3-(1-hydroxyethyl) deuteroporphyrin IX **(Figure 1)** [15]. Compared to first-generation PS, e.g., Photofrin and hematoporphyrin derivative (HpD), HMME has a known structure, higher photoactivity, stronger photodynamic efficiency, lower toxicity, and a faster clearance rate. Moreover, HMME is less costly than other photoactive drugs [16-20]. In previous studies it has been shown that in the presence of light HMME is effective at killing several types of cancer cells [19-22] and some Gram-positive and Gram-negative bacteria [23, 24]. However there are no reported studies on the capacity of this porphyrin to cause photodynamic inactivation (PDI) of *C. albicans*. Therefore, we conducted this study to assess the potential of HMME to mediate PDI of drug-resistant and drug-sensitive strains of *C. albicans* and evaluated the effects of treatment on cell macromolecular structure, DNA and protein.

Material & methods

- ***C. albicans* strains & culture conditions**

A standard *C. albicans* strain (ATCC 10231) and an azole-resistant clinical isolate of *C. albicans* were obtained from the First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China. The strains were grown aerobically on Sabouraud dextrose agar (SDA, Nisuvi Sehuu Biotech, China) at 37°C for 48 h. The colonies were transferred into 15 ml Sabouraud dextrose broth (SDB, Nisuvi Sehuu Biotech, China) and incubated overnight at 37°C. Cell pellets were collected by centrifugation (4000 rpm for 10 min, Thermo Fisher D-37520, Germany) and washed three times with

sterile phosphate-buffered saline (PBS, pH 7.0). The pellets were resuspended to a cell density of 1×10^7 colony forming units (CFU)/ml before the experiments.

- **PS & light source**

Drug-grade HMME was purchased from Shanghai Xianhui Pharmaceutical Co., China. A 1 mM stock solution was freshly prepared by dissolving the PS in PBS and stored in the dark. The stock solution was filtered through a 0.22-μm filter disk and diluted to the desired concentration with PBS before use. All illuminations were performed with white light from a 150 W xenon lamp (Ceaulight CEL-HXF300, China) with a wavelength range 400–780 nm selected by an optical filter (Ceaulight CEL-UVIRCUT PD-145, China). To avoid sample heating, the light was passed through a 1-cm water filter. The fluence rate at the level of the samples was 40 mW cm^{-2}, as measured by a power meter (Ceaulight CEL-NP2000, China).

- **PDI on *C. albicans***

Samples of the yeast suspension (2 ml, 1×10^7 CFU/ml) were centrifuged at 4000 rpm for 10 min. The pellets were resuspended in 2 ml of PBS containing HMME at various concentrations (0.01–10 μM) and incubated at 37°C in the dark for 30 min in a shaking incubator (100 rpm). The samples were transferred to sterile 35-mm polystyrene culture dishes and irradiated for 30 min (total energy dose of 72 J cm^{-2}). After irradiation, yeast suspensions were centrifuged at 4000 rpm for 10 min. The pellets were resuspended, serially diluted 10-fold with PBS and 20 μl of each dilution was spread in triplicate on SDA. Colonies were counted after 24 h incubation at 37°C. The fraction of surviving yeast was calculated as the CFU/ml after exposure to light divided by the CFU/ml before light exposure. All experiments were performed three times.

- **Genomic DNA purification & electrophoresis**

To determine if PDI of *C. albicans* occurred through DNA damage, genomic DNA was extracted and analyzed by agarose gel electrophoresis. After PDI treatment (10 μM HMME and 72 J cm^{-2} white light), genomic DNA was immediately extracted

using a Genomic DNA Purification Kit (Promega, USA). DNA samples were mixed with 6 × loading-buffer (0.25% w/v bromophenol blue, 40% w/v sucrose, 1.15% acetic acid, 40 mM Tris, 1 mM EDTA) and analyzed by electrophoresis in a 1% agarose gel in Tris/Borate/EDTA buffer (TBE; 90 mM Tris-HCl, 90 mM boric acid, and 2 mM EDTA, pH 8) at 2.9 V cm^{-1} for 1.5 h. Ethidium bromide (1 μg/ml) was incorporated into the agarose gel, and a Lambda DNA/*Hind*III digest marker with 125–23,130-bp DNA fragments (Promega, USA) was used as a molecular weight marker.

- **Protein extraction & SDS-PAGE**

Whole-cell protein extraction was performed according to a previously described method [25]. After PDI treatment, yeast suspensions were centrifuged at 4000 rpm for 10 min. The pellets were washed twice with PBS and resuspended in 200 μl sample buffer containing 0.06 M Tris-HCl, pH 6.8, 2% (w/v) SDS, 5% (v/v) β-mercaptoethanol, 10% (v/v) glycerol, 1 mM phenylmethylsulfonylfluoride, and 0.5% (w/v) bromophenol blue. The samples were boiled for 20 min, and 10 μl of each sample was loaded onto a 10% (w/v) polyacrylamide gel and subjected to electrophoresis at 80 V for 10 h. The reservoir buffer consisted of 0.25 M Tris-HCl, 1.92 M glycine, and 1% (w/v) SDS. A Biostep Prestained Protein Marker (Tanon, China) with a range of proteins 10–170 kDa was used as molecular weight marker. The gel was stained with 0.05% (w/v) Coomassie Brilliant Blue R 250 for 4 h and destained in 10% (v/v) acetic acid and 20% (v/v) methanol.

- **Fluorescence labeling**

For intracellular localization of HMME, the yeast cells were incubated with HMME and a DNA-specific fluorescent dye, Hoechst 33342 (Sigma-Aldrich, China). After PDI treatment, yeast suspensions were centrifuged at 4000 rpm for 10 min. The pellets were resuspended in 2 ml Hoechst 33342 (1 μg/ml) in PBS and incubated in the dark at room temperature for 10 min in a shaking incubator (100 rpm). Labeled cells were washed three times with PBS, spotted on glass slides and immobilized by

the coverslips. Cell imaging was conducted on a confocal microscope (Olympus FluoView FV1000, Japan). Images were captured with CFI VC 60× oil immersed optics. Confocal images of HMME and Hoechst 33342 fluorescence were collected using solid-state diode lasers, with 543 and 351 nm excitation wavelengths, respectively, and with appropriate emission filters.

- **Transmission electron microscopy (TEM)**

TEM samples were prepared according to a previously described method [26]. After PDI treatment, yeast cells were centrifuged at 4000 rpm for 10 min and fixed in 2.5% glutaraldehyde at 4°C for 2 h. The pellets were washed with PBS three times and fixed in 1% osmium tetroxide at 4°C for 2 h. Thereafter, the pellets were dehydrated with ethanol gradients and embedded in Epon 812 epoxy resin (SPI-Chem, USA) at 60°C for 24 h. Thin-section samples of 50–70 nm were prepared using a LKB-V ultratome (LKB, Sweden). The samples were stained with uranyl acetate and lead citrate for 15 min, respectively. Finally, the samples were viewed and digitally photographed using a TEM (Hitachi H-7650, Japan).

- **Scanning electron microscopy (SEM)**

After PDI treatment, yeast suspensions were transferred into the wells of a sterile 24-well polystyrene microplate (Corning, USA) that contained sterile glass coverslips and incubated at 37°C for 1 h. The coverslips were gently washed with PBS three times and fixed in 2.5% glutaraldehyde at 4°C for 2 h. Then, the coverslips were washed with PBS three times and fixed in 1% osmium tetroxide at 4°C for 2 h. After dehydration with ethanol gradients, the samples were freeze-dried, sputter-coated with gold, and observed using an SEM (Hitachi TM-1000, Japan).

Results

- **PDI**

Before and after irradiation, sample temperatures were 35.1 and 32.3°C, respectively, as measured by a thermocouple (IKA EST-D5, Germany) at room temperature,

indicating that the light had no heating effect on these samples. HMME did not have any dark toxicity towards the two *C. albicans* strains at the concentrations and times tested **(Figure 2)**. Furthermore, direct exposure of these strains to light in the absence of HMME produced no cytotoxic effect (data not shown). In contrast, treatment with 0.01 μM HMME and irradiation with 72 J cm^{-2} white light achieved 0.90 and 0.78 log_{10} reductions in the number of viable *C. albicans* ATCC 10231 and the azole-resistant clinical isolate of *C. albicans*, respectively. The number of viable yeast was further reduced with increasing concentrations of HMME; treatment with 1 μM HMME in the presence of 72 J cm^{-2} white light yielded 4.26 and 3.88 log_{10} reductions in the number of viable *C. albicans* ATCC 10231 and the azole-resistant clinical isolate of *C. albicans*, respectively. Furthermore no viable cells were detected after irradiation in the presence of 10 μM HMME, representing a 7 log_{10} reduction **(Figure 2)**.

- **Photodynamic effect on genomic DNA and whole-cell protein**

As shown in **Figure 3**, genomic DNA isolated from the *C. albicans* strains treated with 10 μM HMME alone or irradiated with light alone migrated the same distance as DNA from untreated cells, suggesting that neither HMME nor white light caused macroscale DNA damage. However, after irradiation of HMME-treated cells, a loss of intensity of intensity of the genomic DNA band was seen on gels, with some smearing indicating that HMME-mediated PDI induced DNA damage in *C. albicans* (**Figure 3)**.

The photodynamic effect on whole-cell protein was examined by SDS-PAGE. We observed no obvious changes in the protein patterns of yeast cells treated with HMME alone or irradiated with light alone compared to that of untreated cells **(Figure 4)**. On the other hand, after treatment with HMME and irradiation with white light, there was a loss of protein bands separated on the gel for both *C. albicans* ATCC 10231 and the azole-resistant clinical isolate of *C. albicans*.

- **Fluorescence confocal microscopy**

Figure 5 shows the confocal fluorescence microscopy images of *C. albicans* cells. Before irradiation, spots of HMME fluorescence were visible in the periphery of most cells, while strong fluorescence in the entire cell was seen in individual cells **(DIC and HMME, L-)**. The nucleus was clearly differentiated as punctate blue fluorescence **(Hoechst 33342 and Merge, L-)**. However after 72 J cm^{-2} irradiation in the presence of HMME all cells showed strong fluorescence in the entire cell **(DIC and HMME, L+)**. HMME fluorescence was observed in the nucleus, indicating that HMME entered the nucleus after PDI treatment. Moreover, Hoechst staining was not confined to the nucleus, and all cells showed blue fluorescence in the entire cell **(Hoechst 33342 and Merge, L+)**.

- **Photodynamic effect on cellular structure**

The yeast cells were analyzed by TEM to determine if the photodynamic effect mediated by HMME caused any morphological changes in cellular structure. Representative results are shown in **Figure 6**. Typical *C. albicans* morphology with a characteristically thick cell wall, intact plasma membrane, and irregularly shaped nucleus was observed for untreated cells **(P-L-)** and for cells treated with HMME alone **(P+L-)** or light alone **(P-L+)**. The ribosomes were visible as dark particles dispersed in the cytoplasm. Treatment with 10 μM HMME and irradiation with 72 J cm^{-2} of white light induced visible damage to the cell wall, membrane, cytoplasm, and nucleus **(P+L+)**. The cell envelopes showed shape changes characterized by cell wall swelling and membrane rupture, and nuclei were not visible.

- **SEM**

Yeast cells were analyzed with SEM to determine whether the photodynamic effect mediated by HMME affected cell surface characteristics **(Figure 7)**. A normal round shape was observed for untreated cells **(P-L-)** and for cells treated with HMME alone **(P+L-)** or light alone **(P-L+)**. In contrast, hollow cracks were observed on the surface of *C. albicans* cells treated with 10 μM HMME and irradiated by 72 J cm^{-2} white light **(P+L+)**.

Discussion

Previous studies have shown that *C. albicans* is sensitive to lethal PDI mediated by a wide variety of PSs **(Table 1)** such as methylene blue (MB) [27], toluidine blue O (TBO) [28], 5-aminolaevulinic acid (5-ALA) [29], rose bengal [30], porphyrins [31-33], and phthalocyanine [34]. MB and TBO are well-studied cationic phenothiazinium dyes and effective PSs that have been demonstrated to mediate PDI of several microorganisms [10]. Although *C. albicans* can be photodynamically inactivated in the presence of either MB or TBO, it is much less susceptible than bacteria, possibly due to differences in the ratio of cell size to volume. *C. albicans* cells are approximately 25–50 times larger than bacterial cells and therefore require more damage to induce cell death [35]. In the present study, 1 μM HMME in the presence of 72 J cm^{-2} white light yielded 4.26 and 3.88 log_{10} reductions in the viability of *C. albicans* ATCC 10231 and the azole-resistant clinical isolate of *C. albicans*, respectively. Comparison of this result with that of previously published data for MB and TBO **(Table 1)** suggests that HMME is more efficacious at mediating PDI of *C. albicans*.

Cationic PSs are more efficient than their neutral or anionic counterparts in the PDI of bacteria [36], with many anionic or neutral PSs becoming more effective against Gram-negative bacteria when they are co-administrated with an outer membrane-disrupting agent such as $CaCl_2$, EDTA, or polymyxin B nonapeptide [37, 38]. Although HMME is an anionic porphyrin derivative our findings demonstrate that it is as efficacious at mediating PDI of *C. albicans* as the cationic porphyrin and phthalocyanine derivatives listed in **Table 1**. To the best of our knowledge, there is no direct evidence that anionic PSs are less efficient than their cationic counterparts in the PDI of *Candida* species.

There are only a few reported studies that have examined PDI efficacy of drug-resistant *C. albicans*. Using a porphyrin-based PS, Dovigo *et al.* found that azole-resistant *Candida* strains were more resistant to PDI than azole-sensitive strains [39]. On the other hand, Mang *et al*. found that fluconazole- and amphotericin

B-resistant *Candida* strains isolated from AIDS patients were equally susceptible to Photofrin-mediated PDI compared to non-resistant strains [40]. Our data demonstrates that the susceptibility of *C. albicans* strains to HMME-mediated PDI is not affected or impaired in any way by their resistance to azole antifungal agents. This finding suggests that HMME would be a good PS to use in PACT of drug-resistant strains.

PDI of microorganisms has been proposed to occur through inactivation of a number of biomolecules. For example, DNA damage would interfere with chromosome segregation, DNA replication, and transcription. Quiroga *et al*. investigated the mechanism of the PDI of *C. albicans* mediated by cationic porphyrin derivatives [26] and found no significant cleavage of genomic DNA after treatment despite efficient yeast cell photoinactivation. The authors hypothesized that either damage to genomic DNA was not involved in the PDI process or that any damage to the DNA resulting from a few lesions was not sufficient to induce DNA strand breaks that could be detected by gel electrophoresis. On the other hand, Lam *et al*. demonstrated that DNA fragmentation occurred with silicon phthalocyanine (Pc 4)-mediated PDI of *C. albicans* [41]. PDI mediated by 0.6 μM Pc 4 produced a DNA fragmentation pattern on electrophoresis gels matching that produced by hydrogen peroxide treatment, with a complete loss of banding and extensive migration of small DNA fragments. In this study, we observed a reduction in the amount of genomic DNA that could be purified from *C. albicans* and a reduction in Hoechst staining of intracellular nucleic acid. In addition the genomic DNA that was detected by gel electrophoresis ran as a faint smear indicating that HMME-mediated PDI induced DNA damage in *C. albicans* consistent with the study by Lam *et al*, although it is likely that the reduction in DNA content was also a result of cellular leakage. We also investigated the HMME-mediated PDI effect on *C. albicans* cellular proteins by SDS-PAGE. After treatment with 10 μM HMME and irradiation with 72 J cm^{-2} white light, there was an evident loss of proteins in both *C. albicans* ATCC 10231 and the azole-resistant clinical isolate of *C. albicans*. Similar findings were also found in the PDI of methicillin-resistant *Staphylococcus aureus* mediated by cationic T4 porphyrin [42]. Dosselli *et al.* hypothesized that the loss of proteins was likely due to the

formation of cross-linked complexes of protein resulting in high-molecular weight aggregates that could be found on the top of the SDS-PAGE gels. However, in the present investigation the high-molecular weight aggregates was not detected in **Figure 4**. Photodynamic inactivation and degradation of isolated bacterial proteins has been demonstrated previously [43] and it is possible that HMME-mediated PDI of *C. albicans* caused degradation of intracellular proteins, however it is also highly likely that intracellular protein was lost by leakage of cellular contents.

The confocal fluorescence microscopy images indicated that most of the HMME did not enter cells during incubation in the dark but bound to the cell envelope, resulting in a weak peripheral fluorescence pattern, although some individual cells did display intracellular HMME. After irradiation by a light dose of 72 J cm^{-2}, all cells showed HMME fluorescence in the entire cell, indicating that the cell membrane was sufficiently damaged to allow HMME to enter cells. Furthermore upon irradiation in the presence of HMME, Hoechst fluorescence was not confined to the nucleus, indicating nuclear rupture and biopolymer release. Our findings are very similar to those obtained for the PDI of *C. albicans* mediated by HpD [44, 45] and porphyrin TriP[4] [33]. HpD uptake by *C. albicans* is insignificant [44], and its photocytotoxic activity mainly occurs through unbound molecules in the aqueous medium. After irradiation, these molecules cause an initial alteration of the cytoplasmic membrane that allows HpD to penetrate into the cell, translocate to the inner membranes, and induce damage of intracellular targets [44]. Lambrechts *et al.* concluded that the plasma membrane became permeable during PDI, but cellular inactivation was attributed to damage of intracellular targets following PS uptake [33]. However, in the PDI of *C. albicans* mediated by Pc 4, Lam *et al*. found that the majority of Pc 4 fluorescence was detected in the cytoplasm and not in the cell wall, plasma membrane, nor the nucleus [41], similar to its distribution in mammalian cells [46]. The differences between the findings of Lam *et al.* and the results we present here suggest that the targets of porphyrin and phthalocyanine photosensitizers in the PDI of *C. albicans* are distinct.

The TEM images of *C. albicans* provided detailed information about the damage induced by HMME-mediated PDI to the plasma membrane and cytoplasmic organelles. HMME-mediated PDI damage to the cell wall, cell membrane, cytoplasm, and nucleus was visible by TEM. The ruptured nucleus was consistent with the nuclear damage inferred from fluorescence confocal microscopy. A previous study reported by Monfrecola *et al*. indicated that 5-ALA in the presence of white light induced membrane ruptures and cell wall swelling of *C. albicans* [29], which was also observed in the present study. Using freeze-fracture electron microscopy, Lambrechts *et al*. concluded that the yeast cytoplasmic membrane is the target of TriP[4]-mediated photoinactivation [33], which was consistent with our TEM results. On the other hand, the SEM images provided direct evidence that damage to the cell envelope was induced during PDI treatment, which could be observed on surface of *C. albicans* cells.

Conclusion & future perspective

The significant burden of dermatophytoses and the worldwide increase in fungal strains resistant to the current antifungals increases the urgency for the development of new therapeutic strategies, such as PACT. The present study assessed the efficacy of HMME to mediate PDI of *C. albicans* strains *in vitro*. HMME effectively photo-inactivated the yeast cells in a concentration-dependent manner but exhibited no significant dark toxicity. The susceptibility of *C. albicans* strains to HMME-mediated PDI was not affected or impaired in any way by the yeast being resistant to azole antifungal agents, suggesting that HMME would be a good PS to use in PACT of drug-resistant *C. albicans* strains. Our data also indicate that HMME-mediated PDI of *C. albicans* occurs through damage to multiple cell targets, including genomic DNA, the cell wall, the cell membrane, the cytoplasm, the nucleus and possibly intracellular protein. These findings provide insights into HMME-mediated PDI of *C. albicans*. HMME-mediated PACT shows promise for development as an antifungal treatment. Of course our studies were conducted with planktonic yeast and there may be differences in the effectiveness against biofilm

grown cells. The incorporation of HMME into liposomes, micelles, or nanoparticles is a promising approach to enhance targeted delivery. The development of these vehicles is particularly important for the potential expansion of PACT for the treatment of deep fungal infections using fiber optic lasers, applied endoscopically, or interstitially.

Financial & competing interests disclosure

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EXECUTIVE SUMMARY

Photodynamic inactivation of *C. albicans*

- Without irradiation, HMME exhibited no significant dark toxicity.
- After irradiated with 72 J cm^{-2} white light, HMME caused dose-dependent inactivation of *C. albicans*.
- 10 μM HMME in the presence of 72 J cm^{-2} white light yielded 7 log_{10} reductions in the number of viable *C. albicans* ATCC 10231 and the azole-resistant clinical isolate of *C. albicans*.

Photodynamic effect on genomic DNA and whole-cell protein

- After treated with 10 μM HMME and irradiated with 72 J cm^{-2} white light, damage of genomic DNA and loss of cellular proteins were observed in both *C. albicans* ATCC 10231 and the azole-resistant clinical isolate of *C. albicans*.

Fluorescence confocal microscopy

- The majority of HMME bound to the cell envelope of *C. albicans* under dark conditions, with little entering the cell.
- After irradiated with 72 J cm^{-2} white light in the presence of HMME, the cell membrane became permeable, and cell inactivation was attributed to membrane damage followed by damage to intracellular targets.

TEM & SEM investigation

- TEM images indicated that 10 μM HMME in the presence of 72 J cm^{-2} white light caused damage to the cell wall, cell membrane, cytoplasm, and nucleus.
- SEM images provided direct evidence that damage to the cell envelope was induced during PDI treatment.

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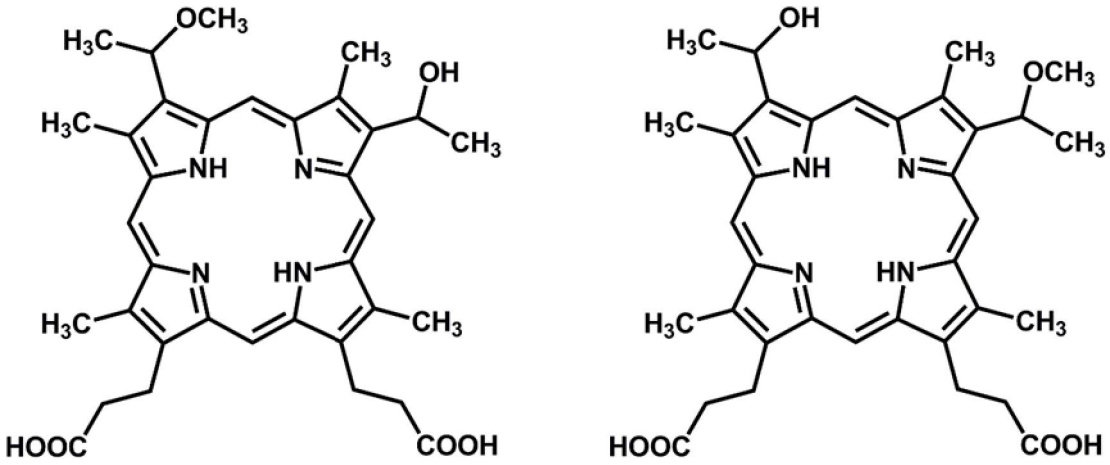

Figure 1. Chemical structure of hematoporphyrin monomethyl ether (HMME).

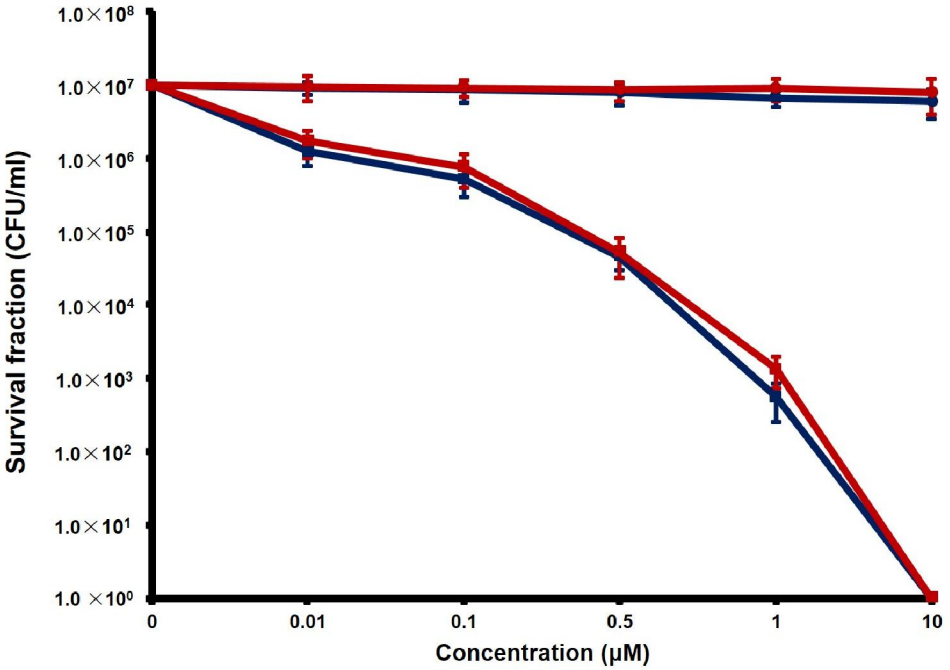

Figure 2. Survival of *C. albicans* ATCC 10231 (blue) and the azole-resistant clinical isolate of *C. albicans* (red) treated with different concentrations of HMME. Filled circles (●) represent the number of yeast surviving after incubation without illumination (dark toxicity), while **filled squares (■)** represent the number of yeast surviving after 30-min irradiation (40 mW cm^{-2}). All the experiments were repeated for three times.

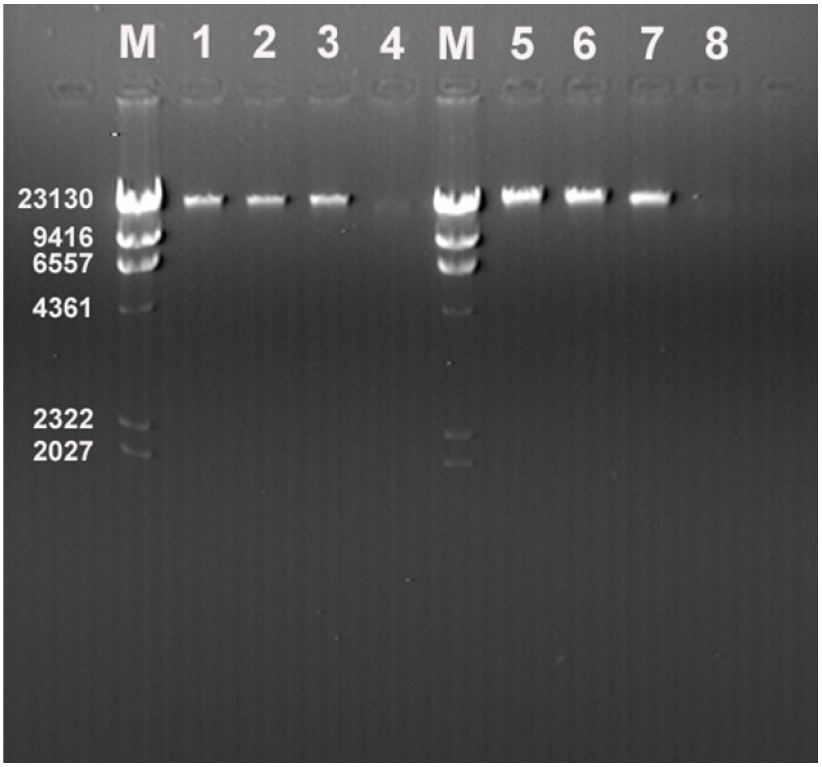

Figure 3. Agarose gel electrophoresis of genomic DNA samples extracted from *C. albicans* (ATCC 10231, lanes 1–4) and the azole-resistant clinical isolate of *C. albicans* (lanes 5–8). Lane M: DNA size maker. **Lanes 1 and 5:** no treatment. **Lanes 2 and 6:** incubated with 10 μM HMME at 37°C for 30 min in the dark. **Lanes 3 and 7:** irradiated with 400–780 nm white light for 30 min (72 J cm^{-2}). **Lanes 4 and 8:** incubated with 10 μM HMME and irradiated with 400–780 nm white light for 30 min (72 J cm^{-2}).

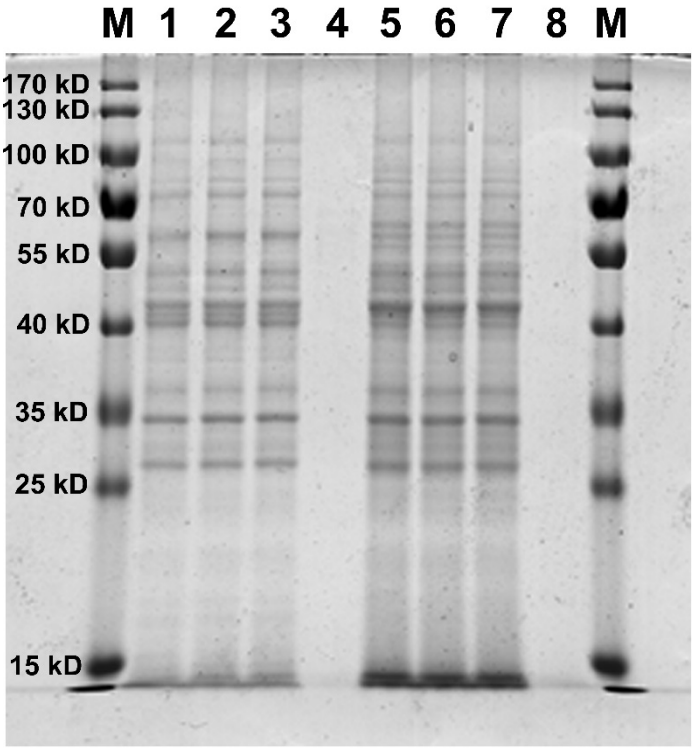

Figure 4. Image of an SDS-PAGE gel with protein samples from *C. albicans* ATCC 10231 (lanes 1–4) and the azole-resistant clinical isolate of *C. albicans* (lanes 5–8). Lane M: DNA weight maker. **Lanes 1 and 5:** no treatment. **Lanes 2 and 6:** incubated with 10 μM HMME at 37°C for 30 min in the dark. **Lanes 3 and 7:** irradiated with 400–780 nm white light for 30 min (72 J cm^{-2}). **Lanes 4 and 8:** incubated with 10 μM HMME and irradiated with 400–780 nm white light for 30 min (72 J cm^{-2}).

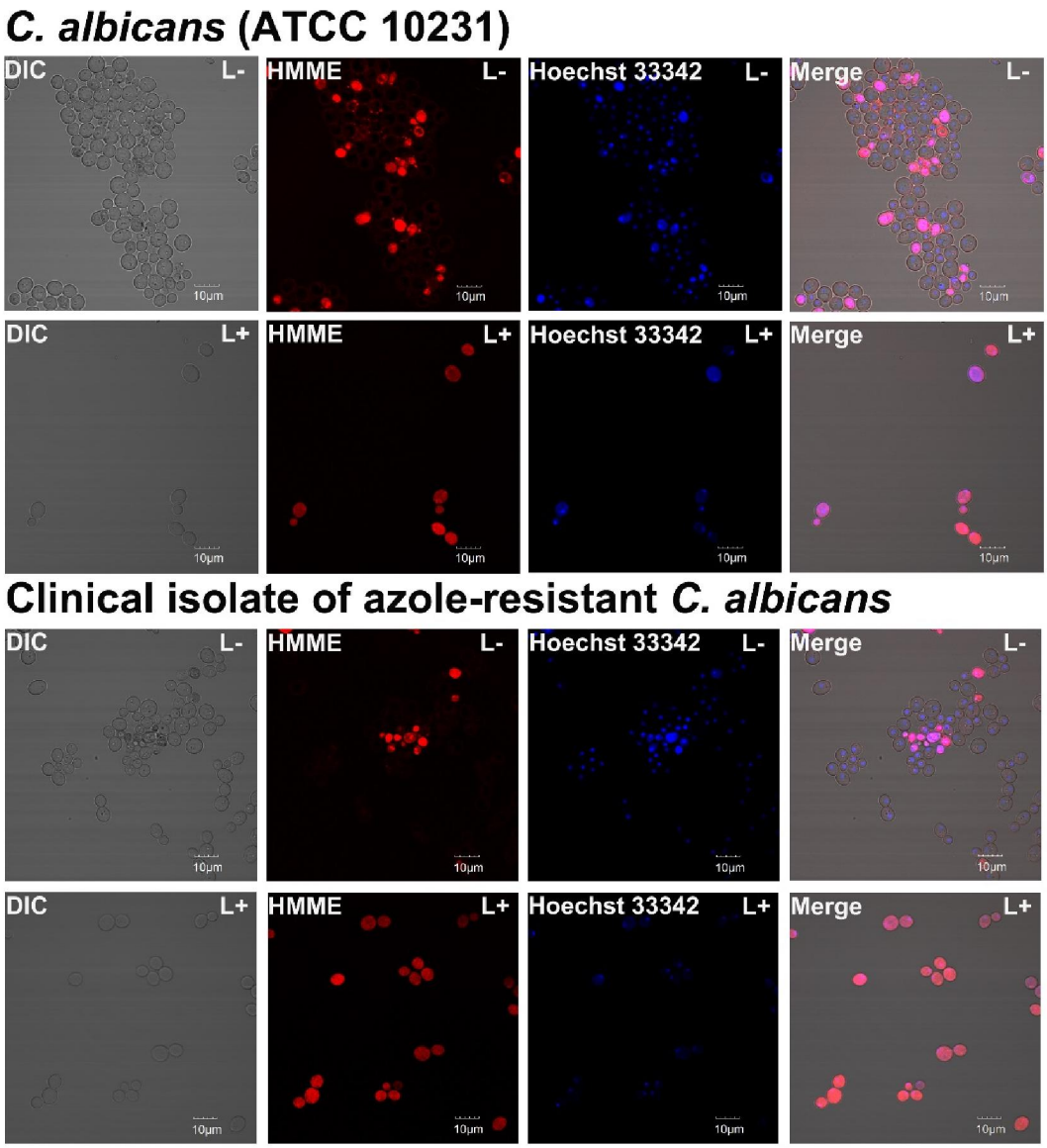

Figure 5. Confocal fluorescence microscopy images of *C. albicans*. (L-): *C. albicans* cells were incubated with 10 μM HMME at 37°C for 30 min in the dark and labeled with 1 μg/ml Hoechst 33342 in the dark at room temperature for 10 min. **(L+):** *C. albicans* cells were incubated with 10 μM HMME at 37°C for 30 min in the dark and irradiated with 400–780 nm white light for 30 min (72 J cm^{-2}). Then the cells were labeled with 1 μg/ml Hoechst 33342 in the dark at room temperature for 10 min.

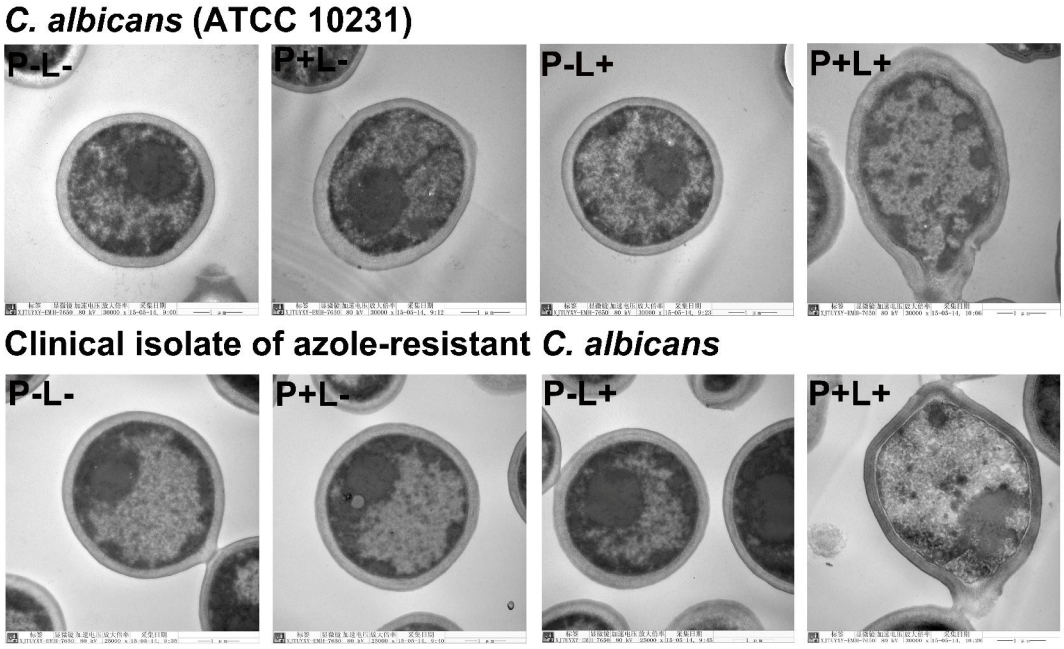

Figure 6. Transmission electron microscopy images of *C. albicans*. (P-L-): no treatment. **(P+L-):** incubated with 10 μM HMME at 37°C for 30 min in the dark. **(P-L+):** irradiated with 400–780 nm white light for 30 min (72 J cm^{-2}). **(P+L+):** incubated with 10 μM HMME and irradiated with 400–780 nm white light for 30 min (72 J cm^{-2}).

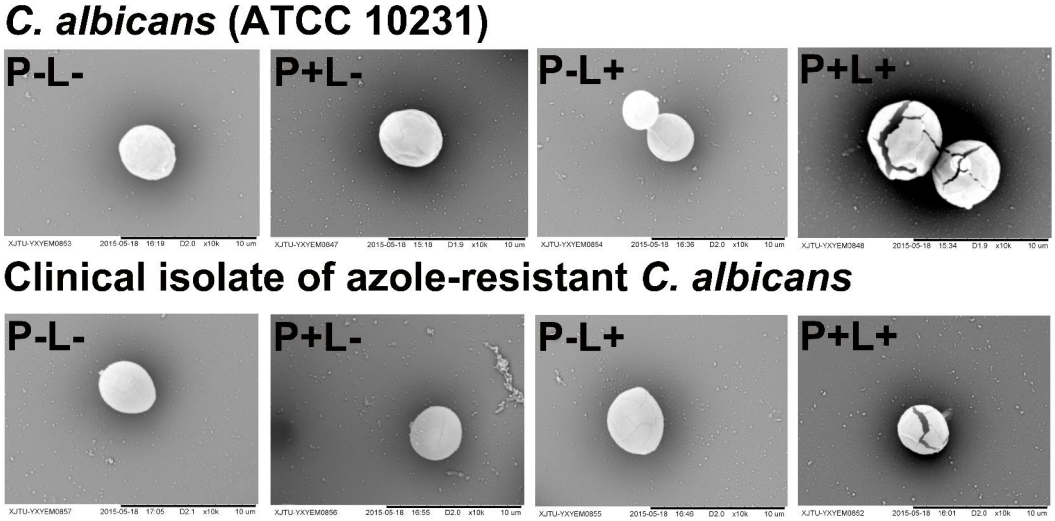

Figure 7. Scanning electron microscopy images of *C. albicans*. (P-L-): no treatment. **(P+L-):** incubated with 10 μM HMME at 37°C for 30 min in the dark. **(P-L+):** irradiated with 400–780 nm white light for 30 min (72 J cm^{-2}). **(P+L+):** incubated with 10 μM HMME and irradiated with 400–780 nm white light for 30 min (72 J cm^{-2}).

Table 1. PDI of *C. albicans* mediated by various photosensitizers.

Photosensitizer	Concentration	Light dose	Reduction of viable cells	Reference
Methylene blue	312 μM	39.5 J cm^{-2}	2.7 log_{10}	[27]
Toluidine blue O	25 μM	180 J cm^{-2}	5.2 log_{10}	[28]
5-ALA	2.98 M	40 J cm^{-2}	~1.0 log_{10}	[29]
Rose bengal	200 μM	42.63 J cm^{-2}	4-6 log_{10}	[30]
Porphyrin XF-73	1 μM	12.1 J cm^{-2}	over 5.0 log_{10}	[31]
Porphyrin Tetra-Py^{+}-Me	10 μM	64.8 J cm^{-2}	6.5 log_{10}	[32]
Porphyrin TriP[4]	25 μM	12.6 J cm^{-2}	5.6 log_{10}	[33]
Phthalocyanine $ZnPPc^{4+}$	1 μM	54 J cm^{-2}	4.0 log_{10}	[34]
HMME	1 μM	72 J cm^{-2}	~4.0 log_{10}	This study
HMME	10 μM	72 J cm^{-2}	7 log_{10}	This study

Porphyrin XF-73: dicat-ionic 5, 15-bis-[4-(3-trimethylammoniopropyloxy)-phenyl]-porphyrin; **Porphyrin Tetra-Py+-Me:** 5, 10, 15, 20-tetrakis (1-methylpyrid-inium-4-yl) porphyrin tetra-iodide; **Porphyrin TriP[4]:** 5-phenyl-10, 15, 20-Tris(*N*-methyl-4-pyridyl)porphyrin chloride; **Phthalocyanine $ZnPPc^{4+}$:** zinc(II) 2, 9, 16, 23-tetrakis[4-(*N*-methylpyridy-loxy)]phthalocyanine.