

# Inactivation of methicillin-resistant *Staphylococcus aureus* (MRSA) by liposome-delivered photosensitising agents

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## Abstract

The uptake of two photosensitising agents (hematoporphyrin and chlorophyll *a*) by a highly pathogenic bacterium, namely methicillin-resistant *Staphylococcus aureus* (MRSA), has been studied by using unilamellar liposomes of different size, fluidity and electric charge as carriers. Optimal results are obtained by using hematoporphyrin embedded in fluid cationic vesicles composed by the monocationic lipid *N*-[1-(2,3-dioleoyloxy)propyl]-*N,N,N*-trimethylammonium methylsulfate (DOTAP), which yield an endocellular concentration of photosensitiser much higher than that obtained by incubation of the cells with the free porphyrin, yet promote a tighter binding and a more efficient photoinactivation of MRSA. Apparently, the photosensitiser is successfully transferred from the liposome to the bacterial cells when the presence of the tetrapyrrolic derivative does not appreciably perturb the native three-dimensional organisation of the lipid vesicle, such as it occurs with hematoporphyrin. On the other hand, chlorophyll, which causes a marked structural alteration of the DOTAP vesicles as shown by electron microscopy and fluorescence anisotropy measurements, does not show any detectable photocytotoxicity toward MRSA, contrary to what observed for the free dye.

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## 1. Introduction

Photodynamic therapy (PDT) represents a well-established therapeutic modality, which was originally developed and recently approved for the treatment of a variety of solid tumours [1,2]. The technique involves the combination of a tumour-localised and intrinsically non-toxic photosensitiser with harmless visible light wavelengths, which are specifically absorbed by the photosensitising agent; as a result, reactive oxygen species are generated, which induce the specific killing of the malignant cells and tissues

[3]. A relatively novel application of PDT has been made possible by the preparation of photosensitisers whose molecule is engineered to promote a very fast interaction with bacterial cells, hence a highly preferential inactivation of such pathogenic agents in comparison with the main constituents of host tissues, such as fibroblasts and keratinocytes [4]. These findings opened the way to the use of PDT for the treatment of localised microbial infections [5,6].

Most photosensitisers which are presently under investigation for antimicrobial PDT belong to the family of phenothiazines [7], porphyrins [8], chlorins [9] and phthalocyanines [10]. The available information suggest that the presence of positively charged substituents at the periphery of the aromatic skeleton of the photosensitiser

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strongly enhances the cytotoxic activity against bacteria and fungi, especially when a mixed pathogenic flora is present [5]. The positive charge can be located either directly in the photosensitizer molecule or in a covalently associated moiety, such as polylysine [9,11]. However, this requirement presently limits the number and type of photosensitizers which can be used for the treatment of microbial infections.

In an aim to expand the type of photosensitizing dyes which can be efficaciously used as antimicrobial photodynamic agents, we decided to study the photosensitized inactivation of a well-known antibiotic-resistant Gram-positive bacterium, namely methicillin-resistant *Staphylococcus aureus* (MRSA), by using two non-cationic liposome-incorporated dyes, such as hematoporphyrin (HP) and chlorophyll (Chl). Liposome-delivered photosensitizers have been often adopted in anti-tumour PDT and proven to yield a more pronounced and selective targeting of the neoplastic lesion [13]. Thus, it appeared of interest to investigate the effect of liposome carriers on the affinity of the photosensitizing agent for bacterial cells and the efficiency of photoinduced bacterial killing. Toward this purpose, we selected liposomal vesicles with a different degree of fluidity at the physiological temperature, as well as with different electric charge or size. Whereas HP has been previously used as an antimicrobial agent [12], no detailed information are available as regards the photocytotoxic activity of chlorin derivatives against the most important microbial pathogens. Chlorins are characterized by an intense absorption band in the red spectral region which is bathochromically shifted as compared with the lowest energy band of porphyrins; this should guarantee an efficient photodynamic activity to a greater depth in human tissues [2].

## 2. Materials and methods

### 2.1. Chemicals

Hematoporphyrin (HP) was supplied by Frontier Scientific (Logan, Utah) as a powder with a purity of about 90%. Its concentration was determined in absolute ethanol using  $\epsilon = 140,000 \text{ M}^{-1} \text{ cm}^{-1}$  at 410 nm. Chlorophyll *a* (Chl) was obtained from Aldrich and appeared to be free from Chlorophyll *b* by HPLC analysis [14]; its concentration was measured in dimethylformamide (DMF) using  $\epsilon = 68,693 \text{ M}^{-1} \text{ cm}^{-1}$  at 410 nm.

The monocationic lipid *N*-[1-(2,3-dioleoyloxy)propyl]-*N,N,N*-trimethylammonium methylsulfate (DOTAP), as well as DL- $\alpha$ -dipalmitoyl-phosphatidyl-choline (DPPC) and L- $\alpha$ -dimiristoyl-phosphatidyl-choline (DMPC) were purchased from Sigma Aldrich.

Small unilamellar vesicles of DOTAP, DPPC and DMPC (diameter = 50–100 nm) were prepared by the ethanol injection procedure, as modified by Kremer et al. [15], namely by co-dissolving HP or Chl and the desired phospholipid in ethanol. Typically, 0.75 ml of the ethanolic solution was slowly injected into 10 ml of PBS at 20 °C

(DOTAP), 56 °C (DPPC) and 37 °C (DMPC). The liposomal suspensions, thus, obtained were dialysed for 3 h against 10 mM phosphate-buffered saline (PBS), pH 7.4. The concentration of the photosensitizer in the final liposomal suspension (7 mM in phospholipids) was 20  $\mu\text{M}$  as determined by spectrophotometric analysis.

Sodium dodecyl sulfate (SDS) was obtained from Merck. All other chemicals and solvents were commercially available reagents of at least analytical grade, unless otherwise stated. BHI (brain heart infusion) was purchased from Difco.

### 2.2. Bacterial strains

The methicillin-resistant strain *S. aureus* MRSA 110 was grown aerobically at 37 °C in brain heart agar (BHA, Difco, Detroit). The cells in the stationary phase of growth were harvested by centrifugation of broth culture (200 g for 15 min), washed twice with PBS and diluted in the same buffer to an absorbance of 0.7 at 650 nm, corresponding to  $10^8$ – $10^9$  bacterial cells/ml.

### 2.3. Photokinetic studies

All the irradiation studies were performed by using white light emitted from a Teclas fluorescent lamp (Lugano, Switzerland), equipped with an UV and an infrared-reflecting filter. The light beam was driven to the target by optical fibers (external diameter = 8 mm).

The rate of HP or Chl photobleaching was followed spectrophotometrically by measuring the variations of the absorption spectrum in the 350–700 nm wavelength interval upon exposure of the photosensitizer (5–10  $\mu\text{M}$ ) in both homogeneous solutions and aqueous dispersions of liposomal vesicles to the Teclas lamp which was operated at a fluence rate of 100 mW/cm<sup>2</sup>. The solutions/dispersions were placed in a quartz cuvette of 1 cm optical path and were gently stirred during irradiation.

### 2.4. Determination of quantum yield for singlet oxygen generation

The quantum yield of singlet oxygen generation by both HP or Chl was measured using 9,10-dimethyl-anthracene (DMA) as a substrate since this compound is known to react with <sup>1</sup>O<sub>2</sub> being converted to the non-fluorescent 9,10-endoperoxide with 100% efficiency (no physical quenching), so that a measurement of the residual DMA concentration as a function of the irradiation time allows one to calculate the amount of photogenerated <sup>1</sup>O<sub>2</sub> [16]. Typically, 2 ml of a DMF solution containing 10  $\mu\text{M}$  DMA and 4  $\mu\text{M}$  photosensitizer were placed in a quartz cuvette of 1 cm optical path and gently stirred during exposure to the Teclas lamp (100 mW/cm<sup>2</sup>) at a temperature of 37 °C.

The kinetic analysis of the decrease in DMA concentration was performed spectrophotofluorimetrically at various

time intervals up to 30 min. The fluorescence emission was measured in the 380–550 nm wavelength range ( $\lambda_{\text{exc}} = 360$  nm).

### 2.5. Photosensitised inactivation of bacterial cells

In a typical experiment, 2 ml of a cell suspension of *S. aureus* MRSA 110 containing approximately  $2 \times 10^8$  CFU (colony-forming units) per ml were transferred into a well. Then, 2 ml of a PBS solution, buffered at pH 7.4, containing either the free or liposome-incorporated photosensitiser at a concentration of 20  $\mu\text{M}$  was added to the well. Control studies showed that no effect on bacterial cell survival was induced by addition of PBS + 5% ethanol medium, as well as by incubation of the cells with HP or Chl in the dark. The samples were incubated in the dark for 30 min and 2 h with the free or liposome-associated photosensitiser, respectively, and then irradiated at 20 °C with the Teclas lamp using a fluence rate of 100  $\text{mW}/\text{cm}^2$ . At the end of the irradiation, unirradiated and irradiated bacterial cells were serially 10-fold diluted with PBS and the number of colonies found after 24 h incubation at 37 °C were counted. Preliminary experiments showed that incubation of the bacterial cells with identical amounts of liposomal vesicles devoid of photosensitiser had no detectable effect on survival. Analogously, no cytotoxicity was induced by visible light irradiation in the presence of empty liposomes.

### 2.6. Photosensitiser binding studies

Samples containing  $10^8$  MRSA cells per ml prepared by dilution from a previously washed bacterial suspension were incubated in the dark for 30 min or 2 h with 10  $\mu\text{M}$  photosensitiser. The cells were then recovered by centrifugation and the pellets were dissolved in 2% aqueous SDS (1 ml). Identically treated samples were washed one or three times with PBS and then dissolved in 2% aqueous SDS. After 18 h incubation at room temperature, 0.6 ml were used to evaluate the photosensitiser concentration spectrophotofluorimetrically after a suitable dilution in 2% aqueous SDS. The fluorescence emitted in the 600–800 nm spectral range upon excitation at 526 nm for HP and 410 nm for Chl was measured for each sample. This was converted into photosensitiser concentration (nmoles/ $10^8$  cells) by interpolation with a calibration plot previously obtained for known concentrations of photosensitiser in 2% SDS vs. fluorescence intensity. The nanomoles of photosensitisers were referred to the number of cells incubated.

### 2.7. Transmission electron microscopy (TEM)

Samples were negatively stained with 1% uranyl acetate. Specimens were transferred to thin carbon films supported by 400 mesh copper grid and pre-treated with glow discharge. Typically, 1% uranyl acetate was used as a negative stain. Observations were made using a Hitachi-H600 (Tokyo, Japan) electron microscope.

### 2.8. Fluorescence anisotropy studies

Fluidity changes of liposomal membranes under different experimental conditions were evaluated by studying the temperature dependency of the fluorescence excitation anisotropy of liposome-bound HP and Chl. Stock liposomal suspensions with a final photosensitiser concentration of 10  $\mu\text{M}$  were prepared. Steady-state fluorescence anisotropy values were obtained at 520 nm ( $\lambda_{\text{em}} = 620$  nm) for Hp and at 410 nm ( $\lambda_{\text{em}} = 640$  nm) for Chl by measurements of  $I_{VV}$  and  $I_{VH}$ , i.e., the fluorescence intensities polarised parallel and perpendicular to the vertical plane of polarisation of the excitation beam, respectively. The fluorescence anisotropy is defined by the equation  $= (I_{VV} - GI_{VH}) / (I_{VV} + 2GI_{VH})$ , where  $G$  equals  $I_{HV}/I_{HH}$  and is the correction factor for instrumental artefacts. All the experiments were performed in the 10–45 °C temperature range.

## 3. Results

As shown in Fig. 1(a), Chl incorporated into DOTAP liposomes exhibited the visible absorption spectrum typical of monomeric tetrapyrrolic derivatives with prominent bands peaking at 410 and 630 nm. Repeated spectroscopic

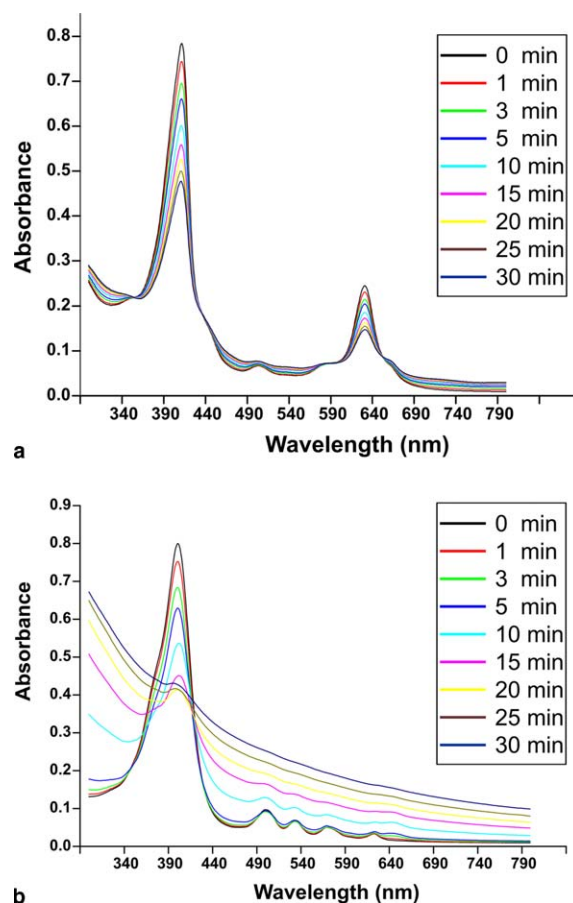


Fig. 1. Visible absorption spectrum of (a) 10  $\mu\text{M}$  DOTAP liposome-incorporated chlorophyll and (b) 10  $\mu\text{M}$  DOTAP liposome-incorporated hematoporphyrin at different irradiation times (white light, 100  $\text{mW}/\text{cm}^2$ ).

analysis showed no apparent release or aggregation of the chlorin for at least 5 days. Upon visible light irradiation, the chlorin underwent a gradual photobleaching, at least up to 30 min exposure to light. The occurrence of such a photoprocess is typical of several tetrapyrrolic compounds [17]. Isosbestic points are clearly detectable at selected wavelengths in the spectrum, which should indicate the progressive photoinduced conversion of Chl to one photoproduct. This is likely to be represented by a porphyrin formed through photooxidative dehydrogenation of one pyrrole ring [18]. Similarly, HP appeared to be embedded in a monomeric state into DOTAP liposomes (Fig. 1(b)) and to be photobleached by visible light irradiation. The extent of the HP photodegradation process was clearly more pronounced as compared with Chl. The observed increase in light scattering and strong hypochromicity upon prolonging the irradiation time (Fig. 1(b)) is possibly related with the photoinduced formation of polymeric material [18]. Electron microscopy observations indicated that, under our experimental conditions, DOTAP yielded a fairly homogeneous population of small unilamellar vesicles (average size 100 nm); no major modification of the liposome structure was induced by the presence of 20  $\mu\text{M}$  HP, whereas the introduction of 20  $\mu\text{M}$  Chl into the liposome was accompanied by a rearrangement of the native lipid organisation, with the possible formation of aggregated particles and/or rupture of the lipid bilayer. On the other hand, the three-dimensional organisation of DPPC and DMPC liposomes, loaded with either Chl or HP, was essentially identical with that observed for the same photosensitiser-free liposomes.

Both Chl and HP, when administered in either a homogeneous aqueous solution or a liposomal dispersion, were readily taken up by MRSA cells, as shown in Fig. 2(a) and (b). Apparently, the efficiency of DOTAP vesicles in delivering the embedded photosensitisers to the bacterial cells was markedly greater as compared with DPPC and DMPC vesicles. On the other hand, free Chl was also efficiently taken up by the bacterial cells, the incorporation yield being very similar to that obtained with DOTAP liposomes (Fig. 2(a)). In any case, a fraction of the initially bound Chl appeared to be tightly associated with the MRSA cells since about 30% (DOTAP-delivered) or 50% (free) Chl was still recovered from the cells after three subsequent washing steps. A very similar binding pattern was found for HP: also in this case, the free or DOTAP-associated porphyrin yielded the largest recovery from MRSA cells, while the binding was fairly stable as shown by the amount of HP which was extracted from one- or three-times washed cells (Fig. 2(b)). On the contrary, the affinity of DPPC- and DMPC-incorporated HP and Chl for MRSA cells was very low since both sensitisers were fully removed after three-times washing.

The mode of Chl or HP delivery had a pronounced influence on the efficiency of the subsequent photosensitised inactivation of the bacterial pathogen. As shown in Fig. 3(a), Chl delivered via liposomes had no detectable

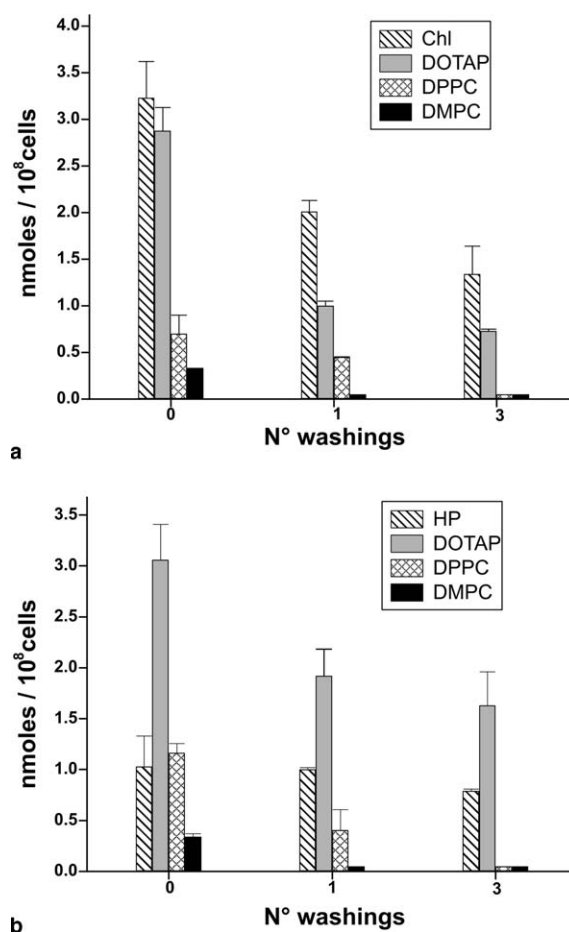


Fig. 2. Accumulation of (a) free or liposomes-bound chlorophyll and (b) free or liposome-bound hematoporphyrin by MRSA cells after 2 h incubation with 10  $\mu\text{M}$  photosensitiser. The recovery was measured spectrophotofluorometrically after different washing steps. The incubation of MRSA with free photosensitiser was performed for 30 min.

effect on the survival of MRSA cells, independently of the released amount or the nature of the vesicles. On the contrary, the chlorin incubated with bacteria in a homogeneous aqueous medium caused an extensive (>5 log) decrease in MRSA survival already after irradiation times as short as 10 min. This behaviour is at variance with that displayed by HP, whose phototoxicity was strongly enhanced by its delivery via DOTAP liposomes, whereas a modest enhancement was observed when DMPC liposomes were used. Delivery of the porphyrin by DPPC liposomes increased HP phototoxicity only at long irradiation times (Fig. 3(b)).

Remarkably, the two tetrapyrrole derivatives were characterised by a widely different quantum yield for singlet oxygen generation, which ranged from 0.10 for Chl to 0.65 for HP in homogeneous medium. The value measured for HP under our experimental condition was in excellent agreement with that found by other authors [19].

The anisotropy plots as a function of temperature for HP associated to DPPC and DMPC liposomes showed a trend typical of the gel–liquid phase transition of the two lipids (Fig. 4(b)). The transition mid-points ( $T_c$ ) were



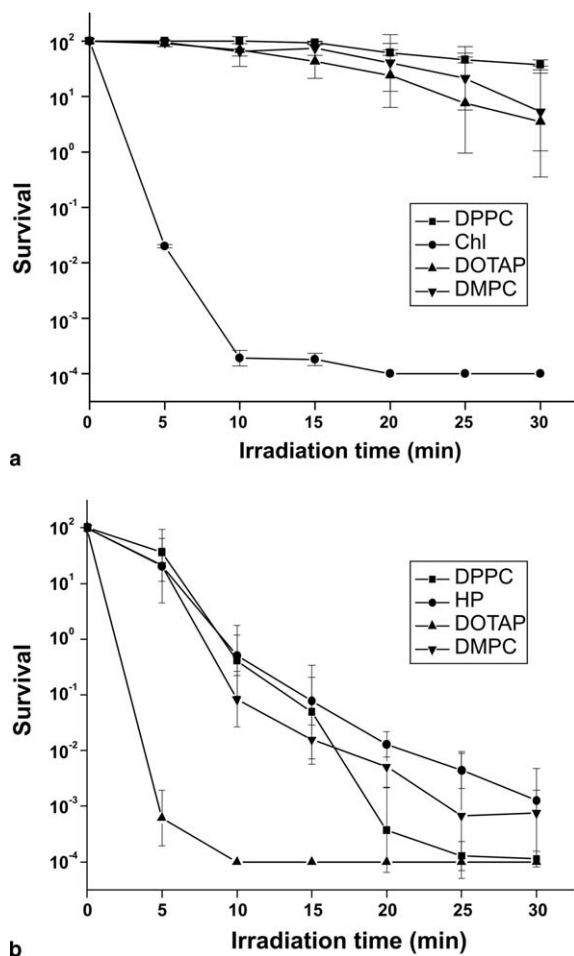


Fig. 3. Survival of MRSA cells irradiated for different period of time with white light ( $100 \text{ mW/cm}^2$ ) after 2 h incubation with  $10 \mu\text{M}$  liposome-bound (a) chlorophyll or (b) hematoporphyrin. The incubation of MRSA with free photosensitiser was performed for 30 min.

slightly shifted to lower  $T$  than those typically reported in the literature ( $41.5$  and  $21^\circ\text{C}$ , respectively). This could be a consequence of a preferential localisation of HP in the inner liposomal monolayer which exhibits lower  $T_c$  values, due to a higher curvature [20]. Since DOTAP lipids are in the fluid state in the temperature range examined by us ( $T_c < 0^\circ\text{C}$ ) [21], HP anisotropy did not reveal any transition phase in this case. Chl exhibited a high degree of fluorescence anisotropy when incorporated into the three liposomal vesicles, as compared to HP (Fig. 4(a)). This suggests that the photosensitiser was characterised by a more rigid microenvironment than HP. Probably Chl partitions in the internal, lipid hydrophobic core of the vesicles whereas HP is known to preferentially localise at the lipid/water interfaces, in close contact with the solvent [20,22]. The limitation to Chl motility is especially evident in the case of DOTAP vesicles, probably as a consequence of particle aggregation, as evidenced by TEM studies. Incorporation of Chl also affects the structure of DMPC lipid bilayer: in this case, the  $T_c$  of the phase transition is shifted to a higher temperature ( $28^\circ\text{C}$ ) than that typical of the lipid ( $21^\circ\text{C}$ ).

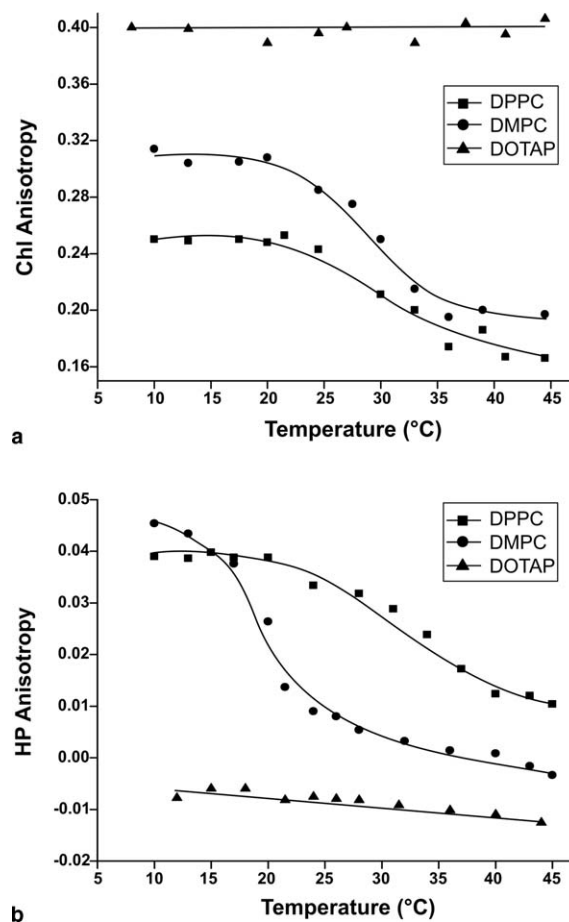


Fig. 4. Effect of temperature on the fluorescence anisotropy of chlorophyll (a) and hematoporphyrin (b) incorporated into different liposomal vesicles. The fluorescence excitation was at  $410 \text{ nm}$  ( $\lambda_{\text{em}} = 640 \text{ nm}$ ) for chlorophyll and  $520 \text{ nm}$  ( $\lambda_{\text{em}} = 620 \text{ nm}$ ) for hematoporphyrin.

#### 4. Discussion

The delivery of photosensitising agents to bacterial cells can be modulated by their association with a variety of suitable carriers, including antibiotic peptides, polylysine moieties, or antibodies [21]. On the other hand, the use of lipid-based structures to enhance the affinity of drugs for microbial pathogens represents an essentially unexplored field. Our present findings suggest that under selected experimental conditions also liposomal vesicles can be used to achieve this purpose. However, at least in the case of MRSA, both the interaction of the photosensitiser with the bacterial cells and the efficiency of the photo-inactivation process appear to be significantly affected by the properties of the liposome, as well as by the nature of the incorporated photosensitiser. Thus, even though a limited number of photosensitisers has been examined so far, the present evidence indicates that optimal results are obtained by using vesicles which combine fluidity (at the physiological temperatures) with the presence of positively charged heads, such as it occurs in the case of DOTAP liposomes. Clearly, this delivery system yields large endo-

cellular concentrations of two different porphyrin-type derivatives, the recoveries of DOTAP liposome-delivered Chl and HP being comparable with those obtained upon addition of the same compounds in a homogeneous aqueous solution. Furthermore, the photosensitisers released via DOTAP liposomes appear to be tightly bound with MRSA cells since they are not readily removed by repeated washing steps. On the other hand, a fluid but neutral liposome, such as DMPC, and a solid liposome, such as DPPC, promote a markedly smaller accumulation of the photosensitising agents by the bacterial cells, coupled with their extensive wash-out from the cells, which suggests the occurrence of a weaker binding.

The greater photosensitiser accumulation in MRSA cells promoted by cationic liposomes could be due to an electrostatic interaction with the numerous negatively charged groups which are present at the surface of bacterial cells, similar to what previously observed for the avid uptake of such liposomes by HeLa cells [23].

However, it seems that a large amount of cell-bound photosensitiser is not sufficient to guarantee a high degree of photosensitivity. In actual fact, MRSA cells loaded by DOTAP-incorporated Chl do not exhibit any appreciable decrease in survival even after exposure to relative drastic irradiation protocols. It appears unlikely that the low photocytotoxicity observed for DOTAP-Chl is due to the small yield for the generation of singlet oxygen, namely the most reactive intermediate which has been proposed to play a major role in porphyrin photosensitisation of biological systems, including microbial pathogens [24]. This hypothesis is supported by the observation that MRSA undergoes an extensive photoinduced killing when comparable Chl endocellular concentrations are obtained upon incubation of the cells with non-liposome-bound chlorin; moreover, the photophysical properties of DOTAP-incorporated Chl should not be appreciably altered, since the chlorin is found to be largely monomeric in the liposomal vesicles. It is well known that photophysical properties, such as the triplet quantum yield or lifetime of porphyrins are strongly diminished by aggregation [24] and this process is often favoured by photosensitiser compartmentalisation [18].

Rather, the mode of photosensitiser association with the liposomal carrier could play a major role. As shown by our ultrastructural and fluorescence anisotropy studies, the presence of Chl brings about a profound alteration in the three-dimensional architecture of lipid vesicles, as well as a drastic increase in rigidity. This could interfere with the Chl-cell interaction preventing the transfer of the photosensitising agent from the liposome to sites which are critical for cell survival upon visible light-irradiation.

On the contrary, HP appears to be endowed with a high degree of motility even when it is associated with the liposomes, whose structure is not appreciably modified by the presence of the porphyrin. As a consequence, the latter porphyrin can orientate itself toward relatively polar sites in the cytoplasmic membrane, which would be in agree-

ment with its low *n*-octanol/water partition coefficient, i.e., 8 [25]. Previous studies [26] showed that an amphiphilic phthalocyanine with a similar partition coefficient induced an extensive mortality of MRSA cells through the fast inactivation of typical membrane-marker enzymes, such as NADH or succinic dehydrogenase. Modulation of the cell killing photoefficiency by the partitioning properties of the sensitiser into the carrier is particularly evident in the case of HP. Actually, DMPC-bound HP is more active than both free and DPPC-bound HP (Fig. 3) even though the drug is taken up by MRSA cells to a much lower extent ( $\sim 1/3$ , Fig. 2). The poor photoactivity of free HP can be easily explained as due to drug aggregation in the aqueous medium, as also confirmed by the shape of the absorption spectra (not shown). As regards the liposome-associated dye, HP mostly localises at the inner lipid/water interfaces and shows no tendency to diffuse into pure lipid domains in DPPC liposomes, a significant loading of the outer monolayer being observed only after an increase in the fluidity of the system [20,22]. A significant partition of HP in the outer leaflet of DMPC liposomes would be consistent with a greater release of the incorporated drug to the cells. In addition to a high degree of lipid fluidity, the DOTAP system is endowed with the capacity of tightly binding to the cells, due to the positive charge. Thus, HP-containing DOTAP appear to perform a dual role in the systems investigated by us: (a) to guarantee a large and tight uptake of the photosensitising agent by bacterial cells; and (b) to induce a more efficient photosensitised cell killing as compared with other modalities of HP delivery.

In conclusion, the present research suggests that the use of suitable liposomal vesicles as delivery systems for antimicrobial PDT agents can be useful for the following reasons: (a) non-cationic photosensitisers as well can be used as efficient killing agents of microbial cells; (b) the synergic effect of positively charged and highly fluid components of the lipophilic carrier optimises the sensitiser uptake by microbial cells, as well as the overall photoactivity. In particular, the sensitiser must be localised in external, polar domains of the lipid vesicles in order to facilitate its release to the microbial cell membrane.

## 5. Abbreviations

HP	hematoporphyrin
Chl	chlorophyll <i>a</i>
DPPC	DL- $\alpha$ -dipalmitoyl-phosphatidyl-choline
DMPC	L- $\alpha$ -dimyristoyl-phosphatidyl-choline
DOTAP	<i>N</i> -[1-(2,3-dioleoyloxy)propyl]- <i>N,N,N</i> -trimethyl ammonium methylsulfate
DMF	<i>N,N</i> -dimethylformamide
SDS	sodium dodecyl sulfate
MRSA	methicillin-resistant <i>Staphylococcus aureus</i>
BHA	brain heart agar
PBS	phosphate-buffered saline
DMA	9,10-dimethyl-anthracene
CFU	colony-forming units

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## References

- [1] T.J. Dougherty, C.J. Gomer, B.W. Henderson, G. Jori, D. Kessel, M. Korbelik, J. Moan, Q. Peng, Photodynamic therapy, *J. Natl. Cancer Inst.* 90 (1998) 889–905.
- [2] E.S. Marmur, C.D. Schmults, D.J. Goldberg, A review of laser and photodynamic therapy for the treatment of nonmelanoma skin cancer, *Dermatol. Surg.* 30 (2004) 264–271.
- [3] M. Ochsner, Photophysical and photobiological processes in the photodynamic therapy of tumours, *J. Photochem. Photobiol. B* 39 (1997) 1–18.
- [4] M. Soncin, C. Fabris, A. Buseti, D. Dei, D. Nistri, G. Roncucci, G. Jori, Approaches to selectivity in the Zn(II)-phthalocyanine-photosensitized inactivation of wild-type and antibiotic resistant *Staphylococcus aureus*, *Photochem. Photobiol. Sci.* 1 (2002) 815–819.
- [5] G. Jori, S. Brown, Photosensitized inactivation of microorganisms, *Photochem. Photobiol. Sci.* 3 (2004) 403–405.
- [6] T. Maisch, R.M. Szeimies, G. Jori, C. Abels, Antibacterial photodynamic therapy in dermatology, *Photochem. Photobiol. Sci.* 3 (2004) 907–917.
- [7] M. Wainwright, Methylene blue derivatives-suitable photoantimicrobials for blood product disinfection? *Int. J. Antimicrob. Agents* 16 (2000) 381–394.
- [8] M. Merchat, G. Bertolini, P. Giacomini, A. Villanueva, G. Jori, Meso-substituted cationic porphyrins as efficient photosensitizers of Gram-positive and Gram-negative bacteria, *J. Photochem. Photobiol. B* 32 (1996) 153–157.
- [9] N.S. Soukos, L.A. Ximenez-Fyvie, M.R. Hamblin, S.S. Sacransky, T. Hasan, Targeted antimicrobial photochemotherapy, *Antimicrob. Agents Chemother.* 42 (1998) 2595–2601.
- [10] G. Bertoloni, F. Rossi, G. Valduga, G. Jori, J. Van Lier, Photosensitizing activity of water- and lipid-soluble phthalocyanines on *Escherichia coli*, *FEMS Microbiol. Lett.* 71 (1990) 149–156.
- [11] F.M. Lauro, P. Pretto, L. Covolo, G. Jori, G. Bertoloni, Photoinactivation of bacterial strains involved in periodontal diseases sensitized by porphycene-polylysine conjugates, *Photochem. Photobiol. Sci.* 1 (2002) 560–564.
- [12] H. Ashkenazi, Y. Nitzan, D. Gal, Photodynamic effects of antioxidant substituted porphyrin photosensitizers on gram-positive and gram-negative bacteria, *Photochem. Photobiol.* 77 (2003) 186–191.
- [13] Z. Huang, A review of progress in clinical photodynamic therapy, *Technol. Cancer Res. Treat.* 4 (2005) 283–293.
- [14] A.R. Battersby, E. McDonald, Synthesis of porphyrins, chlorins and corrins, in: K. Smith (Ed.), *Porphyrins and Metalloporphyrins*, Elsevier, Amsterdam, 1985, pp. 61–121.
- [15] J.M.H. Kremer, M.W.J. Van der Esker, C. Pathmamanoharan, P.H. Wiersema, Vesicles of variable diameter prepared by a modified injection method, *Biochemistry* 16 (1977) 3932–3935.
- [16] E. Gross, B. Ehrenberg, F.M. Johnson, Singlet oxygen generation by porphyrins and the kinetics of 9,10-dimethylanthracene photosensitization in liposomes, *Photochem. Photobiol.* 57 (1992) 808–813.
- [17] M.R. Detty, S.L. Gibson, S.J. Wagner, Current clinical and preclinical photosensitizers for use in photodynamic therapy, *J. Med. Chem.* 47 (2004) 3897–3915.
- [18] D.G. Whitten, Photochemistry of porphyrins and their metal complexes in solution and organized media, *Rev. Chem. Intermed.* 2 (1978) 107–138.
- [19] E. Reddi, M. Cecon, G. Valduga, G. Jori, J.C. Bommer, F. Elisei, L. Zatterini, U. Mazzucato, Photophysical properties and antibacterial activity of meso-substituted cationic porphyrins, *Photochem. Photobiol.* 75 (2002) 462–470.
- [20] F. Ricchelli, G. Jori, S. Gobbo, M. Tronchin, Liposomes as models to study the distribution of porphyrins in cell membranes, *Biochim. Biophys. Acta* 1065 (1991) 42–48.
- [21] M.R. Hamblin, B. Ortel, Future directions-photosensitizer targeting and new disease indications, in: P.G. Calzavara, R.M. Szeimies, B. Ortel (Eds.), *Photodynamic Therapy and Fluorescence Diagnosis in Dermatology*, Elsevier, Amsterdam, 2001, pp. 339–366.
- [22] R.B. Campbell, S.V. Balasubramanian, R.M. Straubinger, Phospholipid-cationic lipid interactions: influences on membrane and vesicle properties, *Biochim. Biophys. Acta* 1512 (2001) 27–39.
- [23] F. Ricchelli, S. Gobbo, Porphyrins as fluorescent probes for monitoring phase transitions of lipid domains in biological membranes. Factors influencing the microenvironment of hematoporphyrin and protoporphyrin in liposomes, *J. Photochem. Photobiol. B* 29 (1995) 65–70.
- [24] K. Berg, Mechanisms of cells damage in photodynamic therapy, in: H. Honigsmann, G. Jori, A.E. Young (Eds.), *Fundamental Basis of Phototherapy*, OEMF, Milan, 1996, pp. 181–207.
- [25] D. Kessel, Sites of photosensitization by derivatives of hematoporphyrin, *Photochem. Photobiol.* 44 (1986) 489–494.
- [26] A. Segalla, C.D. Borsarelli, S.E. Braslavsky, J.D. Spikes, G. Roncucci, D. Dei, G. Chiti, G. Jori, E. Reddi, Photophysical, photochemical and antibacterial photosensitizing properties of a novel octacationic Zn(II)-phthalocyanine, *Photochem. Photobiol. Sci.* 1 (2002) 641–648.