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Research Paper

Analytical Studies on Photochemical Behavior of Phototoxic Substances; Effect of Detergent Additives on Singlet Oxygen Generation

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Purpose. Monitoring of reactive oxygen species (ROS) generation from photoirradiated compounds would be effective for the prediction of the phototoxic potential. The aim of this investigation was to clarify the possible role of biomimetic vehicle systems on the photochemical properties of phototoxic compounds, focusing on the singlet oxygen generation.
Materials and Methods. Nine phototoxic and one non-phototoxic compounds (200 μM), dissolved in Tween 20, sodium laurate, or sodium dodecyl sulfate (SDS) micellar solution, were exposed to UVA/B light (250 W/m^2), and singlet oxygen generation was monitored by RNO bleaching methodology. Photochemical properties of photosensitizers were also evaluated by UV measurement, and the interaction of photosensitizers with surfactant micelles was assessed by Z-potential and NMR spectroscopic analyses.
Results. All phototoxic compounds tended to generate singlet oxygen under light exposure in the all micellar solutions tested. There appeared to be some differences in photoreactivity of both cationic and anionic photosensitizers among the micelles tested, whereas ROS data on anthracene, dissolved in three micellar solutions, were found to be quite similar. Photosensitizers exhibited no significant changes in UV spectral patterns among the dissolving micellar solutions. Addition of cationic photosensitizer at the final concentration of 100 μM into 100 mM SDS solution resulted in the 20 mV increase of zeta potential and transition of NMR spectral pattern, which would reflect the electrostatic interaction with anionic micelles.
Conclusion. Based on the data obtained, the photoreactivity of photosensitizing molecules, especially cationic and anionic photosensitizers, strongly depends on the physicochemical properties of the microenvironment.

KEY WORDS: photosensitizer; phototoxicity; singlet oxygen; surfactant; UV.

INTRODUCTION

Phototoxic skin responses, after topical or systemic administration of drugs, have been identified as one of significant side effects (1). Several classes of drugs exhibit this type of side effect, including antibacterials (2,3), thiazide diuretics (4), non-steroidal anti-inflammatory drugs (NSAIDs) (5), quinolones (6) and tricyclic antidepressant (7,8). Drug-induced phototoxic reactions can be categorized as photoirritant, photogenotoxic or photoallergic, and some drugs can cause all three types of reactions (9). To predict the potential of these phototoxic responses and photochemical reactions, the development of effective methodology to evaluate the photochemical/biological properties have been attempted over the past few years (10). Upon these researches, a lot of screening methods for recognizing photosensitizing drugs have been suggested, including measurement of UV absorption, photohemolysis model (11), measurement of oxygen consumption in *Bacillus subtilis* (12), cutaneous phototoxic reaction model using human reconstituted epidermis Episkin (13), and 3T3 neutral red uptake (3T3 NRU) phototoxicity test (14).

Previously, we reported that a lot of phototoxic or photosensitive compounds have an ability to generate ROS under light exposure, resulting in the induction of the photodegradation and/or oxidative stress against cell membrane and DNA (15). According to the results, the primary event in any photosensitization process is the absorption of a photon, and the following free radical (Type I reaction) and singlet oxygen generation (Type II reaction) by photo-excited drug molecules may appear to be the principal intermediate species in the phototoxic response (16). Herein, we proposed that ROS assay would be effective to classify chemicals as phototoxic and/or

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ABBREVIATIONS: DNA, deoxyribonucleic acid; DMSO, dimethyl sulfoxide; NMR, nuclear magnetic resonance; NRU, neutral red uptake; NSAIDs, non-steroidal anti-inflammatory drugs; ROS, reactive oxygen species; SDS, sodium dodecyl sulfate; UV, ultraviolet.

photosensitive compounds on the basis of their photochemical properties. Although the ROS assay might be highly productive for the photochemical evaluation, it is still unclear whether the experimental conditions of the present ROS assay could reflect the biological environment.

In the possible pathway of phototoxic skin responses, a photoreactive substance may reach the skin following topical application or indirectly, via the blood flow, after systemic administration, and then photochemical reaction, targeting the lipid, DNA or proteins, would occur under light exposure (17). Epidermal cell membrane have a complex lipid composition which includes fatty acids, phospholipids, ceramide, and cholesterols, and some components tend to exhibit the non-covalent association with the phototoxic compounds (18,19). Although molecular properties of phototoxic compounds, focusing on membrane interaction and penetration, have been investigated using biomimetic micelles, the photochemical behavior of phototoxic compounds in the presence of micelles, liposome or other biomimetic systems has not been fully elucidated. The aim of the present study is to clarify the effect of micelles on the photoreactivity of photosensitizers. We investigated the generation of singlet oxygen from the photo-irradiated phototoxic/photosensitive compounds, dissolved in 20 mM sodium phosphate buffer containing Tween 20, sodium laurate, or sodium dodecyl sulfate (SDS). In addition, the UV spectral patterns of phototoxic compounds in the presence of micelles, focusing on the UVA/B region, were measured, and an interaction of photosensitizing compounds with micelles was also evaluated by Z-potential and NMR spectroscopic analyses. We could demonstrate that the photochemical properties of photosensitizers are variable depending on the solution environment, possibly due to the interaction of micelles.

MATERIALS AND METHODS

Chemicals

Sulisobenzone, a non-phototoxic compound, and nine photosensitizers; amiodarone, anthracene, chlorothiazide, diclofenac, furosemide, haloperidol, imipramine, omeprazole and tamoxifen, were purchased from Sigma (St. Louis, MO). Sodium laurate, sodium dodecyl sulfate (SDS), Tween 20, *p*-nitrosodimethylaniline, imidazole, and nitroblue tetrazolium were obtained from Wako Pure Chemical Industries (Osaka, Japan).

UV Spectral Analysis

All tested compounds were dissolved in 20 mM sodium phosphate buffer (NaPB, pH 7.4) at the final concentration of 20 μM. UV-Vis absorption spectra were recorded with a JASCO V-560 double-beam spectrophotometer (JASCO, Tokyo, Japan) interfaced to a PC for data processing (software: Spectra Manager). Spectrofluorimeter quartz cells with 10-mm pathlength were employed.

Measurement of Size and Zeta Potential of Micelles

The particle size and zeta potential of micelles were measured by Zetasizer Nano ZS (Malvern Instruments, Worcestershire, UK). Samples were diluted appropriately with 20 mM NaPB (pH 7.4) for the measurements. The particle size distribution of micelles was determined using a dynamic light scattering method at 37°C. The light source was a He–Ne laser with a wavelength of 633 nm and the scattering angle was 90°. Zeta potential measurements were done at 25°C using a second generation phase analysis light scattering method (M3-PALS) according to the manufacturer's protocol.

Irradiation Conditions

Each tested compound was stored in an Atlas Suntest CPS+ solar simulator (Atlas Material Technology LLC, Chicago, IL) equipped with a xenon arc lamp (1500 W). UV special filter and window glass filter were installed to adapt the spectrum of the artificial light source to natural daylight. The irradiation test was carried out at 25°C with an irradiance of 250 W/m^2.

Determination of Singlet Oxygen

Singlet oxygen was determined following the Kraljic and El Moshni procedure (20), and it was measured in an aqueous solution by spectrophotometrically monitoring the bleaching of RNO at 440 nm using imidazole as a selective acceptor of singlet oxygen. Samples containing the compounds under examination (1–400 μM), *p*-nitrosodimethylaniline (50 μM) and imidazole (50 μM) in 20 mM NaPB (pH 7.4) were irradiated with UVA/B for different periods in 96-well plate, and then the UV absorption at 440 nm was measured by a SpectraMax plus 384 microplate spectrophotometer (Molecular Devices, Kobe, Japan). Determination of singlet oxygen was also carried out for all samples with UV light protection as dark control.

Log *D* Calculation

Log *D* at pH 7.4 (log $D_{7.4}$) were calculated based on the log *P* and p*K*a value as follows;

$$\text{For acidic compounds, } \log D_{7.4} = \log P - \log\left(1 + 10^{7.4-\text{pka}}\right)$$
$$\text{For basic compounds, } \log D_{7.4} = \log P - \log\left(1 + 10^{pKa-7.4}\right)$$

NMR Measurement

^{1}H-NMR spectra of photosensitizers, using D_2O (99.95% D, Wako Pure Chemical Industries) containing Tween 20 (final concentration, 0.5%) or SDS (100 mM) as a solvent, were recorded on a JEOL, JNM-LA 300 (Nihon Denshi, Tokyo, Japan).

Data Analysis

For statistical comparisons, a one-way analysis of variance (ANOVA) with the pairwise comparison by Fisher's least significant difference procedure was used. A *P* value of less than 0.05 was considered significant for all analyses.

RESULTS AND DISCUSSIONS

Solubilization of Photosensitizers in Micellar Solutions

Surfactants are amphiphilic molecules, like lipid, and some of the same rules governing lipid behavior also apply to

the surfactant (21). This would be a part of reasons why surfactants have a far-ranging use in membrane studies. Surfactant micelles and vehicles has also been used as structural and functional models of photochemical utilization of light energy through the photoionization of molecules solubilized in these systems, and practically they were applied to the study of the photoexcitation and electron transfer of chlorophyll which can be incorporated into micelles and vehicles (22). In the present study, photochemical properties of nine photosensitizing molecules; amiodarone (18), anthracene (23), chlorothiazide (11), diclofenac (24), furosemide (4), haloperidol (25), imipramine (7), omeprazole , and tamoxifen (15), and one non-phototoxic compounds, sulisobenzone (13), were investigated in the micellar solution (Fig. 1). Although some compounds were poorly soluble in 20 mM NaPB (pH 7.4), all compounds tested were found to be soluble in all micellar solutions tested, such as Tween 20 (0.5%), sodium laurate (100 mM), and SDS (100 mM) dissolved in 20 mM NaPB (pH 7.4). Average size and zeta potential of micelles tested were summarized in Table I. The mean size of SDS and sodium laurate micelles was two to three-times smaller than that of Tween 20 ($p<0.01$), and zeta potentials of anionic micelles were low since the ionic headgroups of the anionic surfactant frequently present a net charge. The negatively charged headgroups can bind with cations from the aqueous buffer, for example calcium or sodium ions, via ionic interactions. If cationic–amphiphilic substances are added to the anionic micellar solutions, the lipophilic parts of the molecules are incorporated into the hydrocarbon chain region of the monolayer. The cationic parts of the compounds are located within the anionic headgroup region at the surfactant-water interface and displace cations from their ionic binding sites. The spatial position of a solubilized compound in micelles also depends on its polarity: nonpolar molecules will be solubilized in the micellar core, and substances with intermediate polarity will be distributed along the surfactant molecules in certain intermediate positions.

Table I. Particle Size and Zeta Potential of Micelles

Micelles	Particle Size (μm)	Zeta Potential (mV)
Tween 20	7.7±0.7	−10.6±2.7
Sodium laurate	3.8±0.1	−44.5±0.9
Sodium dodecyl sulfate	2.3±0.1	−29.8±2.3

Each surfactant was dissolved in 20 mM NaPB (pH 7.4). Data represent mean ± SD for five experiments.

UV Spectral Patterns of Photosensitizers in Micellar Solutions

The UV absorption spectra of tested compounds, at the concentration of 20 μM, were recorded in three micellar solutions, and the wavelength and absorbance of the long-wave peak were noted (Table II). The absorption spectra of anthracene, a neutral compound; chlorothiazide, a diuretic

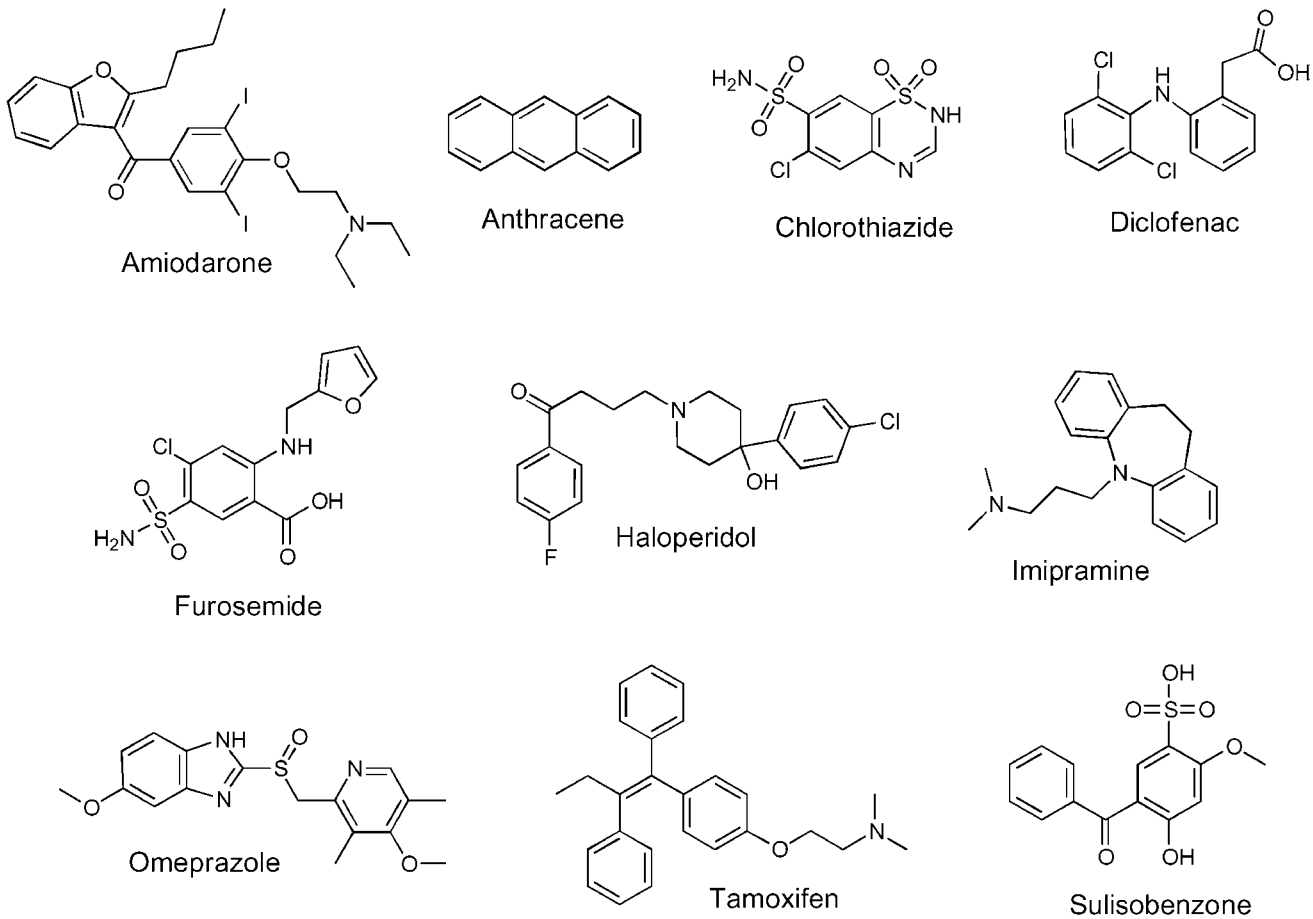

Fig. 1. Structures of nine known-photosensitizers and a non-phototoxic sunscreen (Sulisobenzone).

Table II. Effect of Coexistent Micelles on UV Spectral Patterns of Photosensitizers

Compounds	λ_{max} (nm) & Molar Extinction Coefficient ($l\ mol^{-1}\ cm^{-1}$)		
	Tween 20	Sodium Laurate	SDS
Amiodarone	303 (5.4×10^3)	308 (5.3×10^3)	307 (5.4×10^3)
Anthracene	379 (1.8×10^3)	378 (1.8×10^3)	378 (1.7×10^3)
	360 (1.9×10^3)	359 (1.9×10^3)	359 (1.9×10^3)
	342 (1.4×10^3)	341 (1.3×10^3)	342 (1.4×10^3)
	326 (7.5×10^2)	326 (6.4×10^2)	325 (8.8×10^2)
	294 (6.4×10^2)	294 (4.1×10^2)	293 (7.5×10^2)
Chlorothiazide	313 (9.5×10^3)	311 (8.7×10^3)	311 (8.3×10^3)
	296 (9.6×10^3)	294 (9.4×10^3)	295 (9.5×10^3)
Diclofenac	[290 (8.8×10^3)]	[290 (8.5×10^3)]	[290 (8.4×10^3)]
Furosemide	332 (4.8×10^3)	330 (4.6×10^3)	331 (4.9×10^3)
Haloperidol	[290 (74)]	[290 (1.6×10^2)]	[290 (2.0×10^2)]
Imipramine	[290 (4.0×10^3)]	[290 (4.0×10^3)]	[290 (3.8×10^3)]
Omeprazole	294 (1.5×10^4)	295 (1.6×10^4)	294 (1.6×10^4)
Tamoxifen	[290 (5.6×10^3)]	[290 (6.1×10^3)]	[290 (5.5×10^3)]
Sulisobenzone	319 (6.7×10^3)	320 (6.8×10^3)	319 (6.5×10^3)

UV absorption spectrum of each compound (10 μM) was measured in 20 mM phosphate buffer (pH 7.4) containing Tween 20 (0.5%), sodium laurate (100 mM), or sodium dodecyl sulfate (100 mM). If the peak and shoulder wavelengths were shorter than the lower limit of UVB (290 nm), the absorbance at 290 nm was noted in brackets.

drug; and tamoxifen, an anti-breast cancer drug, are shown in Fig. 2. These photosensitive compounds showed strong absorption in UVA/B range, and the their lowest energy bands have maxima at 379 (anthracene), 313 (chlorothiazide) and 276 nm (tamoxifen) in 0.5% Tween 20. UV spectral patterns of tested compounds were found to be slightly different among the vehicle system, however no significant UV transition or bathochromic shift was observed.

According to the Jagger's report (26), solar radiation reaches the surface of the earth after passage through the atmosphere where the higher energy part is absorbed, resulting in the cut-off UVC region. Herein, spectrum of solar radiation is composed of UVA, UVB and visible light. Almost all tested photosensitizers showed the significant absorption of UVA/B (Fig. 2), suggesting that they may absorb photon energy and be excited under exposure to sun light. Although sulisobenzone has been identified to be non-phototoxic, it also showed strong UVA/B absorption. In this context, absorbing photon energy might not always be used for photochemical reaction, and UV spectral patterns of tested compounds could not completely represent their photoreactivity or phototoxic potential.

Generation of Singlet Oxygen from Photoirradiated Compounds

The generation of singlet oxygen was detected by spectrophotometric measurement of *p*-nitroso-dimethylaniline (RNO) bleaching, followed by decrease of the absorbance of RNO at 440 nm (20). Although singlet oxygen does not react chemically with RNO, the RNO bleaching is a consequence of singlet oxygen capture by the imidazole ring which results in the formation of a trans-annular peroxide intermediate capable of inducing the bleaching of RNO. In Fig. 3, only four compounds (anthracene, chlorothiazide, tamoxifen and sulisobenzone) are presented for the sake of clarity, and data show the kinetics of RNO bleaching after irradiation (250 W/m^2) in the presence of photosensitizing compounds. Sulisobenzone (200 μM), a strong UV absorber, was unable to generate singlet oxygen to significant levels, and the order of singlet oxygen-forming ability was as follows: chlorothiazide > tamoxifen ≒ anthracene > sulisobenzone in 0.5% Tween 20 (Fig. 3a); and tamoxifen > chlorothiazide ≒ anthracene > sulisobenzone in 100 mM SDS (Fig. 3b). Thus, photoreactivity of tamoxifen and

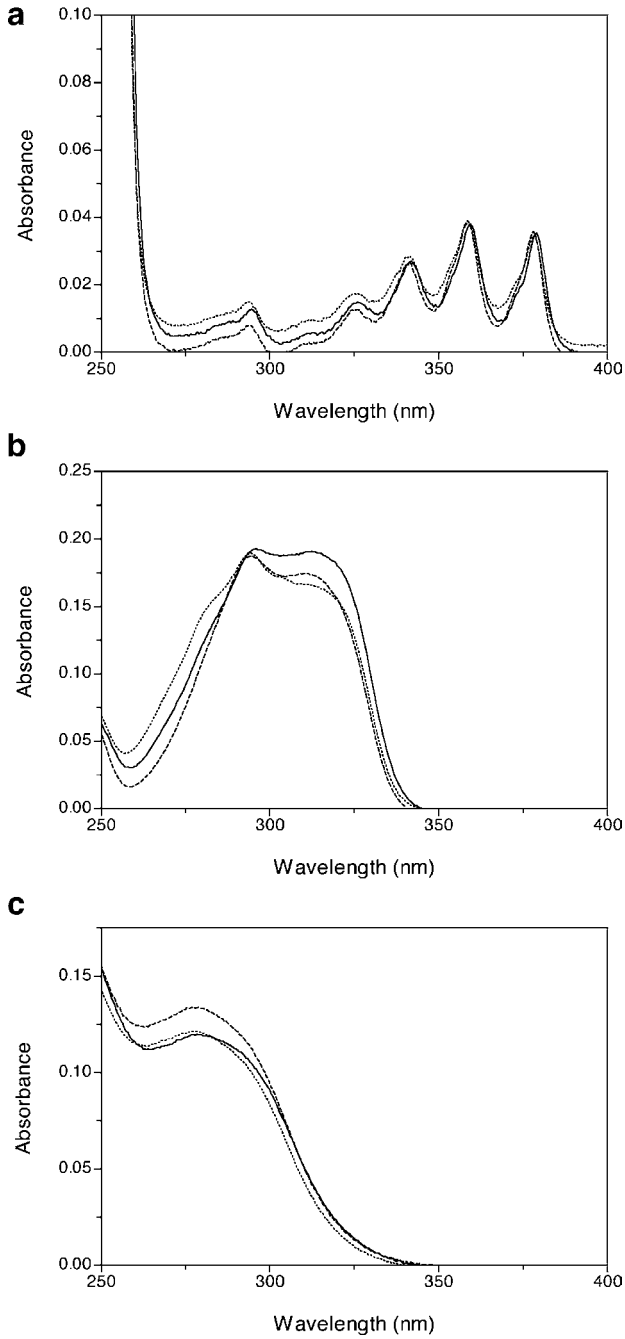

Fig. 2. UV/VIS spectral patterns of photosensitive compounds. Each photosensitizer, such as **a** anthracene, **b** chlorothiazide, and **c** tamoxifen, was dissolved in 20 mM NaPB (pH 7.4) containing micelles, including Tween 20 (*solid line*), sodium laurate (*dashed line*), and SDS (*dotted line*).

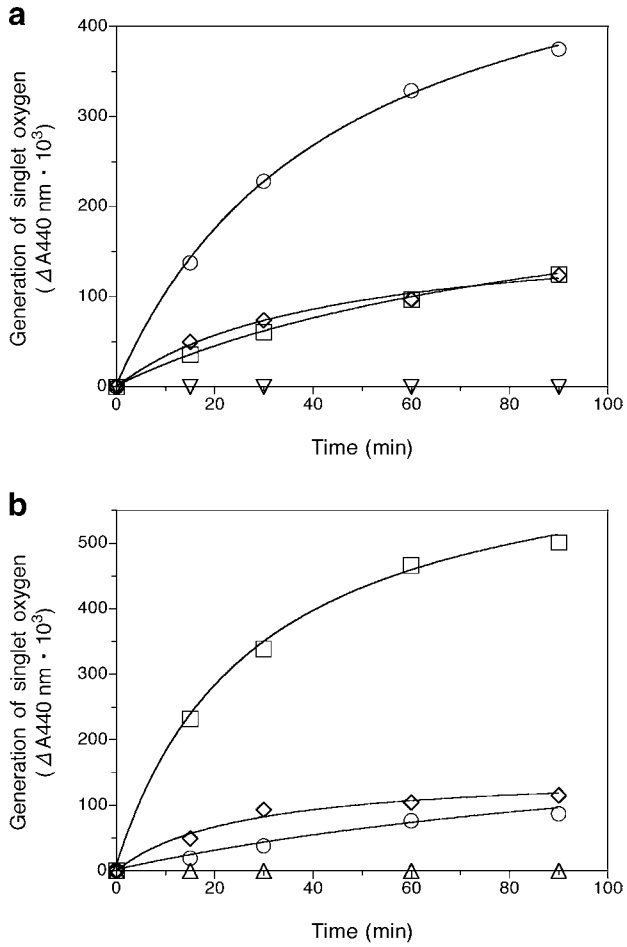

Fig. 3. Generation of singlet oxygen from photo-irradiated photosensitizers. Each tested compound (200 μM) was dissolved in 20 mM NaPB (pH 7.4) containing Tween 20 (**a**) and SDS (**b**), and exposed to UVA/B (250 W/m^2) for the indicated periods. *Open squares*, Tamoxifen; *open circle*, Chlorothiazide; *open diamond*, Anthracene; and *open triangle*, Sulisobenzone.

chlorothiazide was found to be variable depending on the vehicles.

Generation of singlet oxygen from irradiated photosensitizers seems to be concentration-dependent (Fig. 4), however they did not show any RNO bleaching without any light exposure (data not shown). Anthracene exhibited the similar data on ROS generation in three micellar solutions tested (Fig. 4a), however, addition of anionic micelles resulted in the significant changes in photochemical properties of tamoxifen and chlorothiazide (Fig. 4b and c). The capacity of the test compounds (200 μM) in aqueous solution to generate singlet oxygen is shown in Table III. All phototoxic/photosensitive compounds showed the ability to generate singlet oxygen, whereas singlet oxygen from sulisobenzone was negligible regardless of the microenvironment. On the basis of the obtained data and their molecular properties, such as p*K*a and log $D_{7.4}$ values, ionic and amphiphilic photosensitizers tended to change their photochemical properties depending on the microenvironment. Interestingly, generation of singlet oxygen from imipramine in 0.5% Tween 20 was negligible, however the exchange of surfactant into SDS led to dramatical increase in the photoreactivity. The variation of ROS generation from cationic photosensitizers, as well as haloperidol, was also observed, and there appears to be some relationship for the acidity of surfactant used. On the contrary, anionic photosensitizers, including chlorothiazide, diclofenac and furosemide, in the presence of anionic micelles were found to be three to four

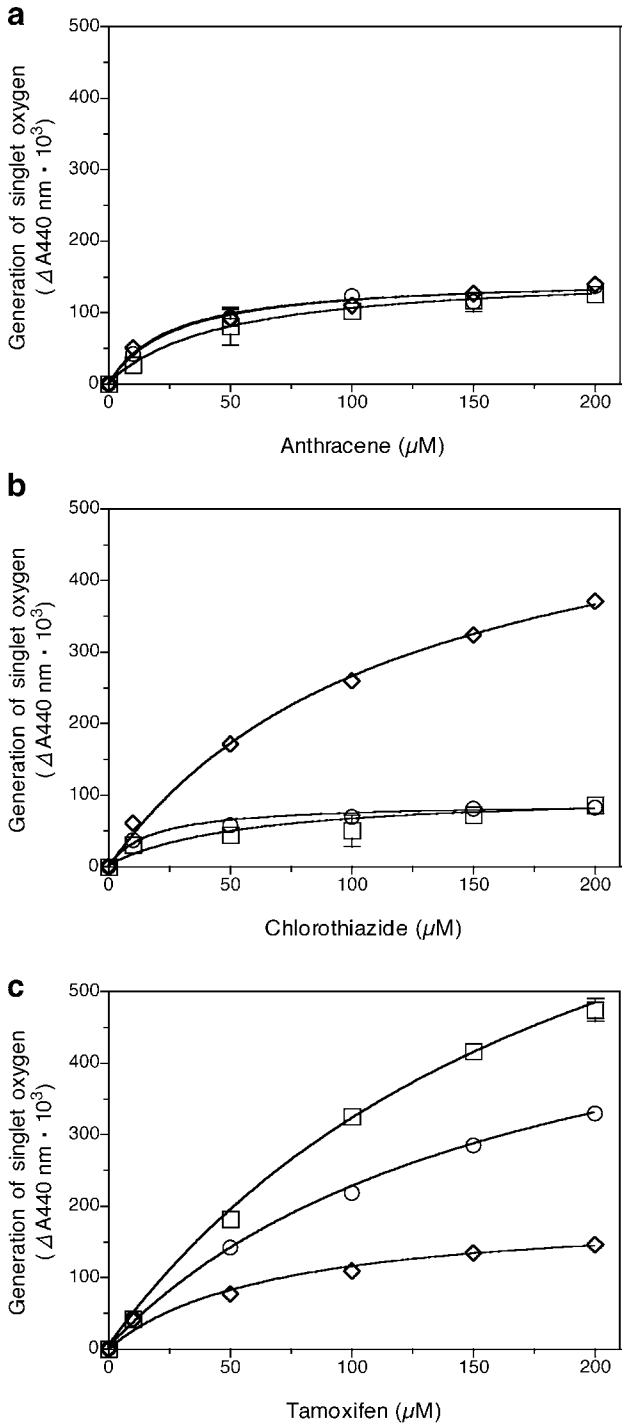

Fig. 4. Concentration-dependent generation of singlet oxygen from the photo-irradiated photosensitizers. Each compound tested, **a** anthracene, **b** chlorothiazide, and **c** tamoxifen, was dissolved in 20 mM NaPB (pH 7.4) containing micelles, including Tween 20 (*open diamond*), sodium laurate (*open circle*), and SDS (*open square*), at the indicated concentrations, and then exposed to UVA/B (250 W/m^2) for 1 h. Data represent mean ± SD of four experiments.

Table III. Generation of Singlet Oxygen from Photoirradiated Compounds

Compounds	p*Ka*[a]	Log $D_{7.4}$	Singlet Oxygen ($\Delta A440 \cdot 10^3$)[b]		
			Tween 20	Sodium Laurate	SDS
Amiodarone	9.4 (B)	6.9	295±10	492±11	684±50
Anthracene	–	4.5	91±7	97±8	083±10
Chlorothiazide	6.8 (A)	−1.6	339±10	75±4	80±5
	9.4 (A)				
Diclofenac	4.0 (A)	1.1	491±3	203±12	113±5
Furosemide	3.3 (A)	−0.1	347±14	164±5	111±50
	10.0 (A)				
Haloperidol	8.6 (B)	2.1	056±10	061±3	140±40
Imipramine	9.4 (B)	2.7	N.D.	063±4	130±12
Omeprazole	4.0 (B)	2.1	400±8	215±3	239±50
	8.7 (A)				
Tamoxifen	8.9 (B)	6.6	111±1	296±5	451±10
Sulisobenzone	6.3 (A)	−3.5	N.D.	N.D.	N.D.

[a] *A* Acid; *B* base. [b] Each tested compound (200 μM) was dissolved in 20 mM NaPB (pH 7.4) containing micelles, and exposed to UVA/B (250 W/m^2) for 1 h.

times less photoreactive, as compared to those in 0.5% Tween 20. Thus, the physicochemical properties may influence the photochemical process and, in particular, the different charge of the micellar surface might strongly influence the photoreaction, possibly due to the interaction with cationic photosensitizers.

Interaction Between Anionic Micelles and Photosensitizers

In the light of these results, we attempted to investigate in more detail the binding of photosensitizers with the surface of micelles. To evaluate the interaction between the micelles and the compounds, zeta potential of SDS was measured in the presence and the absence of the compounds (Fig. 5). In the absence of the drugs, the surface charge of SDS micelles was very negative (−30 mV) due to the presence of anionic group. An increase in the proportion of the positive charges on the micellar surfaces was observed with increase in the concentration of tamoxifen, whereas anthracene and chlorothiazide did not show any transition in zeta potential. In addition to Z-potential measurements, interaction of tamoxifen with micelles was also evaluated by NMR spectral analysis (Fig. 6). All ^{1}H-NMR spectra of 0.5% Tween 20, 100 mM sodium laurate and 100 mM SDS micelles containing tamoxifen (200 μM) were recorded at a sample temperature of 293 K. On the basis of the spectra recorded, tamoxifen incorporated into SDS micelles gave aromatic proton peaks that were shifted upfield or downfield by ~0.2 ppm relative to tamoxifen/Tween 20 micelles. The slight transition of aromatic proton peaks was also observed in tamoxifen/sodium laurate micelles (data not shown). These observations would indicate the changes in microenvironment of tamoxifen through the interaction with anionic micelles. Upon these findings, tamoxifen was found to have a high affinity to SDS micelles to interact their surfaces, and this might be a part of reason why the photochemical properties of cationic photosensitizers had changed in anionic micellar solution. With respect to anionic photosensitizers, there would be an ionic repulsion of SDS micelles, and this also led to the variation in the photoreactivity of anionic molecules, depending on the solvent systems.

Some cationic and amphiphilic compounds have been reported to interact with cell membrane, as well as anionic micelles (27,28). The cell membrane contains a variety of

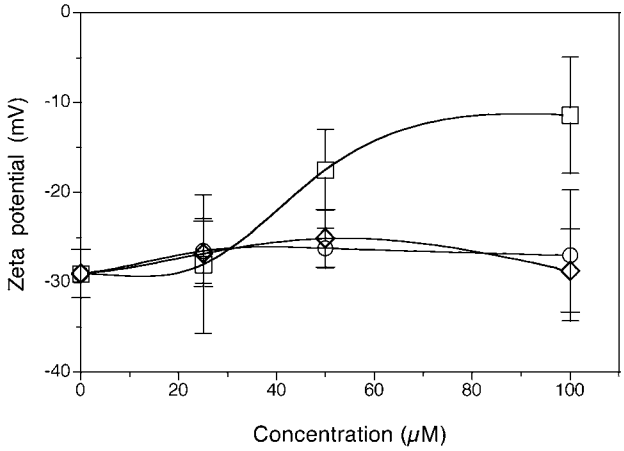

Fig. 5. Zeta potential of SDS micelles in the presence of anthracene, chlorothiazide and tamoxifen with various concentrations. *Open square*, Tamoxifen; *open circle*, Chlorothiazide; and *open diamond*, Anthracene.

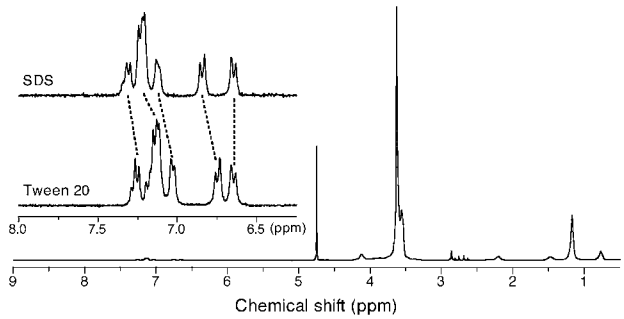

Fig. 6. ^{1}H-NMR spectra of micelles incorporated with tamoxifen in D_2O. Full spectrum of 0.5% Tween 20/tamoxifen was shown in *bottom*, and aromatic proton peaks of SDS/tamoxifen and Tween 20/tamoxifen were magnified.

biological molecules, primarily proteins and lipids, and the major lipids in mammalian membranes are phospholipids, glycolipids and sterols (29,30). The relative composition of each depends upon the type of cells, however, in the majority of cases, phospholipids are the most abundant. The outer cell membrane and the membranes surrounding inner cell organelles are bilipid layers, and the membrane phospholipid molecules create a spherical three dimensional lipid bilayer shell around the cell. In the formation of a bilipidlayer, the tails of the phospholipids orient towards each other creating a hydrophobic environment within the membrane, while the ionic head groups are immersed in an aqueous environment and bind with cations. The potency of cationic amphiphilic compounds to displace cations on the ionic headgroups of phospholipids would depend on their affinity to the lipid monolayer, and the photochemical behavior of cationic compound would change after electrostatic interaction with the cell membrane, as well as the anionic micelles we used. Among the membrane models utilized, micellar systems can be considered an interesting alternative to study the interactions of compounds with membranes because of the relative simplicity of these systems (21,31). Herein, determination of ROS in the anionic micellar solution might partly reflect the photochemical behavior of cationic photosensitizers on the surface of cell membrane, and the ROS assay system in the micelle-based vehicles would be more effective to predict the phototoxic potential of pharmaceutical substances. In addition, mechanisms of drug-induced phototoxic responses are so complicated (17). In some phototoxic drugs, other radical species, such as superoxide and hydroxy radicals, and the interaction with proteins, DNA or other biomolecules are involved in the phototoxic cascades (32,33). In this context, further clarification of possible phototoxic pathways is important, and which could be helpful for further modification and optimization of ROS assay to improve the predictability of phototoxic risk and to avoid the misleading data.

CONCLUSION

In this investigation, we demonstrated that photoreactivity of ionic photosensitizers was variable depending on the dissolving micellar solution, whereas anthracene, a neutral compound, exhibited similar photoreactivity in all micellar solutions tested. No significant UV transitions, neither bathochromic nor hyperchromic effect, were observed in the photosensitizers dissolved in the micellar solutions, therefore the variation in ROS data was not attributed to the changes in the UV-absorption and following photosensitizing properties of tested compounds. Based on the data obtained, some basic compounds, under exposure to UVA/B light, were found to generate much more single oxygen in accordance with the acidity of surfactant used. These findings, as well as the result from zeta potential and NMR measurements, suggested that interaction between anionic micelles and cationic photosensitizers affects their photochemical behavior. The main conclusion that can be drawn from this work, taken together with the biological conditions of skin membrane in which phototoxic reaction occur, is that the use of anionic surfactant might be effective for the ROS assay to predict the phototoxic potential of pharmaceutical substances in the biomimetic environment.

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REFERENCES

1. A. M. Kligman and R. Breit. The identification of phototoxic drugs by human assay. *J. Invest. Dermatol.* **51**:90–99 (1968).
2. N. J. Wainwright, P. Collins, and J. Ferguson. Photosensitivity associated with antibacterial agents. *Drug Saf.* **9**:437–440 (1993).
3. J. A. Wiebe and D. E. Moore. Oxidation photosensitized by tetracyclines. *J. Pharm. Sci.* **66**:186–189 (1977).
4. F. Vargas, I. Martinez Volkmar, J. Sequera, H. Mendez, J. Rojas, G. Fraile, M. Velasquez, and R. Medina. Photodegradation and phototoxicity studies of furosemide. Involvement of singlet oxygen in the photoinduced hemolysis and lipid peroxidation. *J. Photochem. Photobiol. B* **42**:219–225 (1998).
5. L. Becker, B. Eberlein-Konig, and B. Przybilla. Phototoxicity of non-steroidal anti-inflammatory drugs: in vitro studies with visible light. *Acta Derm. Venereol.* **76**:337–340 (1996).
6. P. Ball and G. Tillotson. Tolerability of fluoroquinolone antibiotics. Past, present and future. *Drug Saf.* **13**:343–358 (1995).
7. G. Viola, G. Miolo, D. Vedaldi, and F. Dall'Acqua. In vitro studies of the phototoxic potential of the antidepressant drugs amitriptyline and imipramine. *Farmaco* **55**:211–218 (2000).
8. N. Canudas and C. Contreras. Isolation and identification of the photodegradation products of the photosensitizing antidepressant drug clomipramine. Phototoxicity studies on erythrocytes. *Pharmazie* **57**:405–408 (2002).
9. J. H. Epstein and B. U. Wintroub. Photosensitivity due to drugs. *Drugs* **30**:42–57 (1985).
10. C. Arlett, L. Earl, J. Ferguson, N. Gibbs, J. Hawk, L. Henderson, B. Johnson, W. Lovell, H. D. Menage, and S. Navaratnam British Photodermatology Group Workshop. Predictive in vitro methods for identifying photosensitizing drugs: a report. *Br. J. Dermatol.* **132**:271–274 (1995).
11. E. Selvaag. Evaluation of phototoxic properties of oral antidiabetics and diuretics. Photohemolysis model as a screening method for recognizing potential photosensitizing drugs. *Arzneimittelforschung* **47**:1031–1034 (1997).
12. J. C. Beani, R. Gautron, P. Amblard, F. Bastrenta, L. Harrouch, P. Jardon, and J. L. Reymond. Screening for drug photosensitization activity by measuring the variations in oxygen consumption of Bacillus subtilis. *Photodermatology* **2**:101–106 (1985).
13. P. Portes, M. J. Pygmalion, E. Popovic, M. Cottin, and M. Mariani. Use of human reconstituted epidermis Episkin for assessment of weak phototoxic potential of chemical compounds. *Photodermatol. Photoimmunol. Photomed.* **18**:96–102 (2002).
14. H. Spielmann, M. Liebsch, B. Doring, and F. Moldenhauer. First results of an EC/COLIPA validation project of in vitro phototoxicity testing methods. *Altex* **11**:22–31 (1994).
15. S. Onoue and Y. Tsuda. Analytical studies on the prediction of photosensitive/phototoxic potential of pharmaceutical substances. *Pharm. Res.* **23**:156–164 (2006).
16. C. S. Foote. Definition of type I and type II photosensitized oxidation. *Photochem. Photobiol.* **54**:659, 1991 (1991).
17. D. E. Moore. Drug-induced cutaneous photosensitivity: incidence, mechanism, prevention and management. *Drug Saf.* **25**:345–372 (2002).
18. A. M. Sautereau, C. Tournaire, M. Suares, J. F. Tocanne, and N. Paillous. Interactions of amiodarone with model membranes and amiodarone-photoinduced peroxidation of lipids. *Biochem. Pharmacol.* **43**:2559–2566 (1992).
19. M. Budai, Z. Szabo, A. Zimmer, M. Szogyi, and P. Grof. Studies on molecular interactions between nalidixic acid and liposomes. *Int. J. Pharm.* **279**:67–79 (2004).
20. I. Kraljic and S. E. Mohsni. A new method for the detection of singlet oxygen in aqueous solutions. *Photochem. Photobiol.* **28**:577–581 (1978).

21. C. O. Rangel-Yagui, A. Pessoa Jr., and L. C. Tavares. Micellar solubilization of drugs. *J. Pharm. Pharm. Sci.* **8**:147–165 (2005).
22. M. Monici, N. Mulinacci, P. Baglioni, and F. F. Vincieri. Flavone photoreactivity. UV-induced reactions in organic solvents and micellar systems. *J. Photochem. Photobiol. B* **20**:167–172 (1993).
23. J. E. Weinstein and K. D. Polk. Phototoxicity of anthracene and pyrene to glochidia of the freshwater mussel Utterbackia imbecillis. *Environ. Toxicol. Chem.* **20**:2021–2028 (2001).
24. S. Encinas, F. Bosca, and M. A. Miranda. Phototoxicity associated with diclofenac: a photophysical, photochemical, and photobiological study on the drug and its photoproducts. *Chem. Res. Toxicol.* **11**:946–952 (1998).
25. G. P. Thami, S. Kaur, and A. J. Kanwar. Delayed reactivation of haloperidol induced photosensitive dermatitis by methotrexate. *Postgrad. Med. J.* **78**:116–117 (2002).
26. J. Jagger. *Solar-UV actions on living cells*, Praeger Scientific, New York, 1985.
27. Y. N. Abdiche and D. G. Myszka. Probing the mechanism of drug/lipid membrane interactions using Biacore. *Anal. Biochem.* **328**:233–243 (2004).
28. S. Kobayashi, K. Takeshima, C. B. Park, S. C. Kim, and K. Matsuzaki. Interactions of the novel antimicrobial peptide buforin 2 with lipid bilayers: proline as a translocation promoting factor. *Biochemistry* **39**:8648–8654 (2000).
29. P. W. Wertz. Epidermal lipids. *Semin. Dermatol.* **11**:106–113 (1992).
30. H. J. Yardley and R. Summerly. Lipid composition and metabolism in normal and diseased epidermis. *Pharmacol. Ther.* **13**:357–383 (1981).
31. M. Fresta, S. Guccione, A. R. Beccari, P. M. Furneri, and G. Puglisi. Combining molecular modeling with experimental methodologies: mechanism of membrane permeation and accumulation of ofloxacin. *Bioorg. Med. Chem.* **10**:3871–3889 (2002).
32. J. P. Martin Jr., K. Colina, and N. Logsdon. Role of oxygen radicals in the phototoxicity of tetracyclines toward Escherichia coli B. *J. Bacteriol.* **169**:2516–2522 (1987).
33. K. Hirakawa, S. Kawanishi, and T. Hirano. The mechanism of guanine specific photooxidation in the presence of berberine and palmatine: activation of photosensitized singlet oxygen generation through DNA-binding interaction. *Chem. Res. Toxicol.* **18**:1545–1552 (2005).