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Alterations in plasma membrane of glioblastoma cells by photodynamic action of merocyanine 540

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Abstract

Photodynamic action of merocyanine 540 (MC540) on the plasma membrane of human glioblastoma(U-87MG) cells has been investigated. Plasma membrane was labeled with lipid specific probe 1,(4-trimethylammonium),6-diphenyl-1,3,5-hexatriene. Steady-state anisotropy, decay time and time-dependent anisotropy of TMA-DPH in U-87MG cells have been measured as a function of light dose. A decrease in the steady-state anisotropy and decay time of TMA-DPH in MC540-treated cells was observed upon light irradiation. The time-dependent anisotropy measurements showed a decrease in the limiting anisotropy (r) and an increase in the rotational relaxation time (ϕ) of the probe upon photosensitization of cells. Analysis of these data using wobbling in cone model for probe rotation in the membrane indicated an increase in the cone angle (θ_c) and a decrease in the order parameter (S). Protein specific probe N-(1-pyrene)-maleimide was used to study the effect of photosensitization on the plasma membrane proteins. An increase in the rotational relaxation time and a decrease in the ratio of excimer to monomer fluorescence intensity of PM was observed on photosensitization. Photodynamic action of MC540 also caused an inhibition of protein SH groups and Na^+-K^+-ATPase activity of plasma membrane. Our results demonstrate that the photodynamic action of MC540 decreases the order of the lipid bilayer and reduces the mobility of the proteins in the plasma membrane of cells.

Keywords: Membrane dynamics; Fluorescence spectroscopy; Photosensitization; Protein mobility; Lipid-protein interaction

1. Introduction

Photosensitizing ability of MC540 is being exploited in autologous bone marrow purging and sterilization of blood as MC540 binds preferentially to the tumor cells, enveloped viruses, virally and malarially infected cells [1–4]. Photosensitization of cells by MC540 induces a number of biochemical processes which may lead to structural and functional damage to different cellular sites and consequently causes cell death. Cell membrane has been proposed to be an important site for photodynamic cellular damage by MC540. Earlier studies on liposomes, erythrocyte ghosts and leukemic cells have shown that the photosensitization causes lipid peroxidation and inactivation of plasma membrane enzymes [5–10]. Plasma membrane-bound enzymes were found highly susceptible but intracellular enzymes were not affected on photosensitization [7]. The membrane-bound enzymes

Abbreviations: MC540, merocyanine 540; EMEM, Eagle's minimum essential medium; FCS, foetal calf serum; BS, bovine serum; PBS, phosphate-buffered saline; DTNB, 5-5′dithio-2-bis nitrobenzoic acid; ATP, adenosine-5′-triphosphate; TMA-DPH, 1, (4-trimethylammonium),6-diphenyl-1,3,5-hexatriene; PM, N-(1-pyrene)maleimide; TCA, trichloroacetic acid.

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were found to be more photosensitive than the solubilized forms of the same enzymes. Previous studies on MC540 photosensitization were carried out mostly either in model membranes or in leukemic cells. Although structural alterations induced by photodynamic action of MC540 in erythrocyte membrane have been reported [8], very little is known about such changes in intact cells. Recently, it has been shown that MC540 binds to and photosensitizes glioma cells [11,12]. The present study was undertaken to investigate the photoinduced structural alterations in the plasma membrane of glioblastoma (U-87MG) cells using lipid- and protein-specific fluorescent probes.

2. Materials and methods

2.1. Chemicals

Merocyanine 540 (MC540), sodium pyruvate, 5-5′dithio-2-bis nitrobenzoic acid (DTNB), ouabain, adenosine 5′-triphosphate (ATP), were obtained from Sigma Chemical Co., St. Louis, MO (USA). Dispase was obtained from Boheringer Mannheim (Germany). Eagle's minimum essential medium (EMEM) was purchased from Hi-Media, Bombay (India). Foetal calf serum (FCS) was procured from Northumbria Biological Ltd., Cramlington (UK). Fluorescent probes 1,(4-trimethylammonium),6-diphenyl-1,3,5-hexatrine (TMA-DPH) and N-(1-pyrene)maleimide (PM) were obtained from Molecular Probes Inc. Eugene, OR (USA). Bovine serum (BS) was prepared in the laboratory. All other chemicals were obtained from commercial sources.

2.2. Cell culture

U-87MG cells obtained from American Type Culture Collection (ATCC) were grown in Nunc plastic tissue culture flasks. The cells were maintained at 37°C in EMEM supplemented with 1 mM sodium pyruvate, 5% FCS, 5% BS, 2.2 g/l Hepes, 50000 units/l benzyl penicillin, 3500 units/l streptomycin and 2.2 mg/l nystatin. Cells were routinely subcultured after 96 h; however, the growth medium was changed at 72 h of growth and the experiments were performed at 120 h.

2.3. MC540 treatment

The cells in monolayer were incubated with a specified quantity of MC540 in EMEM containing 1% serum for 1 h at 37°C. Cell monolayer was then washed with PBS (pH 7.4) to remove extracellular MC540. Cells were released with dispase and resuspended in PBS containing 5 mM glucose for further experiments. After addition of MC540, all procedures were performed in the dark.

2.4. Light exposure

Cells were irradiated using two 40-W cool daylight fluorescent tubes (Phillips, India) covered with a perspex diffuser sheet. This source has its major emission in the wavelength range 400 to 700 nm. The fluence rate at the position of cells was about 1 W/m^2 as measured by Kyoritsu Illuminometer Model 5200 (Kyoritsu, Electrical Instruments, Tokyo, Japan).

2.5. Labeling of cells with fluorophores

Cells were loaded with TMA-DPH as described by Petty et al. [13]. Briefly, the stock solution (500 μM) of TMA-DPH in dimethylformamide was diluted 250-fold by adding the same to PBS containing 5 mM glucose and the solution was stirred continuously for 1 h. The known amount of this solution was added to a specified volume of cell suspension (2.0×10^6 cells/ml) for fluorescence measurements. The concentration of TMA-DPH was 1 μM and the cell density was 1×10^6 cells/ml during the fluorescence measurements.

Cells were labeled with PM as described by Papp et al. [14]. Appropriate volume of PM stock solution in acetone was added to the cell suspension (2×10^6 cells/ml) to get a final concentration of 15 μM. The cell suspension was incubated with PM for 30 min in a shaker water bath at 25°C. The cells were washed to remove the unreacted PM and were resuspended in PBS containing 5 mM glucose at a density of 1×10^6 cells/ml for fluorescence measurements.

2.6. Fluoresence spectra and steady-state anisotropy measurements

The fluorescence spectra and steady-state anisotropy measurements were made using SLM 8000C spec-

trofluorometer. The excitation wavelength was 337 nm, whereas the emission was monitored at 415 and 396 nm for TMA-DPH and PM respectively. The bandwidths of excitation and emission monochromators were 2 and 4 nm respectively. Glan Thompson calcite prism polarizers were placed in the excitation and emission paths for the measurements of anisotropy. For steady-state anisotropy measurements, samples were excited with vertically polarized light. Vertically (I_{vv}) and horizontally (I_{vh}) polarized fluorescence intensities were measured and the anisotropy was calculated using the relationship [15]

$$r = I_{vv} - GI_{vh}/I_{vv} + 2GI_{vh} \quad (1)$$

where G is the correction factor given by $G = I_{hv}/I_{hh}$. G was determined by measuring the fluorescence intensities I_{hv} and I_{hh} using the horizontally polarized exciting light. MC540-treated cells with and without light irradiation but not labeled with fluorescent probes were used as blanks for dark control and irradiated samples respectively. The blank values corresponding to I_{vv}, I_{vh}, I_{hv} and I_{hh} fluorescence intensities were subtracted before calculating the anisotropy (r).

2.7. Decay time and time-dependent anisotropy measurements

Edinburgh CD 900 time-resolved spectrofluorometer, which utilizes single photon counting method, was used to measure the decay of fluorescence intensity and anisotropy. The light source was N_2 discharge lamp operated at a pressure of 1 bar. The excitation and emission wavelengths for both TMA-DPH and PM were the same as used in the steady-state anisotropy measurements. The bandwidth for both the excitation and emission monochromators was 4 nm. The data were collected in 1024 channels using a multichannel analyzer (MCA). In each experiment the data were acquired to give the peak counts of about 5000. For each sample decay curve, a corresponding lamp profile using a scattering solution was collected. The decay of fluorescence anisotropy was measured by incorporating the Glan Thompson calcite prism polarizers in the excitation and emission monochromators. Samples were excited with vertically polarized light. The decay of vertically (I_{vv}(t)) and horizontally (I_{vh}(t)) polarized fluorescence intensities was measured by acquiring data alternately in two memory segments of MCA. The toggling of the emission polarizer optics between vertical and horizontal positions with simultaneous change in the memory segment in MCA was automatically controlled with a dwell time of 60 s. The decay of anisotropy was generated from the measured time-resolved decay of I_{vv}(t) and I_{vh}(t) using Eq. (1).

2.8. Analysis of fluorescence intensity and anisotropy decay

The decay of fluorescence intensity is represented by a sum of multi-exponentials such as $I(t) = I_0 \sum_i e^{-t/\tau_i}$ which was analyzed by non-linear least square deconvolution procedure [15]. The goodness of fit was judged by χ^2 and residuals. The fractional intensity (f_i) of each component of decay is given by $f_i = \frac{\alpha_i \tau_i}{\sum_i \alpha_i \tau_i}$ where α is preexponential factor and τ is the decay time.

The decay of the fluorescence anisotropy of fluorophore in the membrane has been explained in terms of wobbling in cone model [16–18]. According to this model, the fluorophore is considered to be in a hindered environment and its orientational motion in the membrane is described by the wobbling confined within a cone around the normal of the membrane. Both the rate and range of the rotational motions, which give dynamic and static information respectively about the mobility of the probe, can be determined. The rotational relaxation time (ϕ) refers to the rotational diffusion of the probe, whereas the range of the rotational motions is expressed by the cone angle (θ_c). The decay of the fluorescence anisotropy of fluorophore in the hindered environment like membrane is represented by

$$r(t) = (r_o - r_\infty)e^{-t/\phi} + r_\infty \quad (2)$$

where r_o is the anisotropy at $t = 0$, r_∞ is the limiting anisotropy and ϕ is the rotational relaxation time for the wobbling diffusion within the cone. The relationship between the cone angle and limiting anisotropy is given by:

$$r_\infty / r_o = 1/4\left[Cos\,\theta_c(1 + Cos\,\theta_c)\right]^2 \quad (3)$$

The limiting anisotropy is related to the lipid order parameter (S) by equation

$$r/r_o = S^2 \quad (4)$$

2.9. Determination of SH groups

Membrane SH groups were estimated by the method of Ellman [19]. Cell suspension (1.0×10^6 cells/ml) was incubated with DTNB (0.4 mM in each assay) at room temperature. After 30 min of incubation cell suspension was centrifuged at 1500 rpm for 10 min. Absorbance of the supernatant was measured at 412 nm. Concentration of SH groups was calculated using extinction coefficient $\epsilon = 1.36 \times 10^4$ M^{-1} cm^{-1} at 412 nm.

2.10. Measurement of Na^+-K^+-ATPase activity

Na^+-K^+-ATPase activity of cell homogenate was measured by the method of Deliconstantinos et al. [20] with some modifications. Briefly, the cell homogenate (150 μg protein) was suspended in buffer (100 mM NaCl, 30 mM KCl, 3 mM $MgCl_2$, pH 7.3) with or without ouabain and was incubated at 37°C for 10 min. The reaction was initiated by the addition of ATP (3 mM). Chilled TCA (10%) was added to stop the reaction. After 10 min of standing at ice temperature, the mixture was centrifuged at 2000 rpm. Supernatant was used for colour development to estimate the liberated P_i according to the Fiske and Subbarow method [21]. Na^+-K^+-ATPase activity was calculated as the difference between the P_i liberated in the presence and absence of ouabain.

Each experiment was repeated three to five times and the data reported are the mean ± S.E. of three experiments.

3. Results

3.1. Steady-state anisotropy of TMA-DPH

The fluorescence anisotropy of TMA-DPH in cells was found to be 0.258 ± 0.001. MC540 treatment in the dark for 1 h did not cause any appreciable change in the anisotropy of TMA-DPH in cells. However, cells treated with MC540 and irradiated with light showed a decrease in the anisotropy of TMA-DPH. The change in anisotropy as a function of light dose is depicted in Fig. 1. Cells irradiated for 60 min. exhibited a decrease of 15% as compared to MC540 treated dark controls.

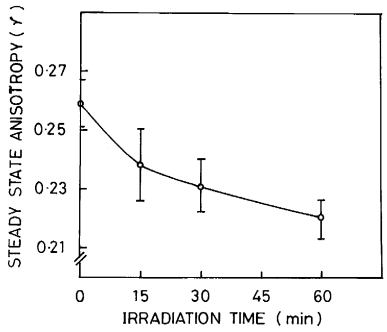

Fig. 1. Steady-state anisotropy of TMA-DPH-labeled cells as a function of irradiation time. Cells were incubated with 15 μg/ml MC540 for 1 h and irradiated for 15, 30 and 60 min. Each data point is a mean ± S.E. from three experiments.

3.2. Decay time of TMA-DPH

The decrease in the steady-state anisotropy of TMA-DPH in cells may arise due to the alterations in the mobility of lipids or due to an increase in the life-time of the fluorophore. To elucidate this, fluorescence decay times were measured as a function of irradiation time and the data are given in Table 1. The decay curve of TMA-DPH in cells was double

Table 1
Fluorescence decay times of TMA-DPH in U-87MG cells

Sample	Decay time (ns)/ (fractional contribution)	
	τ_1/f_1	τ_2/f_2
Cells + MC540	3.02 ± 0.16	8.05 ± 0.29
	(0.29 ± 0.07)	(0.71 ± 0.07)
Cells + MC540 + light (15 min)	2.69 ± 0.11	7.10 ± 0.36
	(0.29 ± 0.02)	(0.71 ± 0.02)
Cells + MC540 + light (30 min)	2.50 ± 0.07	6.56 ± 0.15
	(0.29 ± 0.01)	(0.71 ± 0.01)
Cells + MC540 + light (60 min)	2.41 ± 0.09 *	6.01 ± 0.09 **
	(0.36 ± 0.03)	(0.64 ± 0.03)

Cells were exposed to 15 μg/ml MC540 for 1 h and irradiated with light for different durations. Each data point is a mean ± S.E. from three experiments.
* Significantly different at $P < 0.005$ from dark controls
** Significantly (different at $P < 0.0025$ from dark controls

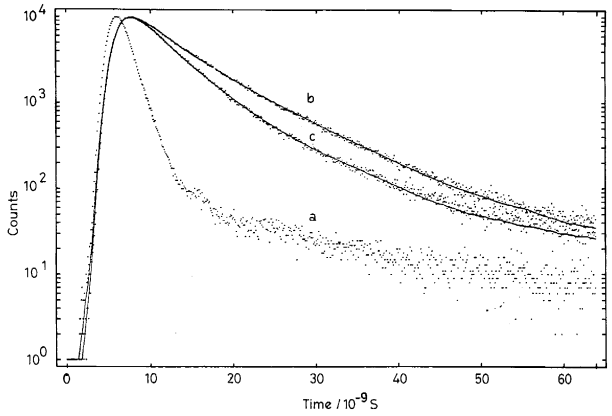

Fig. 2. Fluorescence decay curves of TMA-DPH labeled cells. (a) Lamp profile, (b) MC540-treated cells, (c) MC540-treated and irradiated (60 min) cells. Dots represent the experimental data points and solid line is fitted decay curve. Cells were treated with 15 μg/ml MC540 for 1 h and irradiated for 60 min. TMA-DPH labeling was done as described in Section 2.

exponential. A decrease in the decay time without a significant change in fractional contribution was observed on irradiation of cells; however, the decay curve remained double exponential. A comparison of the decay curve of TMA-DPH in MC540-treated cells in the dark and upon light irradiation is shown in Fig. 2. Photoirradiation of cells reduced the lifetime of TMA-DPH; therefore, the observed decrease in steady-state anisotropy on light irradiation reflects the change in the mobility of the probe in photosensitized cells. The decrease in the decay time could be due to the change in the hydrophobicity of the membrane core.

3.3. Time-dependent anisotropy of TMA-DPH

Analysis of the anisotropy decay curve, using Eq. (2), showed a best fit for a single exponential decay. A decrease in the limiting anisotropy and an increase in the rotational correlation time was observed on irradiation of MC540 treated cells. Order parameter (S) and cone angle (θ_c) were determined from limiting anisotropy (r) using Eq. (3) and Eq. (4)) as mentioned earlier. Irradiation of cells caused a decrease in the order parameter and an increase in the cone angle (Table 2). These data indicate that the mobility of membrane lipids in MC540-treated cells increases on light irradiation.

3.4. Protein SH groups

The photoinduced chemical changes in the plasma membrane proteins were examined by measuring the protein SH groups using DTNB. Fig. 3A and 3B show the effect of MC540 concentration and light dose on membrane SH groups. The cells incubated with different concentrations of MC540 in the dark did not show any appreciable change in DTNB-reactive SH groups. This clearly shows that MC540 does not bind to protein SH groups. It is interesting to note that the cells treated with a fixed concentration of MC540 showed a decrease in DTNB reactive SH groups upon light irradiation (Fig. 3B). The cells irradiated for 15 min exhibited a 37.5% decrease in SH groups; however, with the increase in light dose no further inhibition in DTNB reactive groups was observed.

3.5. Fluorescence spectra and excimer to monomer ratio of PM

The effect of light irradiation on protein SH groups was also studied using a SH-group specific fluorescent probe PM. Fluorescence spectrum of PM bound

Table 2
Limiting anisotropy (r), rotational relaxational time (ϕ), order parameter (S) and cone angle (θ_c) of TMA-DPH in U-87MG cells

Sample	r	ϕ (ns)	S	θ_c
Cells + MC540	0.225 ± 0.006	1.21 ± 0.15	0.757 ± 0.009	34.09 ± 0.71
Cells + MC540 + light (15 min)	0.206 ± 0.01	3.06 ± 0.53	0.725 ± 0.02	36.50 ± 1.60
Cells + MC540 + light (30 min)	0.195 ± 0.007	2.92 ± 0.26	0.705 ± 0.013	37.94 ± 0.96
Cells + MC540 + light (60 min)	0.185 ± 0.017 $^{+}$	4.46 ± 0.75 *	0.686 ± 0.033	39.25 ± 2.45 $^{+}$

Cells were treated with 15 μg/ml of MC540 for 1 h in dark and irradiated with light for different durations. Each data point is a mean ± S.E. from three experiments.
$^{+}$ Significantly different than dark control at $P < 0.0005$
* Significantly different than dark control at $P < 0.005$

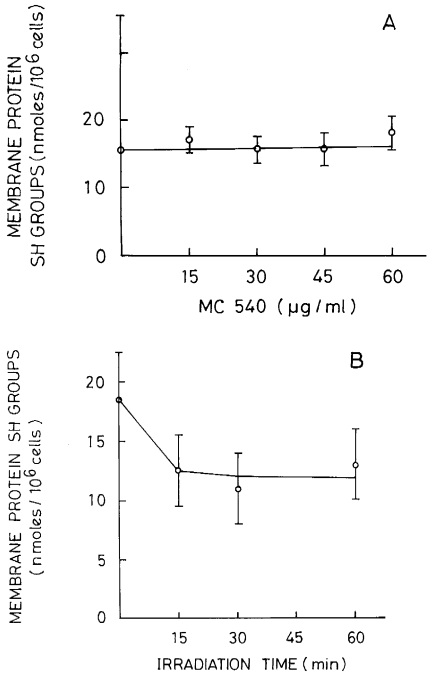

Fig. 3. Membrane SH groups in nmol/10^6 cells as a function of (A) MC540 concentration and (B) time of irradiation. Cells were incubated with MC540 for 1 h in dark and irradiated with light. SH groups were measured using DTNB as described in Section 2. Each data point is a mean ± S.E. from three experiments.

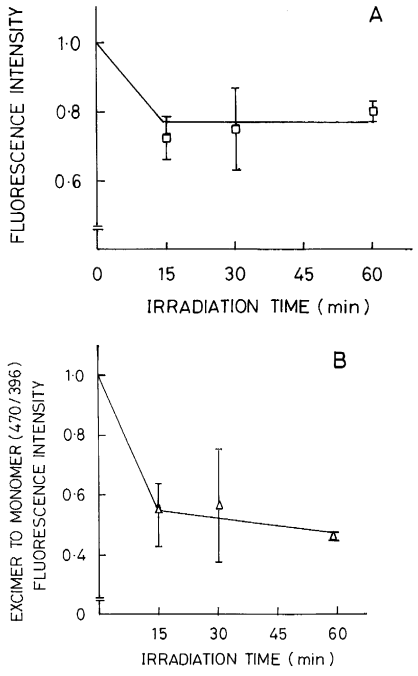

Fig. 4. Relative change in (A) total fluorescence intensity of PM and (B) ratio of excimer to monomer fluorescence intensity of PM as a function of time of irradiation. Cells were treated with 15 μg/ml MC540 for 1 h in dark before irradiation with different doses of light. Each data point is a mean ± S.E. from three experiments.

to cells treated with MC540 exhibited three fluorescence bands at 375, 395 and 415 nm which have been attributed to monomer and a broad shoulder at 470 nm is due to excimer fluorescence. A decrease in the total fluorescence intensity of PM was observed on light irradiation of MC540-treated cells; however, the position of the fluorescence bands was unaffected. A decrease in the ratio of excimer to monomer fluorescence intensity was observed on irradiating the MC540-treated cells. Fig. 4A and B shows the change in the total fluorescence intensity and the ratio of excimer to monomer fluorescence intensity of PM for different doses of light.

3.6. Decay time and time-dependent anisotropy of PM

The decay curve of PM in MC540-treated cells gave a best fit for a triple exponential decay. The lifetimes obtained were 2.95 ± 0.11, 24.81 ± 3.12 and 93.74 ± 4.69 ns with fractional contributions of 0.11, 0.25 and 0.64 respectively. These values were com-

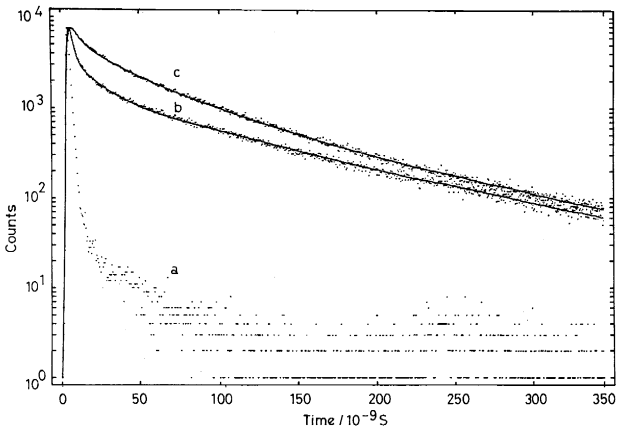

Fig. 5. Fluorescence decay curves of PM labeled cells. (a) Lamp profile (b) MC540-treated cells (c) MC540-treated and irradiated (60 min) cells. Dots represent the experimental data points and solid line is fitted decay curve. Cells were treated with 15 μg/ml MC540 in the dark before irradiation. PM Labeling was done as described in Section 2.

parable with MC540-untreated controls. On light irradiation an increase was observed in the decay time of shorter component while no significant change was observed in the longer components as shown in Fig. 5. Increasing light dose did not cause a significant change in the fractional contributions (Table 3). For the decay of anisotropy the best fit was obtained for a single exponential decay. The rotational correlation time of PM in cells treated with MC540 in the dark was found to be 40.75 ± 4.0 ns. As shown in Table 4 an increase in the rotational correlation time was observed in MC540-treated and light-irradiated cells. These results suggest that photosensitization of cells by MC540 decreases the mobility of plasma membrane proteins.

3.7. Na^+-K^+-ATPase activity

So far, we have shown that the photodynamic action of MC540 on cells causes structural alterations in the plasma membrane proteins. Na^+-K^+-ATPase activity was measured to examine the functional changes caused by photosensitization of cells. Na^+K^+-ATPase activity of cells treated with MC540 ($15\ \mu g/ml$) in the dark did not show any change as compared to untreated controls. The specific activities were 38.75 ± 6.73 and 41.4 ± 3.67 nmol/mg protein/min for MC540-treated and untreated cells respectively. Fig. 6 shows the effect of light irradiation on the enzyme activity in MC540-treated cells. Upon irradiation with low light dose the enzyme activity showed an increase but with higher light dose an inhibition in the enzyme activity was observed.

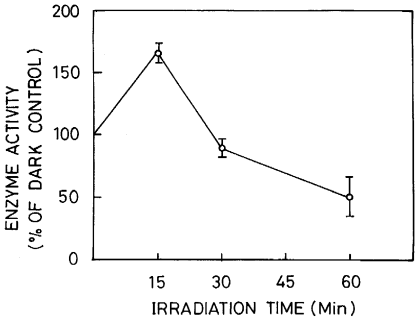

Fig. 6. Na^+-K^+-ATPase activity of U-87MG cells as a function of time of irradiation. Cells were incubated with 15 $\mu g/ml$ MC540 in dark before irradiation with different doses of light. Na^+-K^+-ATPase activity was measured as described in Section 2.

Table 3
Fluorescence decay time of PM in U-87MG cells treated with MC540 with and without irradiation with light

Sample	Decay time (ns)/(fractional contribution)		
	τ_1/f_1	τ_2/f_2	τ_3/f_3
Cells + MC540	2.95 ± 0.11	24.81 ± 3.12	93.74 ± 4.69
	(0.11 ± 0.06)	(0.25 ± 0.05)	(0.64 ± 0.07)
Cells + MC540 + light (15 min)	5.38 ± 0.20	28.80 ± 0.38	91.82 ± 6.18
	(0.09 ± 0.01)	(0.30 ± 0.03)	(0.61 ± 0.03)
Cells + MC540 + light (30 min)	4.62 ± 0.11	26.41 ± 6.80	90.99 ± 1.18
	(0.06 ± 0.01)	(0.26 ± 0.01)	(0.68 ± 0.02)
Cells + MC540 + light (60 min)	5.71 ± 0.73 *	28.74 ± 3.00	85.64 ± 2.92
	(0.10 ± 0.03)	(0.32 ± 0.04)	(0.58 ± 0.02)

Cells were treated with 15 $\mu g/ml$ MC540 for 1 h before irradiation with light. Each data point is a mean ± S.E. from three experiments.
* Significantly different than dark control at $P < 0.005$

Table 4
Time-dependent anisotropy parameters of PM in U-87MG cells treated with MC540

Sample	Cells + MC540	Cells + MC540 + light (15 min)	Cells + MC540 + light (30 min)	Cells + MC540 + light (60 min)
Rotational relaxation time (ns)	40.75 ± 4.01	74.00 ± 7.07	76.66 ± 5.77	79.99 ± 1.01

Cells were treated with 15 $\mu g/ml$ MC540 for 1 h before irradiation with light. Each data point is a mean ± S.E. from three experiments. Significantly different than dark control at $P < 0.0005$.

4. Discussion

In the present study, we have utilized the fluorescence methods to detect the structural changes in the plasma membrane of cells after photosensitization by MC540. The results presented here demonstrate that the fluorescence characteristics of the lipid and protein probes were altered when MC540-treated cells were irradiated with light, suggesting that the photosensitization of cells alters the structural organization of plasma membrane. The decrease in the steady-state anisotropy, limiting anisotropy and order parameter as well as an increase in the cone angle implies a less hindered motion of TMA-DPH molecules in the membranes of photosensitized cells. An increase in the rotational relaxation time is probably due to the increased cone angle, since the time required for the equilibrium in a larger angle will be more [22]. These results reflect a decrease in the plasma membrane rigidity upon photosensitization of cells. Contrary to this finding, previously we have reported that the photosensitization of U-87MG cells by HpD results in an increase in plasma membrane rigidity [23]. This dissimilarity may be due to the differences in the localization of HpD and MC540 in the cell membrane. It has been proposed earlier that HpD binds to the proteins [24,25], whereas MC540 localizes in the cholesterol free lipid domains of the cell membrane [26]. The dissimilarities in the photosensitization of P388 cells by rose bengal and MC540 have also been suggested due to the differential localization of these dyes in the cell membrane [27].

The decrease in the order parameter upon photosensitization of cells may arise due to the formation of lipid peroxides. We have shown earlier that photosensitization of U-87MG cells by MC540 produces a significant amount of lipid peroxidation [28]. The formation of lipid peroxides have been shown to perturb the membrane order [29–31]. The generation and accumulation of lipid peroxides within the hydrophobic core of the lipid bilayer would produce the polar environment which may disturb the packing of the phospholipid acyl chains. Wratten et al. [31] have observed a decrease in the molecular order in the unsaturated fatty acid liposomes on incorporating small amount of lipid peroxides. The physical arrangement of the phospholipids in the membrane depends on the intrinsic shape of the individual lipid molecules. Presence of hydroperoxy or alcohol moeity in lipid acyl chain may change the shape of the phospholipid molecule in such a way that it no longer packs correctly in the bilayer, resulting in the decrease of the molecular order in the membrane. Our results on decrease in membrane order on MC540-mediated photosensitization are in contrast to the results of Feix et al. [8]. This inconsistency could be due to the differences in the experimental conditions. These authors have used isolated erythrocyte membranes; on the other hand, the present study deals with the intact glioma cells. Earlier studies have shown that the effect of lipid peroxidation on the membrane order may not be the same in isolated membranes and intact cells [30].

MC540 treatment in the dark does not inhibit SH groups, which indicates that the dye does not bind to sulfhydryl groups of membrane proteins. The decrease in the SH groups on combined treatment of MC540 and light could be due to the photooxidation of SH groups in the proteins. The loss of SH groups on light irradiation as evidenced by the decrease in total fluorescence intensity of PM in cells and a decrease in the excimer to monomer fluorescence intensity ratio of PM in cells, suggest the cross-linking of membrane proteins. The photoinduced cross-linking of proteins is also manifested as the increase in the rotational relaxation time of PM in cells as a result of light irradiation. Such cross-linking may take place between protein SH groups at close proximity.

Our results on increase in the fluidity of plasma membrane lipid bilayer and decrease in protein mobility due to MC540-mediated photosensitization of cells can be explained on the basis of changes in the lipid–protein interaction in the cell membrane as suggested earlier [32]. It is known that the fluidity of the lipid matrix plays an important role in controlling the dynamic features of the membrane proteins. Membrane proteins are always in a state of equilibrium between the interaction with the lipids and with the aqueous surroundings. Upon decreasing the order of the lipid bilayer, the lipid–lipid interaction would decrease and the interaction of the protein hydrophobic part with the lipid surroundings will increase. As a result of this the proteins may be squeezed in and occupy a new equilibrium position with the decreased mobility. It is also possible that the perturbations in

the lipid–protein interaction lead to altered conformation of the protein which imposes more restrictions on the mobility of the PM-labeled segments. The structural changes in the cell membrane as a result of photosensitization are manifested as the changes in the functional activities of the membrane-bound enzyme Na^+-K^+-ATPase. In summary, the photosensitization of U-87MG cells by MC540 caused an increase in plasma membrane fluidity and a decrease in protein mobility which may be due to the change in lipid–protein interactions.

Acknowledgements

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