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MODEL OF DAMAGE TO CELL MEMBRANES DURING PHOTODYNAMIC THERAPY: PHOTOSENSITIZATION OF PLANAR LIPID BILAYER BY HEMATOPORPHYRIN DIMETHYL ESTER

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The use of photosensitizers, with the ability to accumulate selectively in the tissues of a tumor and to destroy it during illumination, is a promising method of treatment of malignant neoplasms [1, 3, 6]. Hematoporphyrin derivatives possess such properties. It has been suggested that one of the main factors in phototoxic action is damage to cell membranes [7], but a detailed investigation of these processes in vivo is limited by the narrow range of conditions within which the viability of the experimental object is not disturbed. The use of model membrane systems offers wider opportunities. Such investigations have been conducted on suspensions of liposomes [4, 5], but this system can be used to record only averaged effects. To study the mechanism of formation of single membrane defects during photodynamic therapy, it is interesting to model this process on a planar bilayer lipid membrane (BLM).

EXPERIMENTAL METHOD

A BLM was formed from 50 mg/ml of a solution of L-α-phosphatidylcholine ("Sigma," USA; type II-S) in decane (chemically pure, from Reakhim) on holes 0.8 mm in diameter in a sheet of Teflon gauze. The electrical characteristics were recorded by means of Ag-AgCl electrodes. Conductivity was measured by applying a constant voltage of 200 mV to the BLM,

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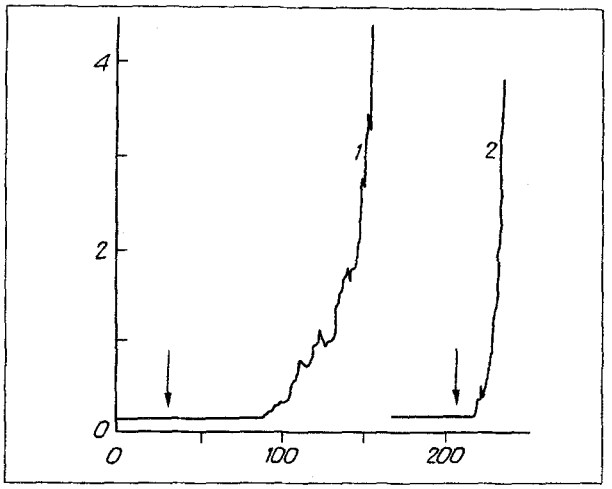

Fig. 1. Changes in conductivity of planar bilayer lipid membranes during illumination in the presence of hematoporphyrin dimethyl ester. Abscissa, time (in sec); ordinate, conductivity, $\times 10^7$ (in $\Omega^{-1} \cdot cm^{-2}$). 1) 1st Membrane; 2) 2nd membrane, formed in same cuvette immediately after destruction of 1st membrane. Trace ends with rupture of membranes. Beginning of illumination indicated by arrows.

and under these circumstances the current was recorded by means of a "Keithley-427" amplifier (USA), with amplification factor of 10^{10} V/A and temporal resolution of 300 msec on an N-306 automatic writer. The difference between the boundary potentials of the membrane was measured by compensation of the intramembranous field, recorded as the second harmonic of the capacitive current [2]. The BLM was illuminated by focused white light from a "Lyumen-3M" source (Institute of Chemical Physics, Academy of Sciences of the USSR), with 150-W xenon lamp, through glass and water filtered, and with an intensity of 200 mW/cm^2. Experiments were carried out in a medium consisting of 100 mM KCl (high purity, Reakhim), 10 mM phosphate buffer (chemically pure, Reakhim), pH 6.7. The dimethyl ester of hematoporphyrin (DMHP) was synthesized and generously provided by A. F. Mironov (Moscow Institute of Fine Chemical Technology). The solutions were prepared in twice-distilled water. All experiments were carried out at room temperature.

EXPERIMENTAL RESULTS

Illumination of BLM in the presence of DMHP caused an increase of conductivity ending with rupture of the membrane (Fig. 1). The increase of conductivity began after a short time (induction period), the duration of which depended on the experimental conditions. If the concentration of DMHP was 2 μM (in both compartments) the duration of the induction period was about 1 min. The duration of the induction period was increased to 2-3 min during illumination through a yellow filter (a 10 mM solution of potassium ferricyanide in a cuvette 1 cm thick), which reduced the intensity of light in the region of Cope's band by many times. The intensity of mixing of the solution in the cuvette had no effect on the duration of the induction period, but if the experiment was carried out with a new membrane each time, formed after rupture of the previous membrane (in the same cuvette), the duration of the incubation period was shortened (Fig. 1).

In control experiments, in which the BLM was illuminated in the absence of DMHP in the cuvette, or during incubation of BLM in darkness, with previous illumination of the DMHP solution, no increase in conductivity of the BLM was observed.

The results described above suggest that due to interaction of excited DMHP molecules with the planar lipid bilayer (directly or through certain intermediates) substances reducing its stability accumulate in the membrane.

To explain the mechanism of injury to the lipid bilayer experiments were carried out in the presence of a quencher of singlet oxygen, sodium azide (up to 25 mM in aqueous solution) or with continuous blowing of argon above the cuvette containing the BLM. Under these conditions the duration of the induction period was unchanged. This suggests that membrane damage is not connected with the action of singlet oxygen. Addition of the antioxidant ionol to the membrane-forming solution

likewise did not affect the duration of the induction period; injury to BLM photosynthesized by DMHP is probably not connected with lipid peroxidation processes.

To record changes in the boundary potential difference of BLM the photosensitizer was added to only one compartment of the cuvette, and the KCl concentration in the electrolyte was 10 mM in both compartments. No changes in the boundary potential of the membrane were observed during the induction period (with an accuracy of 4 mV). This means that either charged products and substances changing the dipole membrane potential do not accumulate in the system or these products are uniformly distributed on both surfaces of the BLM. To test the second hypothesis, the same experiments were carried out but with an ionic strength gradient: the KCl concentration in the compartment with DMHP was 10 mM but in the opposite compartment 100 mM. In such a system, even with a uniform distribution of charged reaction products, a boundary potential difference ought to appear because of the lower value of the surface potential on the surface with the greater ionic strength. However, even under those conditions, no change was observed in the boundary potential difference of the BLM. This suggests that during photosensitized membrane destruction, no charged particles accumulate.

The suggested system provides a model of injury to cell membranes during photodynamic therapy. However, the field of its possible application is not limited to the study of the dimethyl ester of hematoporphyrin, but it can also be used to study membrane effects of other photosensitizers or for screening dyes for their membrane-toxic action.

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