Department of Biophysics, Comenius University, Bratislava, Slovakia

Peroxidation damage of erythrocyte membranes by merocyanine 540

L. Šikurová, A. Cabánik and G. Ruttkay-Nedecký

Photodynamic therapy (PDT) is a new approach to treatment of cancer and virus diseases using photosensitisers and visible light. A promising photosensitising agent is merocyanine 540 (MC 540) – a water-soluble anionic dye that binds preferentially to fluidlike, cholesterol free domains of the cell membrane. This agent is of great therapeutic interest due to its ability to inactivate under illumination selectively neoplastic cells (e.g. leukemia, lymphoma and neuroblastoma cells) in autologous bone marrow grafts, and also to eradicate pathogenic viruses (Herpes simplex virus, human cytomegalovirus, and Friend erythroleukemia virus) in blood [1, 2]. Plasma membranes are believed to be the primary cellular targets of MC 540-mediated phototoxic effects and lipid peroxidation may be a major process of the phototoxicity [3–5]. The advantage of this sensitiser would be the elimination of leukemia cells and viruses without causing excessive damage to normal pluripotent hematopoietic stem cells. Nevertheless, MC 540 can also be taken up, although in lesser extent [6], by the membrane of normal blood cells, what may result in their damage. Despite the use of MC 540 in medicine, only a few publications are devoted to this problem [7, 8]. Therefore, we concentrate on acquiring data on the consequences of the accumulation of MC 540 in the plasma membrane of the normal human erythrocyte, both in the dark and under illumination.

In the present work membrane damage was assessed using TBA (thiobarbituric acid) reactivity, which signals the occurrence of malonaldehyde and other carbonyl by-products of free radical-mediated peroxidation. Typical changes in thiobarbituric acid reactive substances (TBARS) formation in erythrocyte membranes induced by MC 540 ($2.5–25.0 \times 10^{-6}$ mol/l) and laser light (532 nm, 12.2 J/cm^2) are presented in Fig. 1. Our experiments have shown that application of laser light energy alone on erythrocyte membrane suspensions causes only slight insignificant increase in TBARS (Fig. 1). Thus, peroxidation damage of erythrocyte membranes by green laser illumination alone can be considered negligible.

However, the incubation of erythrocyte membranes with MC-540 in the dark induced a small (Fig. 1) but significant ($p < 0.02$) increase in TBARS formation. Fig. 1 shows that toxicity of MC 540 in the dark is not negligible.

At the same time, simultaneous action of MC 540 and laser illumination induced a dramatic increase in TBARS formation in erythrocyte membranes to levels more than twice as compared to the control sample. By varying MC 540 concentration, we have found a non-linear dependence of TBARS level on MC 540 concentration (Fig. 2). In erythrocyte membrane suspensions, stained by MC 540 and illuminated by 532 nm laser light (12.2 J/cm^2), the increase of TBARS in respect to control reached saturation at MC 540 concentration of 15×10^{-6} mol/l (Fig. 2). This effect can be attributed to a limited number of binding sites for MC 540 in erythrocyte membranes. In this way erythrocyte membranes can be defended against further damage by excessive concentrations of MC 540.

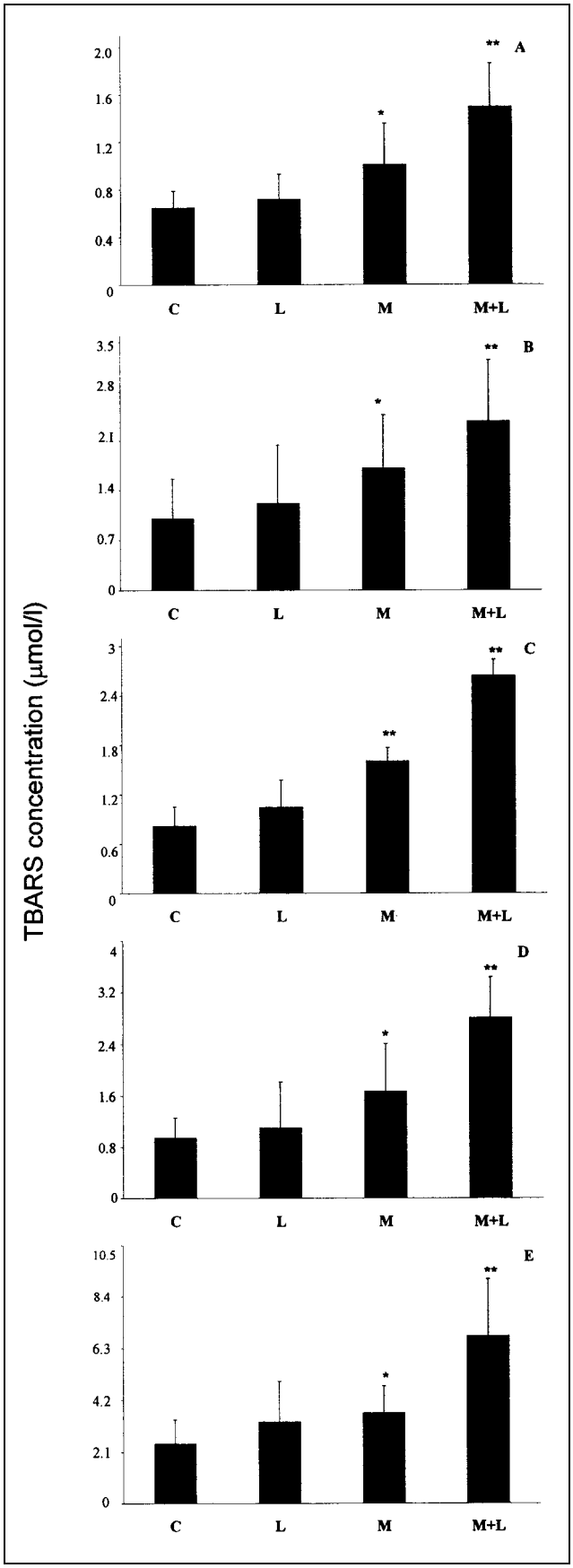

Fig. 1: Effect of MC 540 (A: 2.5 μmol/l; B: 5 μmol/l; C: 10 μmol/l; D: 15 μmol/l; E: 25 μmol/l) and/or light (532 nm; 12.2 J/cm^2) on TBARS formation in erythrocyte membranes. C – Control; L – Light; M – MC 540; M + L – MC 540 and Light. Statistical significances of differences between M or M + L samples and C are indicated by * ($p < 0.02$) and ** ($p < 0.002$), respectively

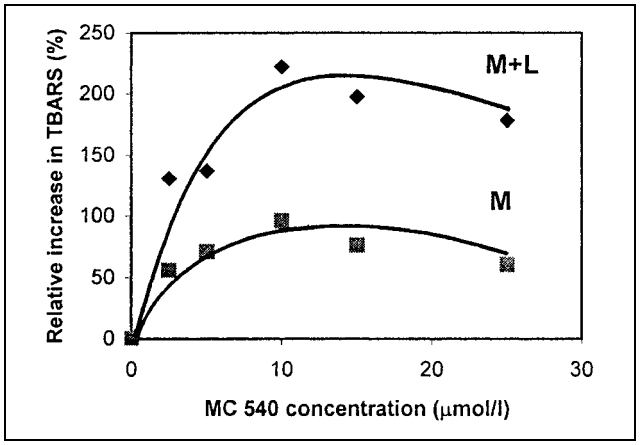

Fig. 2: Relative increase in TBARS formation (in respect to control) in erythrocyte membranes as a function of MC 540 concentration. M – in the dark; M + L – under illumination (532 nm; 12.2 J/cm^2)

In conclusion, peroxidation processes which take place in biological membranes can be escalated to a devastating scale by the photosensitising effects of MC 540 and green laser illumination.

Experimental

Erythrocyte membranes were isolated from human blood of normal (healthy) adult donors according to the standard method of Hanahan and Ekholm [9]. MC 540 from Eastman Kodak Co. was used as a photosensitizing dye in final concentrations ranging from 2.5 to 25×10^{-6} mol/l. The final concentration of erythrocyte membranes (in 10 mM TRIS buffer, pH = 7.4) was 10%. MC 540 was pre-incubated with membranes for 120 min at 21–24 °C in the dark, following 60 min of exposure by a light dose of 12.2 J/cm^2. We used the second harmonic of a Nd:YAG laser (50 mW, 532 nm) from Raise Electronic, as a light source. The lipid peroxide level of erythrocyte membranes was quantitatively assessed in terms of thiobarbituric acid reactive substances (TBARS), using a procedure [4, 10] in which MC540 does not interfere with absorbance measurements. The measurements were carried out at room temperature. Student's paired t-test was applied for testing statistically significant differences in TBARS induced by MC 540 and/or light treatment. The criterion of statistical significance was a p-value less than 0.02.

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Assoc. Prof. Dr. Libuša Šikurová
Dep. of Biophys. Chem. Phys.
Fac. of Mat. Phys.
Comenius Univ.
Mlynská dolina F1
84215 Bratislava
Slovak Republic
sikurova@fmph.uniba.sk