Effects of 1,4-dihydropyridine derivatives on photosensitised cell damage

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Millimolar concentrations of 2,6-dimethyl-1,4-dihydropyridine-3,5-dicarbonic acid (1,4-DHP) derivatives protected human erythrocytes from Al-phthalocyanine tetrasulfonate-sensitized photohaemolysis. The azide-sensitive photooxidation of 1,4-DHP was accompanied by H_2O_2 formation. The 1,4-DHP derivatives studied expressed a low dark toxicity on murine hepatoma MH22 cells *in vitro*. All the derivative studied slightly protected chlorine e_6-sensitized MH22 cells from photodamage, but only one of them downregulated the process of apoptosis to a certain degree.

Key words: photosensitization, 1,4-dihydropyridines, antioxidants, singlet oxygen

INTRODUCTION

In the photodynamic therapy of cancer, the cytotoxic or/and therapeutic effects of photosensitization by haematoporphyrins and metallophthalocyanines arise mainly from the generation of singlet oxygen ($^1O_2$) [1]. It has long been suggested that antioxidants could play a useful role in minimizing the adverse effects of photodynamic reactions, such as skin photosensitization, damage to normal tissues, and erythrocyte lysis. Various groups of antioxidants including thiols, disulfides, flavonoids and polyphenols, which act as $^1O_2$scavengers, may protect from the damaging effect of photosensitized irradiation [2–6]. Among other potentially important but poorly studied groups of compounds, the derivatives of 1,4-dihydropyridine (1,4-DHP) deserve certain interest, in view of their ability to scavenge $^1O_2$ [7]. The effects of 1,4-DHP on cell photosensitization have never been reported. In this paper, we present data on the effects of water-soluble representatives of 1,4-DHP on the photosensitized cell damage.

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MATERIALS AND METHODS

Chemicals

Derivatives of 1,4-DHP (purity >98%) were synthesized at Latvian Institute of Organic Synthesis. Al-phthalocyanine tetrasulfonate and chlorine e_6 were purchased from Porphyrin Products (USA). Tissue culture products were obtained from Biochrom. Culture flasks and plates were from Becton-Dickinson. Other chemicals were from Sigma.

Erythrocyte lysis

The rate of photosensitized lysis of human erythrocytes (2.6×10^6/ml) by 10 μM Al-phthalo-cyanine tetrasulfonate ($\lambda > 590$ nm, 25 W/m^2) and the protective effects of 1,4-DHP derivatives (0.25–1.50 mM) were studied spectrophotometrically as described in our previous studies of polyphenolic antioxidants [6], in 0.01 M K-phosphate (pH 7.0)

containing 0.137 M NaCl, 0.0027 M KCl, 10 mM glucose and 1 mM EDTA, at 25 °C. The oxygen uptake during photosensitized oxidation of 1,4-DHP under the same conditions in the absence of erythrocytes was monitored by a Clark electrode [4].

Cell culture treatment

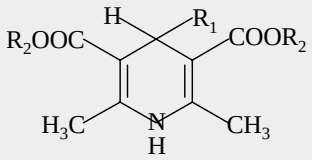
MH22 cells from murine hepatoma were cultivated in DMEM as described in Ref. (8). The cytotoxicity of 1,4-DHP was estimated following 24 h of incubation of cells in the presence of 1,4-DHP in the incubation medium. Cell viability was estimated by MTT assay as described in Ref. (8). For investigation of the effect of 1,4-DHP derivatives on the viability of photosensitized cells, 5 μg/ml of chlorine e_6 in DMEM without serum was added. After incubation in the dark for 11 h, 1,4-DHP were added to the incubation medium. After additional 4 h the cells were exposed for 50 s to light from LED array (660 nm, 10 W/m^2). The ratio of apoptotic and necrotic cells was estimated following 1.5 h of incubation after light exposure as described in Ref. (9). Cell viability was estimated by MTT assay 24 h following light exposure.

RESULTS AND DISCUSSION

The rate of photosensitized oxidation of 1,4-DHP derivatives (compounds **I–III,** Table) by Al-phthalocyanine tetrasulfonate exhibited a linear dependence on the compound concentration range between 0.25–1.5 mM. Sodium azide (3.0 mM) inhibited the reaction by 80–75%. After the complete oxygen consumption, addition of 30 μg/ml of catalase into the reaction mixture caused the return of 50% of oxygen, showing that 1,4-DHP are oxidized by photogenerated $^1O_2$ with the formation of H_2O_2 and evidently of the corresponding pyridines [10]. The decrease in the relative photooxidation rate of 1,4-DHP (**I > II > III**, Table) is caused by the presence of electron-accepting substituents at position 4 of compounds **I, II** (Table). Similarly, such substitution decreases the radical and $^1O_2$ scavenging activity of 1,4-DHP [7,11]. Like polyphenolic antioxidants [6], the 1,4-DHP tested protected human erythrocytes from photohaemolysis. The concentrations for a 2-fold increase in the half time of photosensitized erythrocyte lysis (cI_{50} (erythrocytes)) are listed in Table. Interestingly, there was not clearcut relationship between the protective efficiency and the photooxidation rates of 1,4-DHP (Table). It is necessary to note that the water soluble 1,4-DHP tested, contrary to lipophilic derivatives, do not protect erythrocytes from osmotic and acidic hemolysis [12]. Lipophilic 1,4-DHP are located on the surface of erythrocytes [13]. Thus, water soluble 1,4-DHP **I–III** could play their protective role in the intercellular space.

All the three 1,4-DHP derivatives studied were low-cytotoxic to murine hepatoma MH22 cells *in vitro* in the dark. All of them slightly protected chlorine e_6-photosensitised cells from light-induced death, which is generally accepted to be mediated by $^1O_2$ as well [14]. However, only compound **I** 2–2.5-fold prolonged the progress of apoptosis of the photosensitized cells following light exposure. As compound **I** exhibits a high radical-scavenging activity [15], the effect of this compound on cell death seems to be associated with the influence on apoptosis-driving oxygen radicals.

Table. **Concentrations of 1,4-dihydropyridine derivative for a 10% decrease in MH22 cell viability (ID_{10}) and a 2-fold increase in the half time of photosensitized erythrocyte lysis (cI_{50} (erythrocytes)) and the relative rates of photooxidation of 1 mM of the compound expressed as the percentage of the rate of photooxidation of 1 mM of lipoic acid under identical conditions**



(I) R_1 = H, R_2 = CH_2COO^-

(II) R_1 = COO^-, R_2 = C_2H_5

(III) R_1 = $CONHCH(C_2H_4COO^-)COO^-$, R_2 = C_2H_5

Compound	ID_{10} (MH22 cells), M	cI_{50} (erythrocytes), M	Relative photooxidation rate, %	Relative viability increase of photosensitised cells*
I	$>10^{-3}$	$6 \times 10^{-4} \pm 0.1$	107±10	1.40±0.15
II	10^{-4}	$7 \times 10^{-4} \pm 0.1$	30.5±2.5	1.17±0.20
III	$3x10^{-4}$	$8 \times 10^{-4} \pm 0.1$	8.5±1.0	1.34±0.18

* Chlorine e_6-photosensitized cells were exposed to light in presence of 10^{-4} M of 1,4-DHP derivative. Viability of cells in the absence of the study compounds was assumed to be 1.00.

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1,4-DIHIDROPIRIDINO DARINIŲ POVEIKIS FOTOSENSIBILIZUOTOMS LĄSTELIŲ PAŽAIDOMS

S a n t r a u k a

Milimolinės 2,6-dimetil-1,4-dihidropiridino-3,5-dikarboninės rūgšties darinių koncentracijos apsaugojo žmogaus eritrocitus nuo Al-ftalocianino tetrasulfonatu sensibilizuotos fotohaemolizės. Azidu inhibuojamą 1,4-dihidropiridinų fotooksidaciją lydėjo H_2O_2 susidarymas. Tirti 1,4-dihidropiridino dariniai mažai toksiški pelių hepatomos MH22 ląstelėms *in vitro.* Visi tirti junginiai nedaug apsaugojo ląsteles nuo matomos šviesos sukeltos žūties po fotosensibilizacijos chlorinu e_6, tačiau tik vienas iš jų sulėtino apoptozę.