Bioorganic & Medicinal Chemistry 22 (2014) 4684–4693

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Novel 3,6-bis(imidazolidine)acridines as effective photosensitizers for photodynamic therapy

L. Čižeková [a], A. Grolmusová [a], Z. Ipóthová [a], Z. Barbieriková [b], V. Brezová [b], Ľ. Hunáková [c], J. Imrich [d], L. Janovec [d], I. Dovinová [e], H. Paulíková [a,*]

[a] Department of Biochemistry and Microbiology, Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava, Radlinského 9, 812 37 Bratislava, Slovak Republic
[b] Department of Physical Chemistry, Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava, Radlinského 9, 812 37 Bratislava, Slovak Republic
[c] Cancer Research Institute, Vlárska 7, 83391 Bratislava, Slovak Republic
[d] Department of Organic Chemistry, Pavol Jozef Šafárik University, Moyzesova 11, 041 57 Košice, Slovak Republic
[e] Institute of Normal and Pathological Physiology, Sienkiewiczova 1, 813 71 Bratislava, Slovak Republic

ARTICLE INFO

Article history:
Received 31 March 2014
Revised 1 July 2014
Accepted 9 July 2014
Available online 17 July 2014

Keywords:
Acridine
PDT
Anticancer
Photosensitizers

ABSTRACT

The photoeffect of new proflavine derivatives with DNA-binding and antitumour activities, 3,6-bis ((1-alkyl-5-oxo-imidazolidin-2-yliden)imino)acridine hydrochlorides (AcrDIMs), was studied to evaluate them as potential photosensitizers for photodynamic antitumor therapy. EPR measurements showed that superoxide radical anion and singlet oxygen were produced upon irradiation of AcrDIMs with UV-A light (>300 nm) in the presence of molecular oxygen. This indicates that AcrDIMs may act as photosensitizers. The most active pentyl-AcrDIM and hexyl-AcrDIM displayed photocytotoxic effect toward the mouse lymphocytic leukemia cell line L1210 and human ovarian cancer cells A2780. Antitumor activity of pentyl-AcrDIM increased as high as about 12 times (72 h incubation) after irradiation of A2780 cells (365 nm, 1.05 J/cm^2). The photocytotoxicity seems to be associated with oxidative stress. Concerning the cell cycle, flow cytometry showed an arrest in the *S*-phase already 4 h after irradiation. In a comet assay, no genotoxicity of AcrDIMs was found. Typical morphologic changes and formation of DNA-ladders indicated induction of apoptotic cell death, though no activation of caspase-3 was observed. Investigation of intracellular localization of pentyl-AcrDIM confirmed its partial accumulation in mitochondria and lysosomes. After irradiation of the A2780 cells, colocalization of pentyl-AcrDIM with monodansylcadaverine, a lysosomal dye, was proven, suggesting that lysosomes in the irradiated cells may be involved in the cell death.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Photodynamic therapy (PDT) is a very useful method for treatment of many types of tumors (skin, head, and neck, digestive and urinary system, brain, lung, and intraperitoneal malignancies).[1,2] After irradiation with light of suitable wavelength, particular photosensitizers (PSs) can produce reactive oxygen species (ROS) that may induce the cell death either by apoptosis or necrosis, depending on the type of photosensitizer, light dose, and genotype of cells.[3] PDT has been progressively developing over the last decades and the demand for new photosensitizers, including non-porphyrins, is still growing.

Though acridine derivatives were widely studied in the past as potential anticancer drugs,[4–8] only some of them proved to be interesting for PDT of tumours. Yoshida et al.[9] applied a new combined therapy utilizing acridine orange (AO) for treating periosteal Ewing's sarcoma. They combined a surgical removal of the tumour followed by PDT with AO as photosensitizer and radiodynamic therapy. By comparing accumulation of AO in malignant versus benign musculoskeletal tumours they found that AO was predominantly concentrated in the malignant tumour cells because of a large pH gradient between the intracellular and extracellular pH.[10] Thereafter, a new surgical approach using AO that preserves an excellent limb function in patients with rhabdomyosarcoma was elaborated.[11]

The photo-inducible properties of AO stimulated a search for new acridine derivatives as potential photosensitizers.[12–16] Benchabane et al.[15] investigated cytotoxicity and photo-enhanced cytotoxicity of a series of 3,6-di-substituted acridines. Three derivatives displayed the most important photo-inducible properties, with IC_{50} averaging 0.14–0.57 μM and corresponding to a 100 fold

* Corresponding author. Tel.: +421 59325173; fax: +421 52493198.
E-mail address: helena.paulikova@stuba.sk (H. Paulíková).

http://dx.doi.org/10.1016/j.bmc.2014.07.013
0968-0896/© 2014 Elsevier Ltd. All rights reserved.

increase of their cytotoxicity as compared to experiments performed in the dark. Although these derivatives were far less clastogenic than the parent molecule proflavine, they possessed the weak specificity for tumour cells.

In this field, many new acridine derivatives have been synthesized by our teams to find interesting anticancer drugs.[17–20] Recently, a new group of 3,6-bis((1-alkyl-5-oxo-imidazolidin-2-yliden)imino)acridines (AcrDIMs) has been prepared and investigated in our laboratory.[19] We have confirmed intercalation of these compounds into DNA and inhibition of topoisomerase activity. Studies on different cancer cell lines showed that only derivatives with longer alkyl chains were able to penetrate the cell membrane and affect the cell proliferation.[19]

In this paper, we focused our attention on the study of photocytotoxicity of AcrDIMs. Production of ROS by AcrDIM upon irradiation was investigated by electron paramagnetic resonance (EPR) spectroscopy. The photocytotoxic action of AcrDIMs was tested on a human ovarian cancer cell line A2780 and a mouse lymphocytic leukemia cell line L1210. Oxidative stress induction, cell cycle changes, and cell death associated with intracellular distribution of AcrDIMs after PDT were monitored on the adherent A2780 cell line.

2. Results and discussion

2.1. Light induced generation of ROS

2.1.1. Photoinduced processes of hexyl-AcrDIM investigated by EPR spectroscopy

EPR spectroscopy is a valuable tool for monitoring and identification of paramagnetic species and is widely applied in the investigations of reactive oxygen species generated upon the photoexcitation of various heterocyclic compounds.[21,22] The in situ photochemical EPR experiments conducted in the presence of the spin trapping agent DMPO showed the formation of oxygen centered reactive radical species upon irradiation (λ >300 nm) of hexyl-AcrDIM in the presence of molecular oxygen. The set of EPR spectra measured during continuous UV-A photoexcitation of hexyl-AcrDIM in the aerated DMSO solution containing DMPO is shown in Figure 1a. A minor twelve-line signal could be observed already before irradiation. Its intensity rapidly increased when the irradiation started and declined after reaching a maximum. The signal is characteristic of spin Hamiltonian parameters a_N = 1.274 mT, a_H^β = 1.034 mT, a_H^γ = 0.141 mT, and g = 2.0059, which are in good correlation with hyperfine coupling constants (hfcc) attributed to a ·DMPO–O_2^- spin adduct in DMSO. In the experiments using a higher concentration of the studied derivative, the signal was of high intensity already before irradiation and instantly increased upon exposure, passing the maximum in a few seconds. Prolonged irradiation led to the formation of an additional EPR signal characteristic of spin Hamiltonian parameters a_N = 1.308 mT, a_H^β = 0.813 mT, a_H^γ = 0.180 mT, and g = 2.0059, attributed to ·DMPO–OCH_3, originating probably from solvent (data not shown). Experimental and simulated EPR spectra obtained after 10 s UV-A exposure of aerated DMSO solutions of hexyl-AcrDIM in the presence of DMPO are shown in Figure 1b.

Photoinduced generation of singlet oxygen was monitored in aerated acetonitrile solutions via oxidation of sterically hindered amine 4-hydroxy-2,2,6,6-tetramethylpiperidine(TMP) to semistable nitroxide radicals, 4-hydroxy-2,2,6,6-tetramethylpiperidine-*N*-oxyl (Tempol) and/or 4-oxo-2,2,6,6-tetramethylpiperidine-*N*-oxyl (Tempone).[23,24] The experiments were performed in dried acetonitrile, a solvent suitable for detection of singlet oxygen formed upon photoexcitation, providing higher concentration of dissolved oxygen (9 mM)[25] and sufficient lifetime of singlet oxygen.[26] Continuous irradiation of hexyl-AcrDIM in the DMSO/ACN mixture (1:5; v/v) in the presence of TMP demonstrated a gradual increase of the EPR signal, as shown in Figure 2a. According to previous investigations[23,24], the three-line signal (Fig. 2b), not sufficiently resolved due to line broadening caused by high molecular oxygen concentration in ACN, can be assigned to superposition of two spectra corresponding to two nitroxide radicals, Tempol and Tempone.

EPR investigations of UV-A photoexcitation of aerated hexyl-AcrDIM solutions in aprotic solvents (DMSO, ACN) provided evidence of an efficient molecular oxygen activation via both Type I and Type II photooxidation mechanisms, confirming that AcrDIM derivatives may behave as photosensitizers producing superoxide radical anion and singlet oxygen.

2.2. Photocytotoxic effect on cancer cell lines

2.2.1. Photocytotoxicity

Recently, we have shown[19] that AcrDIMs possessed antitumor activity against leukemia cell lines (HL-60, L1210). Photocytotoxicity of the most cytotoxic AcrDIM derivatives against mouse lymphocytic leukemia cells L1210 and human ovarian cancer cells A2780 found by MTT assay was evaluated in the present study.

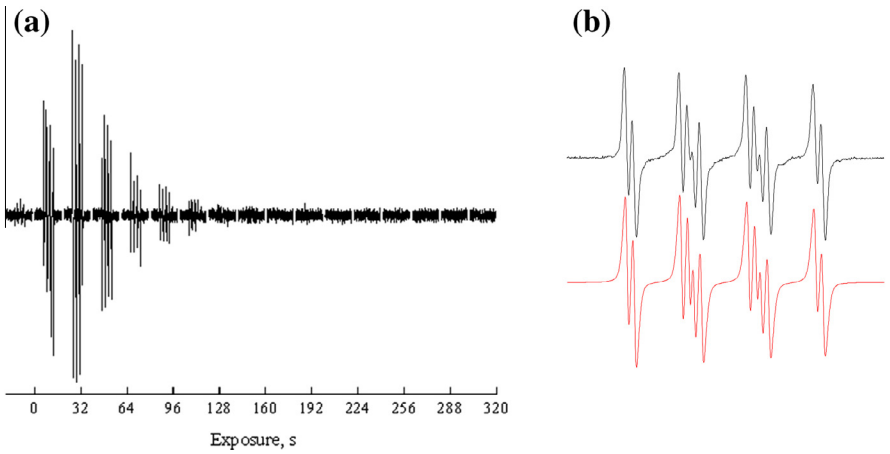

Figure 1. EPR spectra obtained upon the photoexcitation of hexyl-AcrDIM in the DMSO/DMPO/air system. (a) Time course of EPR spectra upon photoexcitation (λ >300 nm) of hexyl-AcrDIM in the DMSO/DMPO/air system (magnetic field sweep, *SW* = 10 mT), (b) experimental (–) and simulated (—) EPR spectrum (*SW* = 6 mT) monitored after 10 s UV-A photoexcitation(λ >300 nm) of hexyl-AcrDIM in the DMSO/DMPO/air system. Spin Hamiltonian parameters from simulation analysis are: ·DMPO–O_2^- a_N = 1.274 mT, a_H^β = 1.034 mT, a_H^γ = 0.141 mT, and g = 2.0059.

Download English Version:

https://daneshyari.com/en/article/1357916

Download Persian Version:

https://daneshyari.com/article/1357916

Daneshyari.com