Photocleavage of pDNA by bis-imidazolidino and bis-thioureido proflavines

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Abstract: New photosensitizers are needed for photodynamic antimicrobial and anticancer chemotherapy. Two new groups of proflavine derivatives have been recently prepared and their action on the cancer cells has been investigated by our research team. In this paper, we studied an effect of UV-A irradiation of two groups of proflavines: 3,6-bis((1-alkyl-5-oxo-imidazolidin-2-yliden)imino)acridine hydrochlorides (AcrDIMs) and 1',1"-(acridin-3,6-diyl)-3',3"-dialkyldithiourea hydrochlorides (AcrDTUs) on a plasmid DNA (pDNA). These compounds induced a photocleavage of pDNA characteristic by generation of free radicals, single strand DNA breaks and formation of an open circular form of pDNA.

Keywords: photocleavage of DNA, photodynamic therapy, proflavine derivatives

Introduction

Photodynamic therapy (PDT) employs a non-toxic substance termed photosensitizer (PS) and a low intensity visible or ultraviolet light producing together reactive oxygen species (ROS) in the presence of oxygen (Castano et al., 2004). Photodynamic antimicrobial and anticancer chemotherapy achieved quick acceptation from clinicians at present. Although porphyrin-based photosensitizers (PS) are traditionally used in PDT, there is a growing attention paid also to non-porphyrin-based PS (Delaey et al., 2000; Allison et al., 2004; O'Connor et al., 2009). Yoshida et al. (2005) applied a new combined therapy for the treatment of a periosteal Ewing's sarcoma by a surgical removal of the tumour, and PDT (utilizing acridine orange-AO). Because of a large pH gradient between intracellular and extracellular pH, AO was predominantly concentrated in the malignant tumour cells (Matsubara et al., 2006). Another *in vitro* and *in vivo* experiment showed that AO and also AO/PDT can inhibit a growth of lung metastases. One of possible inhibition mechanisms here may be a dysfunction of lysosomal enzymes (Satonaka et al., 2011).

Photo-inducible effects of AO noted above stimulated also screening of potential photosensitizing properties of some new acridine derivatives (Yang et al., 2006; Benchabane et al., 2009). In 2008, Di Giorgio's research team synthesized and studied PDT effects of new thiazolo[5,4-a]acridine derivatives, some of which intercalated into DNA after irradiation. In the dark, only three derivatives had cytotoxic effects, while the cytotoxicity of other eleven compounds has been observed after irradiation. In a search for new anticancer drugs, our team synthesized and studied new derivatives from a proflavine family (Kožurková et al., 2008; Vantová et al., 2009; Janovec et al., 2011; Paulíková et al., 2012). Photocytotoxicity of two groups of derivatives, 3,6-bis((1-alkyl-5-oxo-imidazolidin-2-yliden) imino)acridine hydrochlorides (AcrDIMs) and 1',1"-(acridin-3,6)-3',3"-diyldithiourea hydrochlorides (AcrDTUs), was studied by Čižeková et al. (2014) and Grolmusová et al. (2015). Intracellular distribution and cytotoxicity of AcrDIMs against A2780 cells and other cell lines was evaluated by Janovec et al. (2011) and Ipóthová et al. (2013). EPR measurements showed that superoxide radical anion and singlet oxygen were produced after irradiation (UV-A, >300 nm) of AcrDIM and AcrDTU derivatives (Grolmusová et al., 2013; Čižeková et al., 2014).

Investigation of a DNA binding of AcrDIMs and AcrDTUs by UV–VIS and fluorescence spectrophotometric titrations, circular dichroism spectroscopy, and denaturation transition temperature measurements confirmed their affinity toward DNA (Vantová et al., 2009, Janovec et al., 2011). We suppose that ROS generated after illumination of AcrDIMs or AcrDTUs could damage the DNA. In this paper, an ability of two proflavine derivatives (Fig. 1) to induce single- or double-strand breaks of DNA (ssb or dsb) after irradiation of a mixture of pDNA with these compounds was studied.

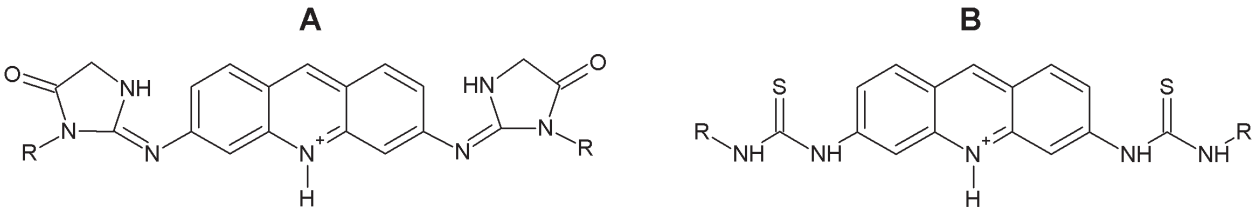

Fig. 1. Structure of 3,6-bis((1-alkyl-5-oxo-imidazolidin-2-yliden)imino)acridine hydrochlorides (AcrDIMs) (A) and 1',1"-(acridin-3,6)-3',3"-diyl-dithiourea hydrochlorides (AcrDTUs) (B), R = ethyl, *n*-propyl, *n*-butyl, *n*-pentyl, and *n*-hexyl.

Experimental

Material and methods

Materials

Sodium dodecyl sulphate (SDS), tris(hydroxymethyl) aminomethane (Tris), ethylenediaminetetraacetic acid (EDTA), RNase, agarose were obtained from Sigma-Aldrich (Germany). Isopropanol, ethanol, glucose, dimethyl sulfoxide (DMSO), and chloroform were obtained from Mikrochem (Slovakia). LiCl, HCl, and sodium acetate (NaAc) were obtained from Lachema (Czech Republic) and NaOH from Slavus (Slovakia).

Isolation of pDNA and photocleavage of DNA

Plasmid DNA (pBlueScript II SK$^+$) was isolated from *Escherichia coli*. Overnight bacterial culture was centrifuged (600× g, 5 min) at 4 °C, and the pellet was re-suspended in a TEG-buffer (25 mmol · L^{-1} Tris, 20 mmol · L^{-1} EDTA, 50 mmol · L^{-1} glucose, pH = 8) and left on ice for 10 min. Then, a 0.2 mol · L^{-1} NaOH/1 % SDS solution was added and left for 3 min at 4 °C. Consequently, an ice-cold sodium acetate solution (3 mol · L^{-1}) was added and incubated for 15 min. A suspension of precipitated proteins and nuclear DNA was centrifuged (4300 g, 10 min) at 4 °C. Ice-cold isopropanol (0.6 mL) was added to the supernatant and kept for 10 min (4 °C). DNA was isolated by centrifugation (4300× g, 15 min). The pellet was re-suspended in 70 % ethanol and centrifuged for 15 min at 14100× g. The pellet was dissolved in LiCl (2 mol · L^{-1}) in 0.5× TE (5 mmol · L^{-1} Tris, 0.5 mmol · L^{-1} EDTA, pH = 8) and incubated with RNase for 30 min at 37 °C. Then, SDS/EDTA/NaAc solution (SDS (0.5 %), EDTA (25 mmol · L^{-1}), NaAc (500 mmol · L^{-1})) was added to stop RNase activity. Consequently, chloroform (0.2 mL) was added to the samples were mixed up and centrifuged for 10 min at 1700× g. Ethanol (96 %, 0.8 mL) was added to the supernatant and the mixture was stored for 24 h at –20 °C to precipitate the plasmid DNA. After centrifugation (15 min, 6700 x g), the pellet was dissolved in 1× TE and stored at –20 °C.

Photocleavage of isolated pDNA (pBlueScript II SK$^+$) was studied using an agarose gel electrophoresis. A mixture of pDNA (0.2 μg) with 500 μmol · L^{-1} AcrDIMs or AcrDTUs was incubated in Tris/HCl (50 mmol · L^{-1}, pH = 7.5) in dark (1 h) or exposed to UV-A light (365 nm, 12.6 J/cm^2) for 90 min. Afterwards, the samples were analysed by gel electrophoresis (95 V, 50 mW, 300 mA, 45 min) in 1 % agarose gel. The gel was visualized by UV trans-illuminator using a Kodak EasyShare Z612 camera.

Results and discussion

Photo-induced cleavage of DNA by proflavine derivatives

Our previous study showed that AcrDIMs intercalated into DNA with binding constants in the range 1.9×10^5—7.1×10^5 M^{-1} determined by UV–VIS spectroscopy (Janovec et al., 2011). EPR investigation of UV-A photoexcitation of aerated AcrDIMs solutions confirmed activation of the molecular oxygen *via* both, Type I and Type II photo-oxidation mechanisms. The results indicate that AcrDIM derivatives behave as photosensitizers producing the superoxide radical anion and singlet oxygen (Čižeková et al., 2014).

Photo-inducible effects of three AcrDIM derivatives (ethyl-, pentyl-, hexyl-AcrDIM) on the plasmid DNA were also investigated. AcrDIMs (500 μM) were incubated with plasmid DNA (0.2 μg pDNA) for 1 h in dark or irradiated for 90 min (12.6 J/cm^2). Irradiation of supercoiled plasmid DNA (SC) in the presence of AcrDIMs led to single strand breaks and formation of an open circular form of pDNA (OC) (Fig. 2). Although ethyl-, pentyl-, and hexyl-AcrDIM had different DNA-binding activity (Janovec et al., 2011), their photo-cleavage activity was similar. The photo-cleavage was investigated in aerated solutions and the results indicated that superoxide radical anion and singlet oxygen were involved in the damage of pDNA.

In 2009, a series of acridin-3,6-diyl dithiourea hydrochlorides (alkyl-AcrDTU) was synthesized (Vantova et al., 2009). High DNA binding affinity via

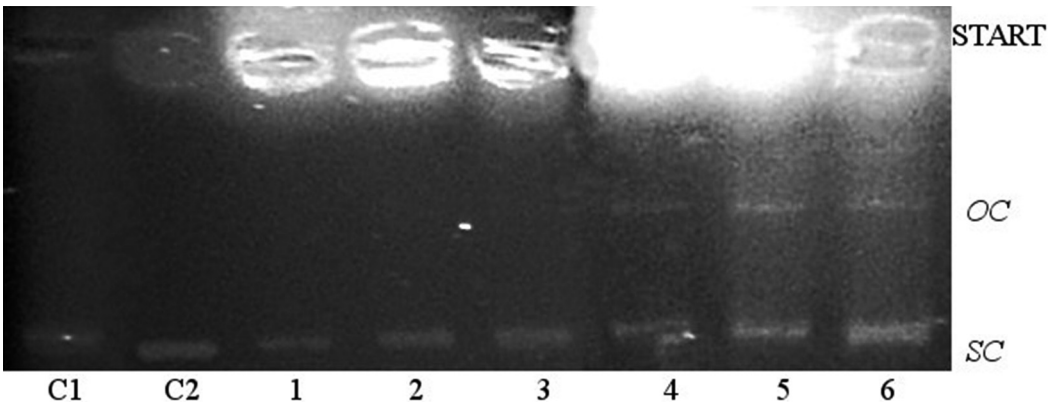

Fig. 2. Photocleavage of pDNA induced by AcrDIMs.
pDNA (0.2 μg) was incubated with 500 μmol · L^{-1} AcrDIMs in Tris/HCl (50 mmol · L^{-1}, pH 7.5) for 1 h either in the dark: (C1) pDNA without AcrDIM, (1) ethyl-AcrDIM, (2) pentyl-AcrDIM, (3) hexyl-AcrDIM or UV-A irradiated (365 nm, 12.6 J · cm^{-2}): (C2) pDNA without AcrDIM, (4) ethyl-AcrDIM, (5) pentyl-AcrDIM, (6) hexyl-AcrDIM (AcrDIMs are positively charged fluorescent compounds, therefore strong fluorescence was recorded near the starting spot). SC — supercoiled plasmid DNA, OC — open circular form of pDNA.

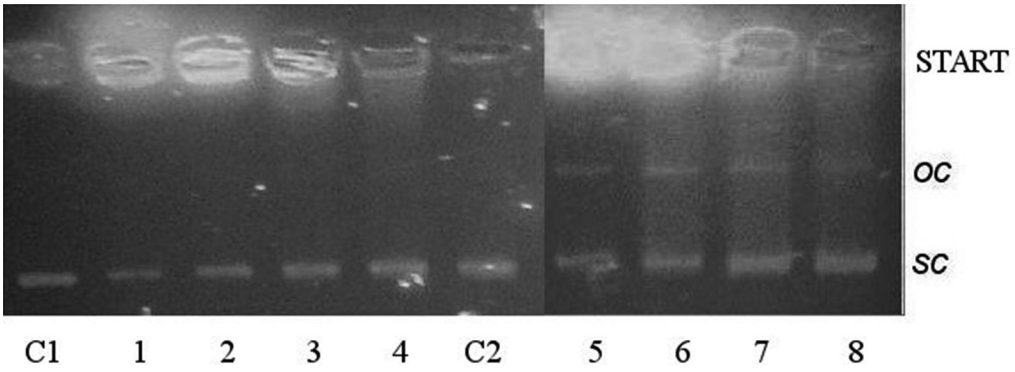

Fig. 3. Photocleavage of pDNA induced by AcrDTUs.
pDNA (0.2 μg) was incubated with 500 μM AcrDTUs in Tris/HCl (50 mM, pH 7.5) for 1 h either in the dark: (C1) pDNA without AcrDTU, (1) ethyl-AcrDTU, (2) propyl-AcrDTU, (3) butyl-AcrDTU, (4) pentyl-AcrDTU or UV-A irradiated (365 nm, 12.6 J · cm^{-2}): (C2) pDNA without AcrDTU, (5) ethyl-AcrDTU, (6) propyl-AcrDTU, (7) butyl-AcrDTU, (8) pentyl-AcrDTU (AcrDTUs are positively charged fluorescent compounds, therefore strong fluorescence was recorded near the starting spot). SC — supercoiled plasmid DNA, OC — open circular form of pDNA.

intercalation of ethyl-, propyl-, butyl-, and pentyl-AcrDTU was confirmed (K = 7.6 – 2.9 × 10^5 M^{-1}). The most cytotoxic/cytostatic activity against sensitive and drug resistant leukemia cell lines was found for pentyl-AcrDTU (IC_{50} = 3.5 μmol · L^{-1}, 48-treatment of HL-60/ADR cells). EPR study confirmed that superoxide radical anion was produced after photoexcitation of AcrDTUs in aerated solutions (Grolmusová et al., 2013; Grolmusová et al., 2015). As shown in Fig. 3, derivatives of AcrDTU induced the single strand breaks, while formation of the open circular form of pDNA (OC) has been observed only after irradiation (Fig. 3).
Our results confirm the induction of single strand breaks of DNA by photo-activated diaminoacridines in accord with the results of the Iwamoto's group (Iwamoto et al., 1993).

Conclusion

Our results confirmed that 3,6-bis((1-alkyl-5-oxo-imidazolidin-2-yliden)imino)acridine hydrochlorides (AcrDIMs) and 1',1"-(acridin-3,6-diyl)-3',3"-dialkyldithiourea hydrochlorides (AcrDTUs) were able of the photo-cleavage of pDNA similarly as did the photosensitizers for PDT. The cleavage of pDNA was supposedly induced by free radicals generated after the compounds were irradiated by UV-A light. Applicability of these derivatives for PDT of tumour cells has been confirmed earlier

(Čižeková et al., 2014). AcrDIMs and AcrDTUs were not investigated as photosensitizers for antimicrobial photodynamic therapy. The high DNA-binding affinity of these compounds and UV-A induced changes indicate that they could be used in the antimicrobial phototherapy.

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