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Research paper

Potentiation of cytotoxic action of *cis*-[$PtCl_2(NH_3)$(1M7AI)] by UVA irradiation. Mechanistic insights

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ABSTRACT

The design of photosensitive platinum antitumor drugs has been shown to be a very promising approach to enhance the low selectivity of antitumor platinum drugs towards cancer tissues. Here, we show that UVA irradiation can substantially potentiate the toxicity of a cisplatin derivative, *cis*-[$PtCl_2(NH_3)$(1-methyl-7-azaindole)], in tumor cells. We used methods of molecular biophysics and cellular biology to investigate the mechanism underlying this effect. We find that the enhanced phototoxicity of *cis*-[$PtCl_2(NH_3)$(1-methyl-7-azaindole)] is connected with the capability of this complex to cleave DNA strands. We demonstrate that production of reactive singlet oxygen, conditioned by the presence of the 1-methyl-7-azaindole ligand, is responsible for the cleavage of DNA strands not only *in vitro* but also in living cells. Our findings also reveal that UVA irradiation of DNA modified by *cis*-[$PtCl_2(NH_3)$(1-methyl-7-azaindole)] may result in a significant increase in the number of more toxic interstrand cross-links in DNA. Collectively, these data provide convincing evidence that the replacement of the ammine in the molecule of cisplatin by the 1-methyl-7-azaindole ligand might lead to a new potential candidate for photoactivated chemotherapy (PACT) against some types of cancer.

1. Introduction

Bifunctional platinum complexes such as cisplatin, carboplatin, and oxaliplatin are currently used as chemotherapeutic agents for the treatment of a variety of cancers. The anticancer activity of these drugs is related to the hydrolytic release of the labile leaving ligands with concomitant generation of active monoaqua and/or diaqua platinum(II) species that can bound nuclear DNA and effectively inhibit transcription machinery [1].

Despite their indisputable success in clinical use, these drugs suffer from several disadvantages including poor activity in certain types of cancer resulting from acquired or intrinsic resistance.

The low selectivity of platinum drugs towards cancer cells and cancerous tissue, together with severe side effects, are the major problems accompanying the clinical use of platinum drugs. In general, there are several possibilities how to improve the cancer selectivity of platinum compounds. Recently, a design of photosensitive platinum prodrugs has been shown to be a very promising approach to enhance the low selectivity of platinum(II) drugs towards cancer tissue to reduce the impact on healthy tissue and, consequently, the severe side-effects. Photoactivated chemotherapy (PACT) seems to be an interesting strategy because it offers control over the drug activation process [2]. Thus, the photoactivatable platinum drugs could find use in treating localized tumors accessible to laser-based fiber-optic devices, where the irradiation can be restricted to cancer tissue.

Among platinum photoactivatable compounds, platinum(IV) prodrugs containing azide ligands have attracted great attention [3–10]. Irradiation of these compounds can cause photodissociation and/or photodecomposition of the azido ligands and reduction of the Pt(IV) to reactive and cytotoxic Pt(II) species [2].

Besides Pt(IV), biological properties of platinum(II) compounds have also been shown to be affected by irradiation with either UV or visible light [11–15]. Interestingly, the cytotoxic effect of ineffective transplatin [16] and its direct analogs [17,18] can also be markedly enhanced by UVA irradiation.

In contrast to transplatin, the biological action of cisplatin is not affected by irradiation with UVA or visible light [16,19]. However, the toxicity against tumor cells, of the second generation cisplatin analog carboplatin and, particularly, its derivatives bearing

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7-azaindole ligands can be markedly potentiated by UVA or visible light [20,21].

Very recently, the cytotoxic properties of a new cisplatin derivative bearing a nonleaving 1-methyl-7-azaindole ligand, *cis*-[$PtCl_2(NH_3)$(1M7AI)] (1M7AI = 1-methyl-7-azaindole, Fig. 1), were investigated along with a molecular and cellular mechanism of its action [22]. The results showed that substitution of 1-methyl-7-azaindole for ammine in cisplatin resulted in an increase in toxic efficiency in cisplatin-resistant cancer cells and enhanced selectivity for tumor cells. In addition, the presence of a 7-azaindole ligand in *cis*-[$PtCl_2(NH_3)$(1M7AI)] suggests that, similarly to carboplatin derivatives containing 7-azaindole ligands, also the cytotoxicity of *cis*-[$PtCl_2(NH_3)$(1M7AI)] could be affected by irradiation with UVA light. Therefore, in the present work, the photocytotoxic potential of the cisplatin analog bearing 1-methyl-7-azaindole ligand, *cis*-[$PtCl_2(NH_3)$(1M7AI)] (Fig. 1), was investigated. The results indicate that UVA can substantially potentiate the cytotoxicity of *cis*-[$PtCl_2(NH_3)$(1M7AI)] and that the mechanism underlying this effect involves Pt-DNA adducts rearrangement as well as ROS generation and DNA cleavage.

2. Experimental

2.1. Material and instrumentation

Cisplatin, dimethyl sulfoxide (DMSO), ethidium bromide (EtBr), agarose, low gelling temperature agarose, N,N′-dimethylformamide (DMF), ethylenediaminetetraacetic acid (EDTA), sodium chloride, sodium pyruvate, HEPES [N-(2-hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid)], sodium hydroxide, 2,2,6,6-tetramethyl-4-piperidone (TMPD), Earle's Balanced Salt Solution (EBSS) and Triton X-100 were from Sigma-Aldrich (Prague, Czech Republic). 1-Methyl-7-azaindole was from Ark Pharm, Inc. (Libertyville, USA). Compound *cis*-[$PtCl_2(NH_3)$(1M7AI)] was prepared and characterized as already described [22]. Stock solutions of *cis*-[$PtCl_2(NH_3)$(1M7AI)] and cisplatin were freshly prepared at a concentration of 5×10^{-2} M in DMF and diluted with water (or cell culture medium) to an appropriate concentration just before use. The final concentration of DMF was always kept at less than 0.1%. The concentrations of platinum in the stock solutions were determined by flameless atomic absorption spectroscopy (FAAS). Plasmid pSP73 (2464 bp) was isolated according to standard procedures. Calf thymus (CT) DNA (42% G + C, mean molecular mass ca. 20,000 kDa) was from Sigma-Aldrich (Prague, Czech Republic). MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was from Calbiochem (Darmstadt, Germany). RPMI 1640 medium, fetal bovine serum (FBS), trypsin/EDTA, agarose and tris (hydroxymethyl) aminomethane (TRIS) were from PAA (Pasching, Austria). Penicillin and streptomycin were from Serva (Heidelberg, Germany). [α-^{32}P]ATP was obtained from MP Biomedicals, LLC (Irvine, CA). Restriction endonuclease EcoRI and Klenow fragment of DNA polymerase I were purchased from New England Biolabs. DNA samples and cells were irradiated using an LZC-4V illuminator (photoreactor) (Luzchem, Canada) with a temperature controller and UVA (3.5 mW cm^{-2}; λ_{max} = 365 nm) tubes. Absorption spectra were measured with a Beckmann DU-7400 spectrophotometer; the fluorescence was measured by using a Varian Cary Eclipse spectrofluorophotometer. FAAS measurements were carried out with a Varian AA240Z Zeeman atomic absorption spectrometer equipped with a GTA 120 graphite tube atomizer. The analysis with the aid of ICP-MS was performed using Agilent 7500 (Agilent, Japan). The gels were dried and visualized using a FUJIFILM BAS 2500 bioimaging analyzer.

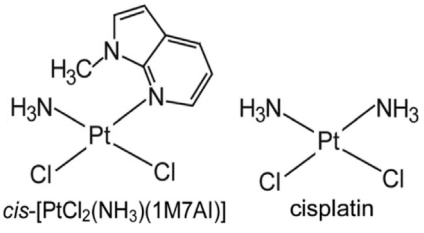

Fig. 1. Schematic representations of structures of *cis*-[$PtCl_2(NH_3)$(1M7AI)] and cisplatin used in this work; 1M7AI = 1-methyl-7-azaindole.

2.2. Cell lines

The human ovarian carcinoma A2780 (cisplatin sensitive) and human prostate adenocarcinoma LNCaP (naturally resistant to cisplatin) cell lines were kindly supplied by Professor B. Keppler, University of Vienna (Austria). The A2780 cells were grown in RPMI 1640 medium supplemented with streptomycin (100 μg mL^{-1}), penicillin (100 U mL^{-1}) and heat inactivated FBS (10%). The LNCaP cells were grown in RPMI 1640 medium supplemented with HEPES (10 mM), sodium pyruvate (1 mM), streptomycin (100 μg mL^{-1}), penicillin (100 U mL^{-1}) and heat inactivated FBS (10%). The cells were cultured in a humidified incubator at 37 °C under an atmosphere of 5% CO_2 and subcultured 2–3 times per week with an appropriate plating density.

2.3. Phototoxicity assessment

Cells were seeded in 96-well tissue culture plates at a density of 10^4 cells/well in 100 μL of growth RPMI medium and left to adhere at 37 °C in a humidified 5% CO_2 atmosphere overnight or 48 h for A2780 or LNCaP cells, respectively. After this period, the medium was removed, cells were carefully washed with PBS, platinum compounds were added at their 0–100 μM concentration in EBSS and cells were incubated for 1 h under cultivation conditions. The final DMF concentration in all wells including untreated controls was 0.1%, which was shown not to affect cell growth. After 1 h, cells were irradiated with UVA (λ_{max} = 365 nm) for 30 min. After irradiation, EBSS with platinum compounds was removed, the cells thoroughly washed with PBS, and then incubated in complete growth RPMI medium for additional 24 h (recovery time). Non-irradiated controls were tested as well. Phototoxicity was determined using standard MTT assay.

2.4. DNA interaction in cell free media

Closed circular pSP73 plasmid DNA (40 μg mL^{-1}) was incubated in $NaClO_4$ (10 mM) in the presence of the Pt complex so that the r_i value was 0.02 (r_i is defined as the molar ratio of the free platinum complex to nucleotide phosphates at the onset of incubation). The samples were then irradiated (UVA, λ_{max} = 365 nm, 3.5 mW cm^{-2}) for the indicated time. Afterward, the samples were analyzed on 1% agarose gel electrophoresis running at 4 °C with a Tris-acetate-EDTA (TAE) buffer and the voltage set at 20 V for 16 h. The gels were then stained with EtBr, photographed with a transilluminator and the intensity of fluorescence associated with bands was quantitated with the AIDA image analyzer software (Raytest, Germany).

2.5. Quantification of Pt-DNA monofunctional adducts

The measurement of an incorporation of [^{14}C]thiourea into DNA under controlled conditions [23,24] was used to quantitate Pt–DNA monofunctional adducts. The measurements were carried out as already described [23,24] with a small modification. The CT DNA was modified for 24 h in the dark with platinum complexes so that the r_b was 0.03 (r_b is defined as the number of molecules of the