Journal of Photochemistry and Photobiology B: Biology 103 (2011) 145–152

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology B: Biology

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

Photosensitization by iodinated DNA minor groove binding ligands: Evaluation of DNA double-strand break induction and repair

Benjamin Briggs [a,b], Katherine Ververis [a], Annabelle L. Rodd [a], Laura J.L. Foong [a], Fernando M. Da Silva [a], Tom C. Karagiannis [a,b,*]

[a] Epigenomic Medicine, Baker IDI Heart and Diabetes Institute, The Alfred Medical Research and Education Precinct, Melbourne, Victoria, Australia
[b] Department of Pathology, The University of Melbourne, Parkville, Victoria, Australia

ARTICLE INFO

Article history:
Received 10 January 2011
Received in revised form 17 February 2011
Accepted 28 February 2011
Available online 5 March 2011

Keywords:
Phototherapy
Photochemotherapy
Iodinated DNA ligand
DNA double-strand break
γH2AX
Trichostatin A

ABSTRACT

Iodinated DNA minor groove binding bibenzimidazoles represent a unique class of UVA photosensitizer and their extreme photopotency has been previously characterized. Earlier studies have included a comparison of three isomers, referred to as *ortho*-, *meta*- and *para*-iodoHoechst, which differ only in the location of the iodine substituent in the phenyl ring of the bibenzimidazole. DNA breakage and clonogenic survival studies in human erythroleukemic K562 cells have highlighted the higher photo-efficiency of the *ortho*-isomer (subsequently designated UV_ASens) compared to the *meta*- and *para*-isomers. In this study, the aim was to compare the induction and repair of DNA double-strand breaks induced by the three isomers in K562 cells. Further, we examined the effects of the prototypical broad-spectrum histone deacetylase inhibitor, Trichostatin A, on *ortho*-iodoHoechst/UVA-induced double-strand breaks in K562 cells. Using γH2AX as a molecular marker of the DNA lesions, our findings indicate a disparity in the induction and particularly, in the repair kinetics of double-strand breaks for the three isomers. The accumulation of γH2AX foci induced by the *meta*- and *para*-isomers returned to background levels within 24 and 48 h, respectively; the number of γH2AX foci induced by *ortho*-iodoHoechst remained elevated even after incubation for 96 h post-irradiation. These findings provide further evidence that the extreme photopotency of *ortho*-iodoHoechst is due to not only to the high quantum yield of dehalogenation, but also to the severity of the DNA lesions which are not readily repaired. Finally, our findings which indicate that Trichostatin A has a remarkable potentiating effect on *ortho*-iodoHoechst/UVA-induced DNA lesions are encouraging, particularly in the context of cutaneous T-cell lymphoma, for which a histone deacetylase inhibitor is already approved for therapy. This finding prompts further evaluation of the potential of combination therapies.

1. Introduction

1.1. Photochemotherapy

Photochemotherapy, the combined use of a photosensitizer and non-ionizing electromagnetic radiation, has been used since antiquity with the ancient Egyptians and Indians using the plants *Ammi majus* and *Psoralea corylifolia*, respectively, combined with solar radiation to treat leukoderma (vitiligo) [1,2]. These plants contain psoralen and other furocoumarins which form the basis of modern photochemotherapy. Currently, photochemotherapy using psoralen (predominantly 8-methoxypsoralen; 8-MOP) and long wavelength ultraviolet radiation (320–400 nm, UVA) – referred to as PUVA therapy, is widely used in dermatology for the treatment of a variety of hyperproliferative skin diseases, particularly psoriasis and cutaneous T-cell lymphoma (CTCL) [3]. For psoriasis and early-stage skin-localized CTCL, 8-MOP is typically administered orally or applied topically followed by exposure of the skin to UVA [3,4]. Numerous mechanisms of action, including lipid peroxidation and activation of biochemical pathways associated with cell-death and apoptosis, have been described for PUVA therapy [5]. However, the well-characterized photochemical reactions involve those associated with DNA in particular, photoadduct formation following photoexcitation of intercalated psoralens [5–7]. Photochemotherapy is also used for advanced leukemic forms of CTCL using a technique known extracorporeal photopheresis (ECP) [4]. This involves exposure of leukapheresed blood to 8-MOP and UVA extracorporeally, followed by re-infusion of the treated peripheral blood mononuclear cells [4]. Together with the mechanisms described above, ECP is thought to be effective due to an immunomodulatory response, involving dendritic cell

* Corresponding author at: Epigenomic Medicine Baker IDI Heart and Diabetes Institute 75 Commercial Road, Melbourne, VIC, Australia. Tel.: +613 8532 1309; fax: +613 8532 1100.
E-mail address: tom.karagiannis@bakeridi.edu.au (T.C. Karagiannis).

1011-1344/$ - see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jphotobiol.2011.02.022

maturation, stimulation of T-regulatory lymphocytes and modulation of T-helper cells [4,8–10].

1.2. Iodinated DNA minor groove binding ligands

The halogenated DNA precursors, iodo- and bromo-deoxyuridine represent a different class of UV sensitizers. Preparation of these pyrimidine analogues involves substitution of the methyl group of thymidine with a halogen. These analogues compete with thymidine during DNA synthesis and result in the incorporation of a halogen atom into DNA. The classical studies indicated that halogenated DNA precursors sensitize DNA-damage and cell-death induced by UVB and ionizing radiation [11,12]. In phototherapy, the mechanism involves photodehalogenation (dissociation of the carbon-halogen bond) resulting in the formation of the highly reactive uracilyl radical [11,12]. This results in the formation of DNA lesions by abstraction of H atoms from deoxyribosyl carbons [11,12]. The major limitation of the analogues is that they are incorporated only in the S-phase of the cell cycle resulting in sensitization of only subpopulation of cells [13]. This prompted the investigation of iodinated DNA minor groove binding bibenzimidazoles.

The photopotency of iodoHoechst 33258 (prepared by direct iodination of the commercially available bibenzimidazole, Hoechst 33258) and of three isomers designated *ortho*-(UV_ASens), *meta*- and *para*-iodoHoechst (differ only in the location of the iodine substituent in the phenyl ring of the bibenzimidazole), has been previously investigated using DNA gel sequencing experiments and in mammalian cell culture systems (Fig. 1) [14–18]. The early DNA sequencing experiments and clonogenic survival assays in V79 fibroblasts indicated that iodoHoechst 33258 was an efficient sensitizer, enhancing DNA strand breakage and cell-death, respectively [14,15]. Subsequent studies compared the UVA photopotency of *ortho*-, *meta*- and *para*-iodoHoechst using plasmid breakage assays and in human erythroleukemic K562 cells [16–18]. Firstly, the findings indicated that photoactivation of the DNA bound ligands results in photodeiodination, yielding phenylHoechst (Fig. 1) [18]. This is consistent with formation of a carbon-centred ligand radical and H-atom abstraction, analogous to the uracilyl radical mediated-damage described for photolyzed halogenated DNA.

The earlier studies also highlighted the remarkable differences in photopotency with the *ortho*-isomer being much more efficient at inducing strand breakage and cytotoxicity than both *meta*- and *para*-iodoHoechst [16–18]. Evaluation of the cross-section for dehalogenation of the three isomers indicated that the yield was 5 and 6-fold higher for *ortho*- compared to *meta*- and *para*-iodoHoechst [18]. However, analysis of the clonogenic survival data on the basis of number of dehalogenation events required for 50% cell-kill, indicated that the high photopotency of *ortho*-iodoHoechst is due to a combination of the greater cross-section for deiodination and also to a greater lethal potency per dehalogenation event [18]. Given the extreme photopotency of *ortho*-iodoHoechst, the analogue has been considered for potential use in receptor-mediated photoimmunotherapy. Proof-of-principle studies using conjugates of *ortho*-iodoHoechst to transferrin and epidermal growth factor (EGF), have demonstrated specific receptor-mediated UVA-induced cytotoxicity in transferrin-expressing K562 cells and EGF receptor-expressing A431 cells, respectively [19].

1.3. Histone deacetylase inhibitors

Histone deacetylase inhibitors have emerged as a new class of anticancer therapeutics with the hydroxamic acid, Vorinostat being approved by the FDA for the treatment of CTCL [20,21]. The acetylation status of the core histones is regulated by two groups of enzymes – histone acetyltransferases (acetylate histones) and histone deacetylases (catalyse the removal of acetyl groups). To date, 18 histone deacetylase enzymes have been identified and they are classified into various classes: class I includes enzymes 1, 2, 3 and 8; class IIA, 4, 5, 7, and 9; Class IIB, HDACs 6 and 10; Class IV, HDAC 11 which possesses properties of both class I and II enzymes [20,21]. The Class III histone deacetylase enzymes, referred to a sirtuins 1–7, have an absolute requirement for NAD^+ are not inhibited by classical broad-spectrum histone deacetylase inhibitors, such as the hydroxamic acid Trichostatin A which inhibit, class I, II and IV enzymes [20,21]. The broad-spectrum histone deacetylase inhibitors result in the accumulation of hyperacetylated core histone and result in a more relaxed, transcriptionally permissive chromatin conformation [22–24]. Further, histone deacetylase inhibitors target numerous nonhistone properties and their overall effects include altered gene expression, cell-death, apoptosis and cell cycle arrest, in cancer and transformed cell lines [20,21].

1.4. Aims

Overall, the previous comparative studies involved an extensive investigation of the photopotency of the *ortho*-, *meta*- and *para*-iodoHoechst isomers in K562 cells, focusing on the different yields of photodeiodination and clonogenic survival [18]. Here our aim was to compare the differences in induction and repair of DNA lesions induced by the three isomers in K562 cells. We used γH2AX as a molecular marker of DNA double-strand breaks to examine the induction and repair of the lesions following combinations of the DNA ligands and UVA-irradiation.

In addition to their intrinsic cytotoxic properties, histone deacetylase inhibitors have been shown to have synergistic or at least additive effects, with other conventional anticancer therapies such radiotherapy or chemotherapeutics [25–32]. Furthermore, a recent study identified that the histone deacetylase inhibitors, sodium butyrate and Trichostatin A augmented the cytotoxic potency of PUVA therapy in various cancer cell lines [33]. Here we investigated the effects of pre-incubation of K562 cells with Trichostatin A on the UVA photopotency of the *ortho* isomer, using γH2AX as a molecular marker of DNA double-strand breaks.

2. Materials and methods

2.1. Cell culture and UVA-irradiation

Human chronic erythroleukemic, K562 cells were grown in suspension in a humidified atmosphere of 5% (v/v) CO_2 at 37 °C in RPMI-1640 medium (Invitrogen, Carlsbad, CA, USA) containing, 10% (v/v) fetal bovine serum (In Vitro Technologies, Victoria, Australia), 20 mM HEPES, pH 7.4, 2 mM L-glutamine and 40 μg/ml gentamicin (complete RPMI). Cells were maintained in exponential growth phase; experiments were performed with cells collected at a density of $5–7 \times 10^5$/ml. All of the experimental procedures were carried out under restricted lighting. As required cells were irradiated with the relevant fluences (0–500 J/m^2) of UVA (8 Watt, black light fluorescent lamps; UVP, Upland, CA, USA), the flux (UVX radiometer, measuring 365 nm, UVP) was adjusted such that irradiations were completed within 2 min.

2.2. Hoechst analogues and Trichostatin A

The bibenzimidazole analogues, *ortho*-, *meta*-, *para*-iodoHoechst and phenylHoechst, the synthesis of which has been described previously, were provided by Professor Roger Martin (Molecular Radiation Biology Laboratory, Peter MacCallum Cancer Centre) [34]. The Hoechst analogues (>97% pure by HPLC analysis with detection at 340 nm), were stored as lyophilized pellets in the dark. Concen-