Toxicology in Vitro 29 (2015) 512–521

Contents lists available at ScienceDirect

Toxicology in Vitro

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

Comparative endothelial profiling of doxorubicin and daunorubicin in cultured endothelial cells

Tomasz Wojcik [a], Elzbieta Buczek [a], Katarzyna Majzner [a,b], Agnieszka Kolodziejczyk [c], Justyna Miszczyk [d], Patrycja Kaczara [a], Wojciech Kwiatek [d], Malgorzata Baranska [a,b], Marek Szymonski [c], Stefan Chlopicki [a,e,*]

[a] Jagiellonian Centre for Experimental Therapeutics (JCET), Jagiellonian University, Bobrzynskiego 14, 30-348 Krakow, Poland
[b] Faculty of Chemistry, Jagiellonian University, Ingardena 3, 30-060 Krakow, Poland
[c] Department of Physics of Nanostructures and Nanotechnology, Jagiellonian University, Reymonta 4, 30-059 Krakow, Poland
[d] Department of Experimental Physics of Complex Systems, The H. Niewodniczanski Institute of Nuclear Physics Polish Academy of Sciences, 31-342 Krakow, Poland
[e] Department of Experimental Pharmacology, Chair of Pharmacology, Jagiellonian University, Medical College, Grzegorzecka 16, 31-531 Krakow, Poland

ARTICLE INFO

Article history:
Received 8 July 2014
Accepted 8 December 2014
Available online 18 December 2014

Keywords:
Doxorubicin
Daunorubicin
Cytotoxicity
Endothelium
High-content screening

ABSTRACT

Although anthracycline antibiotics have been successfully used for nearly half a century in the treatment of various malignancies, their use is limited by their cardiac and vascular toxicities, and the mechanisms of these toxicities are still not entirely clear. Herein, we comprehensively characterized cytotoxic effects of two structurally related anthracyclines, doxorubicin and daunorubicin. In nanomolar concentrations, both drugs induced DNA damage and increased nuclear area that were associated with their accumulation in the nucleus (doxorubicin $\geqslant$50 nM and daunorubicin $\geqslant$25 nM) as evidence by Raman microspectroscopy at 3820–4245 cm^{-1}. At low micromolar concentrations, doxorubicin ($\geqslant$5 μM) and daunorubicin ($\geqslant$1 μM) increased the generation of reactive oxygen species, decreased intracellular reduced glutathione, induced an alteration in endothelial elasticity and caused a reorganization of the F-actin cytoskeleton. In isolated mouse aortic rings, doxorubicin ($\geqslant$50 μM) was less potent than daunorubicin ($\geqslant$5 μM) in impairing the endothelium-dependent response.

In summary, using a comprehensive endothelial profiling approach, we demonstrated clear-cut differences in the potencies to induce endotheliotoxic responses for two structurally similar chemotherapeutics, at a nuclear, cytosolic and membrane levels. Furthermore, our results suggest that the differences in the endothelial toxicities of doxorubicin and daunorubicin are linked to differences in their nuclear accumulation and the DNA damage-triggered response of the endothelium.

© 2015 Published by Elsevier Ltd.

1. Introduction

Anthracyclines are a group of potent and effective chemotherapeutic agents that are used for the treatment of various neoplastic disorders, including breast cancer, Hodgkin's lymphoma, leukaemia and testicular cancer. The clinical usefulness of anthracyclines is limited by their cardiotoxic side effects, which include arrhythmias, ventricular dysfunction and congestive heart failure. These cardiotoxic effects have been widely documented and can manifest decades after the cessation of chemotherapy. Doxorubicin-induced cardiovascular toxicity is dose-dependent, and the incidence of cardiomyopathy rises sharply with cumulative doses exceeding 550 mg/m^2 (Singal and Iliskovic, 1998). Congestive heart failure is observed in 18% of patients following cumulative doses of 550–600 mg/m^2, and as many as 36% of patients who are treated with a cumulative dose above 600 mg/m^2 (Shapiro et al., 1998).

The chemical structure of doxorubicin differs from the structure of daunorubicin only by the presence of a single hydroxyl group in the tetracycline moiety. Both compounds inhibit the growth of a variety of experimental tumours, but doxorubicin has proven more effective than daunorubicin in inhibiting cancer cell proliferation (Meriwether and Bachur, 1972; Sneddon, 1965). In humans, both drugs have shown good efficacy against acute leukaemia, breast cancer, Hodgkin's disease and testicular cancer. Several clinical studies indicated, that doxorubicin was less cardiotoxic, than daunorubicin (Swain et al., 2003; Steinherz et al., 1993).

It has recently been suggested that anthracycline-mediated cardiac toxicity might be also linked to the injurious effects of these drugs on the coronary endothelium. Indeed, the vascular toxicity of anthracyclines has been repeatedly reported in both experimental

* Corresponding author at: Jagiellonian Centre for Experimental Therapeutics (JCET), Jagiellonian University, Bobrzynskiego 14, 30-348 Krakow, Poland.
E-mail address: stefan.chlopicki@jcet.eu (S. Chlopicki).

http://dx.doi.org/10.1016/j.tiv.2014.12.009
0887-2333/© 2015 Published by Elsevier Ltd.

and clinical studies (Soultati et al., 2012). In a pilot study of pediatric cancer patients who completed treatment with doxorubicin or daunorubicin, brachial artery vasomotor reactivity was found to be impaired relative to control patients (Chow et al., 2006). The more comprehensive Childhood Cancer Survivor Study demonstrated that, among adult survivors of childhood acute lymphoblastic leukaemia (ALL), the patients who received chemotherapy without cranial irradiation and the patients who received chemotherapy combined with cranial irradiation exhibited magnitudes of flow-mediated dilation (FMD) impairment that were similar to those observed in controls. These results indicate that chemotherapy cancer survivors have poorer endothelial function 20 years after treatment and that this effect is attributable to anthracycline treatment but not radiotherapy (Dengel et al., 2008). Interestingly, even a single intravenous infusion of doxorubicin directly impairs NO-dependent function in humans as measured by FMD (Duquaine et al., 2003). Moreover, in experimental *in vitro* studies, doxorubicin induces endothelial cell apoptosis that is linked to increased generation of reactive oxygen species (ROS) and increased redox-cycling of the doxorubicin semiquinone radical, which leads to increased intracellular oxidant stress (Wolf and Baynes, 2006) and endothelial cell apoptosis *via* caspase-dependent mechanisms (Kaushal et al., 2004).

Over the last several years, anthracycline toxicity has been linked to various mechanisms of drug action. It has been proposed that anthracycline cardiotoxicity is dependent on direct DNA damage (Gewirtz, 1999), the inhibition of topoisomerase-IIβ (Zhang et al., 2012) or the toxicity of the hydrogen peroxide generated by the redox cycling of the semiquinones of anthracyclines (Konorev et al., 2004). The issue has been discussed in numerous reviews (Gewirtz, 1999; Octavia et al., 2012). Although accumulating evidence indicates the clinically relevant endothelial toxicity of anthracycline-containing chemotherapy, the mechanisms responsible for this toxicity remain far from being understood. Furthermore, to our knowledge, the literature is deficient in terms of comparative studies that have been designed to assess the toxicity profiles of anthracyclines in similar experimental setting, and there are no direct comparisons of the endothelial toxicities of doxorubicin and daunorubicin. Therefore, the aim of the present work was to characterize the nuclear and cellular effects of the two structurally related anthracyclines, doxorubicin and daunorubicin, in human endothelial cells (EA.hy926) using high-content screening automated fluorescence microscopy, Raman microspectroscopy, atomic force spectroscopy and biochemical analysis.

2. Materials and methods

2.1. Ethics statement

All animal procedures and experiments were performed in accordance with national and European legislation, and were approved by the Local Ethical Committee on Animal Testing of Jagiellonian University in Krakow (approval number: 53/2009).

2.2. Cell culture and drug application

The hybridoma EA.hy926 cell line, which was formed by fusion of the HUVEC and A549 human lung carcinoma cell lines, was kindly provided by Dr. C.J. Edgell (Department of Pathology of the University of North Carolina, Chapel Hill, NC, USA) (Edgell et al., 1983). The cells were grown in Dulbecco's Modified Eagle medium (DMEM) containing 4.5 mg per ml glucose, 10% foetal bovine serum, 100 U per ml penicillin, 100 μg per ml streptomycin and 2% HAT supplement (complete culture medium) maintained at 37 °C in a fully humidified atmosphere of 5% CO_2 and passaged three times per week. For the experiments, the cells were plated into 96-well plates at 18,000 cells per well to produce confluence on the first post-plating day, which was also the day on which exposure to the anthracyclines was initiated. In the experiments involving prolonged (5 days) exposure of the cells in culture to the anthracyclines, the cells were plated into 96-well plate at 5000 cells per well. Doxorubicin hydrochloride (DOX) or daunorubicin hydrochloride (DNR) (both anthracyclines were from Sigma–Aldrich Co. St. Louis, MO, USA) were dissolved in the culture medium to obtain the required final concentrations. The cultures were incubated with complete medium containing either no anthracycline (control) or a range of concentrations of DOX or DNR (0.125–20 μM).

2.3. High-content automated fluorescence microscopy

Control and DOX- and DNR-treated cells were incubated for 30 min at 37 °C with dihydroethidium (DHE) dissolved in the culture medium (Life technologies, Carlsbad, CA, USA). After removing the medium containing the fluorescent probe, the cells were rinsed twice with phosphate-buffered saline (PBS), incubated for 10 min at 37 °C with Hoechst 33342 and Cell Mask deep red plasma membrane stain (Life technologies, Carlsbad, CA, USA) and rinsed twice more with PBS. Pretreated, unstained cells were used for background correction. To detect reductions in glutathione, the cells were incubated for 30 min at 37 °C with ThiolTracker violet, rinsed with PBS, followed by fixation with 2% formaldehyde (10 min), permeabilization with 0.1% Triton X-100 (5 min), incubation with CellMask deep red stain and rinsed twice with PBS. The images were captured with a Olympus ScanR automated fluorescence microscope (Olympus Corp., Tokyo, Japan) using the following filters: DAPI for Hoechst 33342 or ThiolTracker violet, Cy3 for DHE or background correction, and Cy5 for Cell Mask stainings. At least 64 fields per well were recorded. All images were stored and processed using a Columbus Image Data Storage and Analysis system (Perkin Elmer, Waltham, MA, USA). The results for individual cells are presented as the means and the standard deviations (SD).

2.4. Raman spectroscopy

EA.hy926 cells were plated onto calcium fluoride slides (CaF_2, 25 mm × 2 mm, Pike Technologies, U.S.) that were placed in the wells of 6-well plates at 5×10^4 cells per well and fed with complete medium containing either no anthracycline (control) or a range of DOX or DNR concentrations (12.5 nM, 25 nM, 50 nm, 1 μM and 10 μM). After 24 h, the cells were fixed with 4% paraformaldehyde (4 min) and stored in isotonic phosphate buffer (pH = 7; 4 °C) until the beginning of data acquisition. Raman mapping was performed with a confocal Raman imaging system (Witec alpha 300) and a 60× water immersion objective (Nikon Fluor, NA = 1). The scattered light was directed to the spectrometer using a 50-μm core diameter multimode fibre that also acted as the pinhole for confocal detection. The spectrometer (WITec UHTS300) was equipped with a back-illuminated CCD camera (Newton EMCCD DU970-BV) and a 600-grooves/mm grating (BLZ = 500 nm). An excitation wavelength of 488 nm was used due to the high lateral and depth resolutions (approximately 0.3 μm and 0.7 μm, respectively). The cells were submerged in PBS during the measurement. The total exposure time was *ca.* 40–60 min per cell (the integration time was 0.7 s per spectrum). Data acquisition was controlled with the Witec alpha 300 software package. All Raman spectra were offset-corrected, and the routine procedure for cosmic ray removal was applied. The ImageJ processing program was applied to calculate the total nuclear area and the nuclear area occupied by the DNR- or DOX-derived fluorescence according to the concentrations of the anthracyclines.

Download English Version:

https://daneshyari.com/en/article/2602485

Download Persian Version:

https://daneshyari.com/article/2602485

Daneshyari.com