Biochemical Pharmacology 143 (2017) 79–89

Contents lists available at ScienceDirect

Biochemical Pharmacology

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

Activation of ALDH1A1 in MDA-MB-468 breast cancer cells that over-express CYP2J2 protects against paclitaxel-dependent cell death mediated by reactive oxygen species

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ARTICLE INFO

Article history:
Received 16 June 2017
Accepted 24 July 2017
Available online 27 July 2017

Chemical compounds studied in this article:
Paclitaxel (PubChem CID: 36314)
Doxorubicin (PubChem CID: 31703)
Staurosporine (PubChem CID: 44259)
Clotrimazole (PubChem CID: 2812)
Dimethylsulfoxide (PubChem CID: 679)
2′,7′-Dichlorofluorescein diacetate (PubChem CID: 101615877)
4-Hydroxynonenal (PubChem CID: 5283344)
Malondialdehyde (PubChem CID: 10964)
2-Thiobarbituric acid (PubChem CID: 2723628)
1,1,3,3-Tetramethoxypropane (PubChem CID: 66019)
3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (PubChem CID: 64965)
Sorafenib (PubChem CID: 216239).

Keywords:
Cytochrome P450 2J2
Breast cancer drug resistance
Reactive oxygen species
Aldehyde dehydrogenase 1A1
14,15-EET
Cell survival

ABSTRACT

Cytochrome P450 2J2 (CYP2J2) expression is elevated in breast and other tumours, and is known to be protective against cytotoxic agents that may be used in cancer chemotherapy. This study evaluated the mechanisms by which MDA-MB-468 breast cancer cells that stably expressed CYP2J2 (MDA-2J2 cells) were protected against killing by the anti-cancer agent paclitaxel. Compared to control cells caspase-3/7 activation by paclitaxel was lower in MDA-2J2 cells, while cell proliferation and colony formation following paclitaxel treatment were increased. Basal lipid peroxidation was lower in MDA-2J2 cells than in control cells, and the paclitaxel-mediated increase in peroxidation was attenuated. The mitochondrial complex III inhibitor antimycin A modulated basal and paclitaxel-activated reactive oxygen species (ROS) formation in control cells; paclitaxel-activated ROS production was also modulated by the NADPH oxidase inhibitor diphenyleneiodonium. Paclitaxel increased the formation of protein adducts by the reactive aldehyde 4-hydroxynonenal that is produced by lipid peroxidation; adduct formation was attenuated in MDA-2J2 cells. ALDH1A1 expression and activity was strongly upregulated in MDA-2J2 cells that was attributed to CYP2J2-derived 14,15-epoxyeicosatrienoic acid (14,15-EET); the 8,9- and 11,12-EET regioisomers did not activate ALDH1A1 expression. Silencing of ALDH1A1 restored the sensitivity of MDA-2J2 cells to paclitaxel, as indicated by a more pronounced decrease in proliferation, and greater increases in caspase activity and formation of ROS to levels comparable with control cells. Similar findings were observed with doxorubicin, sorafenib and staurosporine, that also promoted ROS-mediated cell death that was attenuated in MDA-2J2 cells and reversed by ALDH1A1 gene silencing. These findings implicate ALDH1A1 as an important gene that is activated in MDA-MB-468-derived cells that contain high levels of CYP2J2. ALDH1A1 modulates the production of ROS by anti-cancer agents such as paclitaxel and diminishes their efficacy. Future approaches could adapt this information to facilitate the targeting of ALDH1A1 to promote the efficacy of ROS-generating cytotoxic agents and enhance the treatment of breast cancer.

Abbreviations: ALDH1A1, aldehyde dehydrogenase 1A1; CYP2J2, cytochrome P450 2J2; DCFDA, 2′,7′-dichlorofluorescein diacetate; DMEM, Dulbecco's modified eagle medium; DMSO, dimethylsulfoxide; EET, epoxyeicosatrienoic acid; 4-HNE, 4-hydroxynonenal; MDA-CTL, MDA-MB-468 cells stably transfected with green fluorescent protein; MDA-2J2, MDA-MB-468 cells stably transfected with CYP2J2 and green fluorescent protein; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PBS, phosphate buffered saline; ROS, reactive oxygen species; RT-PCR, real time-PCR; TBARs, thiobarbituric acid-reactive substances; TBS, Tris-buffered saline; UGT, UDP-glucuronosyltransferase.

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http://dx.doi.org/10.1016/j.bcp.2017.07.020
0006-2952/

1. Introduction

The cytochrome P450 2J2 (CYP2J2) epoxygenase is upregulated in a number of cancer types [21], including breast tumours [46]. CYP2J2 converts arachidonic acid to four regioisomeric epoxyeicosatrienoic acids (5,6-EET, 8,9-EET, 11,12-EET and 14,15-EET) that promote tumourigenesis and tumour cell survival by increasing proliferation and decreasing apoptosis [8,22]. Although the activation of prosurvival signalling pathways has been demonstrated in cells that over-express CYP2J2, or that produce high quantities of EETs [49], there is less information on the underlying protection mechanisms.

Reactive oxygen species (ROS) have bimodal actions in the regulation of tumour viability. ROS are present in breast tumours [30,43], which promote cell proliferation and metastatic progression [36]. In contrast, enhanced production of ROS is implicated in tumour cell killing by a range of chemotherapeutic agents [1,2,12 28,32]. Mitochondrial electron transport activity is a major site of ROS generation with other potentially important sites including the enzymes xanthine oxidase and NADPH oxidase [5,31,41]. Antioxidant enzymes, such as glutathione peroxide and catalase, and biotransformation enzymes, such as the aldehyde dehydrogenases (ALDHs) and UGT-glucuronosyltransferases (UGTs), are potentially important in protecting cells against ROS and ROS-derived reactive aldehydes that mediate lipid peroxidation [40].

The present study was undertaken to investigate the mechanism by which CYP2J2 overexpression protects triple-negative MDA-MB-468 breast cancer cells (MDA-2J2 cells) against the cytotoxic chemotherapeutic agent paclitaxel. The principal finding was that expression of ALDH1A1 was markedly enhanced in MDA-2J2 cells and that this enzyme was a major determinant of the efficacy of paclitaxel by conferring protection against ROS-mediated cell death.

2. Materials and methods

2.1. Materials

Paclitaxel, doxorubicin, staurosporine, clotrimazole, dimethylsulfoxide (DMSO), 2′,7′-dichlorofluorescein diacetate (DCFDA), 2-thiobarbituric acid, 1,1,3,3-tetramethoxypropane, 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), malondialdehyde tetrabutylammonium, *trans*-2-hexenal, PD98059 (ERK inhibitor) the protease inhibitor cocktail and Dulbecco's modified eagle medium (DMEM, containing 1 g glucose/L) were obtained from Sigma-Aldrich (Castle Hill, NSW, Australia). Sorafenib was from Toronto Research Chemicals (Toronto, ON, Canada). Fetal bovine serum was purchased from Thermo Scientific (Scoresby, VIC, Australia) and trypan blue, 0.5% trypsin-EDTA and penicillin–streptomycin (5000 U/mL) were from Life Technologies (Mulgrave, VIC, Australia). Phosphate buffered saline (PBS) was from Amresco (Solon, OH, USA). The ALDH activity assay kit, thiobarbituric acid-reactive substances (TBARs) lipid peroxidation kit, the JC-1 Mitochondrial Membrane Potential Assay Kit, 4-hydroxynonenal (4-HNE), diphenyleneiodonium chloride (DPI), antimycin A1 (antimycin) and allopurinol were purchased from Cayman Chemical (Ann Arbor, MI, USA). SB203580 (p38 mitogen-activated protein kinase; p38 MAP kinase inhibitor), and LY294002 (phosphoinositide 3-kinase/Akt inhibitor) were purchased from Sapphire Biosciences (Waterloo, NSW, Australia).

Murine monoclonal antibodies used to detect human ALDH1A1 and β-actin were from BD Bioscience (cat 611194; Heidelberg, Germany) and Santa Cruz Biotechnology (sc-47778; Santa Cruz, CA), respectively. The mouse monoclonal 4-HNE antibody (MAB3249) and recombinant human ALDH1A1 enzyme were from R&D Systems Inc. (Minneapolis, MN, USA). The rabbit anti-human glyceraldehyde 6-phosphate dehydrogenase antibody (14C10, #2118) was from Cell Signalling Technology (Arundel, QLD, Australia) and the goat anti-mouse secondary antibody (LCR 926-68020) was purchased from Millennium Science (Mulgrave, VIC, Australia).

2.2. Cell lines

The human MDA-MB-468 cell line was obtained from American Type Culture Collection (Manassas, VA, USA). MDA-MB-231, A549 and PC3 cell lines were obtained from ATCC, while the SK-BR-3 and MCF10A cell lines were generous gifts from A/Prof Jenny Byrne, Children's Hospital, Westmead, and Prof Christine Clarke, The Westmead Institute, Westmead Hospital, NSW, Australia, respectively. MDA-MB-468 cells were stably transfected with CYP2J2 and green fluorescent protein vectors under the control of the CMV promoter (MDA-2J2 cells) or green fluorescent protein vector alone (MDA-CTL cells), as described previously [4]. Three other clonal cell lines were obtained during the preparation of MDA-2J2 cells (MDA-2J2-I, MDA-2J2-II and MDA-2J2-III). CYP2J2 mRNA was over-expressed to 0.12-, 0.19- and 0.30-fold, respectively, of that in MDA-2J2 cells. Corresponding ALDH1A1 mRNA expression was 0.21-, 0.33- and 0.39-fold, respectively, of that in MDA-2J2 cells. The MDA-2J2 cell line was used in all of the experiments described. CYP2J2 over-expression was confirmed by real time-PCR (RT-PCR) and immunoblotting. All cell lines were maintained in DMEM medium containing 10% fetal bovine serum and 100 U/mL penicillin-streptomycin, were cultured at 37 °C in an atmosphere of 5% CO_2 and used between passage 12 and 20.

2.3. Western blotting

Whole cell lysates were prepared in Laemmli sample buffer containing 50 mM dithiothreitol, sonicated for 5 s (Sonifier 250; Branson Ultrasonics Co., Danbury, CT) and incubated at 95 °C for 5 min. Lysates were electrophoresed on 7.5% sodium dodecyl sulfate–polyacrylamide gels [34], and subjected to western immunoblotting essentially as described previously [9]. Proteins were transferred to nitrocellulose membranes (Whatman GmbH, Dassel, Germany, washed with 5% milk in Tris-buffered saline (TBS; pH 8.8) and then incubated overnight at 4 °C with primary antibody (1:1000 dilution; 1% bovine serum albumin in TBS containing 0.1% Tween). After washing and incubation with the secondary antibody, signals on immunoblots were detected using an Odyssey IP imaging system (LI-COR Biosciences, Lincoln, NE, USA).

2.4. RNA extraction and real-time PCR

Cells were seeded on six-well plates (3×10^5 cells/well), allowed to adhere for 24 h and then incubated in minimal DMEM medium for 18 h. Cells were washed with PBS and total RNA was extracted (Purelink RNA mini kit; Life Technologies) and quantified spectrophotometrically (NanoDrop Technologies; BioLab Pty Ltd., Scoresby, Vic, Australia). RNA samples were treated with RQ1 DNase (Promega Corp; Alexandria, NSW, Australia) prior to RT-PCR for comparison between MDA-468 and MDA-2J2 mRNA expression. Primer sequences and PCR cycling conditions are shown in Table 1. RT-PCR was conducted in a Rotor-Gene 6000 thermal cycler (Corbett Life Science, Mortlake, NSW, Australia) using the express one-step SYBR® GreenER™ Universal qPCR supermix (Life Technologies). PCR conditions were validated by melting