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Perspective

Modulating the function of ATP-binding cassette subfamily G member 2 (ABCG2) with inhibitor cabozantinib

Guan-Nan Zhang[a], Yun-Kai Zhang[a], Yi-Jun Wang[a], Anna Maria Barbuti[a], Xi-Jun Zhu[a,b,1], Xin-Yue Yu[a,c,1], Ai-Wen Wen[a,d,1], John N.D. Wurpel[a], Zhe-Sheng Chen[a,*]

[a] Department of Pharmaceutical Sciences, College of Pharmacy and Health Sciences,St. John's University, Queens, New York, 11439, USA
[b] The Affiliated High School of South China Normal University, Guangzhou, Guangdong, 510630, China
[c] Zhixin High School, Guangzhou, Guangdong, 510000, China
[d] Guangdong Pharmaceutical University, Guangzhou, Guangdong, 510515, China

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ABSTRACT

Cabozantinib (XL184) is a small molecule tyrosine kinase receptor inhibitor, which targets c-Met and VEGFR2. Cabozantinib has been approved by the Food and Drug Administration to treat advanced medullary thyroid cancer and renal cell carcinoma. In the present study, we evaluated the ability of cabozantinib to modulate the function of the ATP-binding cassette subfamily G member 2 (ABCG2) by sensitizing cells that are resistant to ABCG2 substrate antineoplastic drugs. We used a drug-selected resistant cell line H460/MX20 and three ABCG2 stable transfected cell lines ABCG2-482-R2, ABCG2-482-G2, and ABCG2-482-T7, which overexpress ABCG2. Cabozantinib, at non-toxic concentrations (3 or 5 μM), sensitized the ABCG2-overexpressing cells to mitoxantrone, SN-38, and topotecan. Our results indicate that cabozantinib reverses ABCG2-mediated multidrug resistance by antagonizing the drug efflux function of the ABCG2 transporter instead of downregulating its expression. The molecular docking analysis indicates that cabozantinib binds to the drug-binding site of the ABCG2 transporter. Overall, our findings demonstrate that cabozantinib inhibits the ABCG2 transporter function and consequently enhances the effect of the antineoplastic agents that are substrates of ABCG2. Cabozantinib may be a useful agent in anticancer treatment regimens for patients who are resistant to ABCG2 substrate drugs.

1. Introduction

Multidrug resistance (MDR) is one of the major mechanisms responsible for chemotherapeutic failure. MDR is a phenomenon of cancer cells developing resistance to several mechanistically and structurally unrelated antineoplastic drugs [1,2]. Overexpression of ATP binding cassette (ABC) transporters is regarded as one of the major mechanisms of MDR; this leads to increased drug efflux which significantly reduces the intracellular concentration of antineoplastic agents [2,3]. Currently, 48 human ABC transporters have been identified and characterized. ABCB1, or P-glycoprotein (P-gp), was the first to be identified, encoded by the *MDR1* gene [3]. ABCG2 is the second member of subfamily G of the ABC transporters, and is also known as breast cancer resistance protein (BCRP) [4,5]. Unlike ABCB1, which consists of two transmembrane domains (TMDs) and two nucleotide-binding domains (NBDs), ABCG2 is a half-transporter, which has only one NBD and one TMD [6]. Overexpression of the ABCG2 protein is responsible for high levels of resistance to a variety of antineoplatic drugs, including mitoxantrone (MX), anthrapyrazole, topotecan, irinotecan and SN-38 [5,7–11].

It has been demonstrated that overexpression of ABCG2 is responsible for MDR, due to its ability to pump out the substrate anticancer drugs and thus decreasing intracellular concentration of drugs [4,12,13]. Reversing MDR by administering ABCG2 protein inhibitors concurrently with ABCG2 substrate antineoplastic drugs is regarded as a potential pharmacological approach to effective cancer treatment [14–16]. A limited number of ABCG2 inhibitors demonstrate the ability to sensitize drug resistant cells to substrate antineoplastic drugs. Some excellent ABCB1 inhibitors have been found to function as inhibitors of ABCG2 [17–19]. For example, the potent P-gp inhibitor GF120918 is effective in reversing

Abbreviations: MDR, multidrug resistant; ABC transporter, ATP binding cassette transporter; ABCG2, ATP binding cassette subfamily G member 2.

* Corresponding author.
E-mail address: chenz@stjohns.edu (Z.-S. Chen).

[1] Xi-Jun Zhu, Xin-Yue Yu and Ai-Wen Wen are the summer students at St. John's University.

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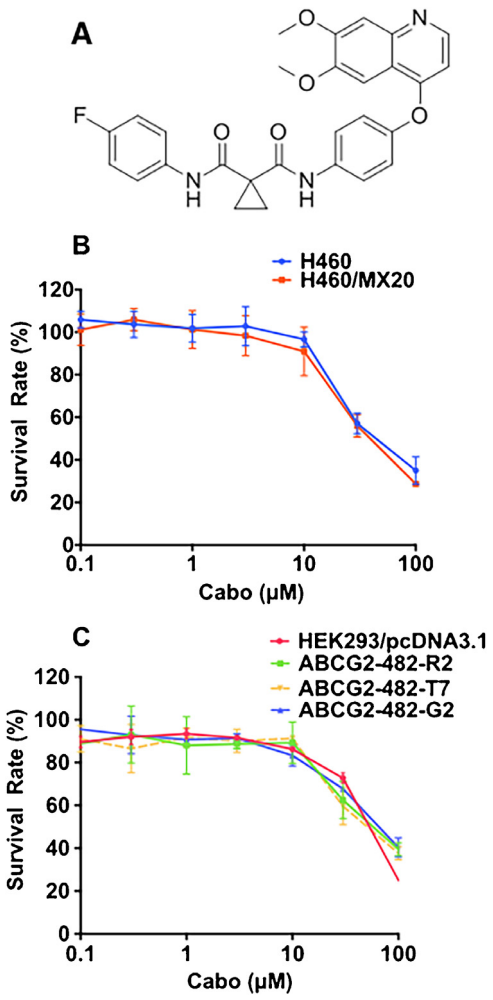

Fig. 1. Cytotoxicity of cabozantinib in parental cell lines and drug resistant cell lines. (A) Structure of cabozantinib. (B) Cytotoxicity of cabozantinib in H460 and H460/MX20 cells. (C) Cytotoxicity of cabozantinib in HEK293/pcDNA3.1, ABCG2-482-R2, ABCG2-482-G2, ABCG2-482-T7 cells.

ABCG2 transporter induced MDR, with an IC_{50} value of 50 nM [20]. Potent dual ABCB1/ABCG2 inhibitors also include tyrosine kinase inhibitors such as imatinib, bafetinib, and nilotinib [17,21,22].

Cabozantinib (XL184) is a tyrosine kinase inhibitor targeting c-Met as well as vascular endothelial growth factor receptor 2 (VEGFR2), which are both dysregulated in various types of cancer [23,24]. On April 25, 2016, cabozantinib was approved by the FDA for the treatment of advanced renal cell carcinoma. Additionally, it has been approved to treat advanced medullary thyroid cancer, and is also being investigated in clinical trials to treat other solid tumors, including renal, prostate, ovarian, and breast cancer [24–28]. The structure of cabozantinib is shown in Fig. 1. In a preclinical study, a lower concentration of cabozantinib was used to inhibit the phosphorylation of HGFR and VEGFR2 in different cancer cell lines [24]. Several phase II studies are being carried out to employ cabozantinib in the treatment of advanced carcinoid and pancreatic neuroendocrine tumors, non-small cell lung cancer, breast cancer, and hepatocellular carcinoma [25,27,29–31]. Patients were given 60 mg of cabozantinib orally per day for the treatment of renal cell carcinoma and non-small-cell lung cancer, and 140 mg per day for the treatment of metastatic medullary thyroid carcinoma [32]. In the present study, we investigate the effect of cabozantinib as a therapeutic compound to increase the chemosensitivity of conventional anticancer drugs through its interaction with the ABCG2 transporter.

2. Materials and methods

2.1. Chemicals

[^{3}H]-MX (4Ci/mmol) was purchased from Moravek Biochemicals, Inc. (Brea, CA). Tween20, NaCl, Tris-HCl, sodium dodecyl sulphate (SDS), Triton X-100, dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-2- yl)-2,5-diphenyltetrazolium bromide (MTT), penicillin/streptomycin, topotecan, SN-38, and MX were purchased from Sigma-Aldrich (St. Louis, MO). Cabozantinib was generously provided by ChemiTex (Brussels Belgique). Fumitremorgin C (FTC) was kindly synthesized by Thomas McCloud, Developmental Therapeutics Program, Natural Products Extraction Laboratory, NCI, NIH (Bethesda, MD). The ABCG2 monoclonal antibody BXP-21 (GTX23380) was purchased from GeneTex (Irvine, CA). The anti-actin monoclonal antibody (sc-8432) was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Dulbecco modified Eagle medium (DMEM), fetal bovine serum (FBS), phosphate buffer saline (PBS), and trypsin 0.25% EDTA were purchased from Hyclone (Waltham, MA). Ammonium molybdate, MES hydrate, antimony potassium tartrate, sodium azide and N-methyl-D-glucamine were purchased from Sigma-Aldrich (St. Louis, MO). Potassium phosphate, EGTA and ATP were products of AMRESCO (Solon, OH). Sulfuric acid solution (37N) was purchased from Fisher Scientific (Pittsburgh, PA). KCl was product of Avantor Performance Materials (Center Valley, PA). Ouabain was purchased from Enzo Life Sciences, Inc. (Farmingdale, NY). Dithiothreitol was a product of Promega Corporation (Madison, WI). $MgCl_2$ was purchased from EMD Millipore (Billerica, MA). Ascorbic acid was a product of VWR International (West Chester, PA). Sodium orthovanadate was purchased from Alfa Aesar (Ward Hill, MA).

2.2. Cell lines and cell culture

The human non-small cell lung carcinoma cell line (NSCLC) H460 was maintained in DMEM based media and its MX resistant cell line, H460/MX20, was maintained in DMEM based media in the presence of 20 nM MX [33]. Wild-type ABCG2-482-R2, mutant ABCG2-482-G2, and mutant ABCG2-482-T7 cells were established as previously described [34]. The HEK293/ABCB1 cell line was generated by transfecting HEK293 cells with vectors that express ABCB1 [19]. Cells were cultured at 37 °C in a humidified incubator containing 5% CO2. When cells were 60% to 80% confluent, they were harvested and used for in vitro studies.

2.3. Cytotoxicity assays

In vitro, the cell sensitivity to each antineoplastic drug was determined by a modified MTT assay [12,35]. Briefly, each well of 96 well plates contained approximately 3000 H460 cells or H460/MX20 cells. Cells were collected, re-suspended in media, and seeded evenly in 96-well plates and incubated for 24 h at 37 °C. Then, 20 μL of a predetermined, fixed concentration of cabozantinib was added into each well. After 1 h of incubation, 20 μL of the respective antineoplastic drugs were added into each well, serially diluted. After 72 h of incubation, 20 μL of 4 mg/mL MTT solution was added to each well. The plates were incubated at 37 °C for 4 h in the presence of the MTT solution. Finally, the MTT-medium was removed and 100 μL DMSO was added to each well to terminate the staining. Absorbance values of the samples were measured at 570 nm using Opsys microplate reader (Dynex Technologies, Chantilly, VA).