Acta Pharmaceutica Sinica B ▮▮▮▮;▮(▮):▮▮▮–▮▮▮

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Acta Pharmaceutica Sinica B

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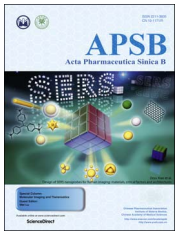

ORIGINAL ARTICLE

Olmutinib (HM61713) reversed multidrug resistance by inhibiting the activity of ATP-binding cassette subfamily G member 2 *in vitro* and *in vivo*

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Received 24 March 2018; received in revised form 30 May 2018; accepted 4 June 2018

KEY WORDS

Olmutinib;
ABCG2;
Multidrug resistance;
Tyrosine kinase inhibitor;
Chemotherapy;
ATPase

Abstract Overexpressing of ATP-binding cassette (ABC) transporters is the essential cause of multidrug resistance (MDR), which is a significant hurdle to the success of chemotherapy in many cancers. Therefore, inhibiting the activity of ABC transporters may be a logical approach to circumvent MDR. Olmutinib is an epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI), which has been approved in South Korea for advanced EGFR T790M-positive non-small cell lung cancer (NSCLC). Here, we found that olmutinib significantly increased the sensitivity of chemotherapy drug in ABCG2-overexpressing cells. Furthermore, olmutinib could also increase the retention of doxorubicin (DOX) and rhodamine 123 (Rho 123) in ABC transporter subfamily G member 2 (ABCG2)-overexpressing cells. In addition, olmutinib was found to stimulate ATPase activity and inhibit photolabeling of ABCG2 with

Abbreviations: ABC, adenosine triphosphate (ATP)-binding cassette; ABCG2, ABC transporter subfamily G member 2; DDP, cisplatin; DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethyl sulfoxide; DOX, doxorubicin; FTC, fumitremorgin C; IAAP, iodoarylazidoprazosin; MDR, multidrug resistance; MTT, 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazoliumbromide; MX, methotrexate; PCR, polymerase chain reaction; Rho 123, rhodamine 123; TKI, tyrosine kinase inhibitor; VRP, verapamil

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Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.

https://doi.org/10.1016/j.apsb.2018.06.002

Please cite this article as: Zhang Zhiqiang, et al. Olmutinib (HM61713) reversed multidrug resistance by inhibiting the activity of ATP-binding cassette subfamily G member 2 *in vitro* and *in vivo*. *Acta Pharmaceutica Sinica B* (2018), https://doi.org/10.1016/j.apsb.2018.06.002

[^{125}I]-iodoarylazidoprazosin (IAAP). However, olmutinib neither altered ABCG2 expression at protein and mRNA levels nor blocked EGFR, Her-2 downstream signaling of AKT and ERK. Importantly, olmutinib enhanced the efficacy of topotecan on the inhibition of S1-MI-80 cell xenograft growth. All the results suggest that olmutinib reverses ABCG2-mediated MDR by binding to ATP bind site of ABCG2 and increasing intracellular chemotherapeutic drug accumulation. Our findings encouraged to further clinical investigation on combination therapy of olmutinib with conventional chemotherapeutic drugs in ABCG2-overexpressing cancer patients.

1. Introduction

Intrinsic or acquired multidrug resistance (MDR) is one of the most common reasons for failure of chemotherapy[1,2]. The common mechanisms that produced MDR in cancer cells include the overexpressing of adenosine triphosphate (ATP)-binding cassette (ABC) transporters, changing in drug targets, alterations in membrane lipids, DNA repair, drug metabolic enzymes, and inhibiting signal pathways of apoptosis[3–5]. One of the predominant mechanisms for MDR is the overexpressing of efflux pumps, which actively extrude a variety of chemotherapeutic drugs from the cancer cells, resulting in drug resistance. To date, in the human genome, 49 members ABC transporters family have been discovered and classified into 7 subfamilies (A–G) based on the sequence similarities as well as structural organization[6]. Among them, ABCB1, ABCC1 and ABCG2 are considered to be accountable for MDR in human cancers[7].

These ABC transporters could utilize the energy to pump out conventional chemotherapeutic drugs, contributing to the failure of chemotherapy. The ABCB1 transporter confers by ATP hydrolysis to reducing the intracellular anticancer drug concentration, resulting in resistance to vincristine, doxorubicin, taxanes and some tyrosine kinase inhibitors (TKIs)[8]. The ABCC1 transporter can pump out a wide spectrum of compounds including anthracyclines, methotrexate, vincristine, epipodophyllotoxins and camptothecins[9]. The ABCG2 transporter was identified from human placenta[10,11]. The ABCG2 expression has been reported in various tumors, such as gastric cancer, colon cancer, lung cancer and melanoma[12]. It could transport a lot of cytotoxic compounds out of cells, such as methotrexate, topotecan and methotrexate[13]. In addition, compared to irinotecan-naive metastases, the *ABCG2* mRNA significantly increased in irinotecan treated hepatic metastases[14].

Developing inhibitors targeted ABC transporters is a promising strategy to overcome MDR. So far, many modulators of ABCG2 have been found and continue to increase. However, there is no commercial available ABCG2 modulator in clinic due to unpredictable adverse reactions and additional toxicity[15]. TKIs are a new class of anticancer drugs that inhibit cancer development, proliferation, metastasis, invasion, angiogenesis. But new resistance to TKIs has been well documented owing to clinical application in great quantities[16]. Some studies have shown that overexpressing ABC transporters were not only developed to MDR but also affected pharmacokinetics (absorption, distribution, metabolism, and excretion) and toxicity of various antineoplastic agents, including TKIs[17]. Recent reports have demonstrated that at clinically accomplishable concentration, some TKIs could inhibit drug efflux function of ABC transporters by directly binding to the drug-binding sites on these transporters, thereby reversing ABC transporter-mediated MDR to conventional chemotherapeutic drugs in cancer cells[18,19]. Therefore, TKIs is possible to be developed as a novel, potent and nontoxic inhibitors of the efflux protein, providing a promising clinical approach to reverse MDR and thereby increasing the success of chemotherapy.

Olmutinib (HM61713) is an EGFR TKI that binds to cysteine residue near the kinase domain. Olmutinib also potently inhibits the growth of cell lines and xenograft tumors harboring *EGFR* T790M and *EGFR* del19, while having little effect on cell lines with EGFRwt[20,21]. In May 2016, olmutinib was approved for advanced EGFR T790M-positive NSCLC patients who were pretreated with EGFR TKIs in South Korea[22]. But there is no previous study reporting that olmutinib could interact with ABC transporters. Here, for the first time, we investigated the chemosensitizing effect of olmutinib in conjunction with conventional chemotherapeutic to overcome ABCG2-mediated multidrug resistance *in vitro* and *in vivo*.

2. Materials and methods

2.1. Chemicals and reagents

The anti-ABCG2 monoclonal antibody (BXP-21) was purchased from Santa Cruz Biotechnology (Paso Robles, USA). Anti-AKT, anti-phospho AKT, anti-phospho ERK and anti-ERK antibodies were obtained from Santa Cruz Biotechnology (Paso Robles, USA). The antibody against GAPDH was from Kangcheng (Shanghai, China). The MX, DOX, Rho 123, topotecan, DDP, VRP, FTC, dimethyl sulfoxide (DMSO) and 3-(4,5-dimethylthiazol-yl)-2,5-diphenyltetrazolium bromide (MTT) were products of Chemical Co. (St. Louis, USA). Olmutinib (HM61713) was purchased from MCE (New Jersey, USA). Dulbecco's modified Eagle's medium (DMEM), RPMI-1640 and fetal bovine serum (FBS) were products of Gibco BRL (Gaithersburg, USA). Penicillin, streptomycin and trypsin were obtained from Hyclone Thermo Scientific (Logan, UT, USA).

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