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Inhibition of P-glycoprotein functionality by vandetanib may reverse cancer cell resistance to doxorubicin

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ABSTRACT

P-glycoprotein belongs to the ATP binding cassette transporters, responsible for the multidrug resistance of cancer cells. These transporters efflux hydrophobic drugs outside cells and decrease their therapeutic efficacy. The aim of this study was to investigate the effect of vandetanib, an oral tyrosine kinase inhibitor of EGFR, VEGFR 2 and RET kinases, on the functionality of P-gp after a 24 h-treatment at therapeutic concentration (2 μM), and its ability to increase the cytotoxicity of chemotherapeutic agents in multidrug resistance cancer cells. In this study we found that IGROV1-DXR and IGROV1-CDDP cells were resistant to doxorubicin and cisplatin respectively, compare to parental cell line IGROV1. The parental sensitive and the two resistant cell lines similarly expressed MRP1 and did not express BCRP. Moreover, in contrast to the IGROV1 and IGROV1-CDDP cells, IGROV1-DXR cell line overexpressed P-gp. Functional activity studies demonstrated that MRP1 was not functional and the MDR phenotype in IGROV1-DXR cells was linked to P-gp functionality. Results also showed that vandetanib reversed resistance to doxorubicin in IGROV1-DXR cells, but not to cisplatin in IGROV1-CDDP cells. After 24 h of treatment, vandetanib increased the accumulation of rhodamine 123 and calcein AM, demonstrating a functional inhibition of the transporter. In IGROV1-DXR cell line, vandetanib reverse resistance to doxorubicin by inhibiting the functionality of P-gp. In conclusion, vandetanib should be an option for drug combination in patients already developing a P-gp mediated multidrug resistance.

1. Introduction

Chemotherapy failure is related to the resistance of tumor cells against anticancer agents (Wu et al., 2011). One of the well-established mechanisms of resistance is the MultiDrug Resistance (MDR) process, linked to increased expression of the ABC (ATP binding cassette) transporters (Leonard et al., 2003). These transmembrane glycoproteins efflux a variety of molecules, including chemotherapeutic drugs, and thus, decrease their efficacy (Gottesman et al., 2002; Perez-Tomas, 2006). Among the ABC transporters responsible for the MDR, the P-glycoprotein (P-gp) acts by pumping substrates out of tumor cells through an ATP-dependant mechanism in a unidirectional manner (Dean et al., 2001). In tumor cells expressing P-gp, the intracellular drug concentration is reduced, decreasing the cytotoxicity of a broad spectrum of hydrophobic drugs including vinca alkaloids, anthracyclines and taxanes (Szakacs et al., 2006). On the other hand, a variety of compounds identified as P-gp inhibitors has been reported to reverse MDR of many chemotherapies, and therefore, to restore the cytotoxicity by blocking their P-gp- mediated transport (Coburger et al., 2009; Germann et al., 1997; Hyafil et al., 1993; Qadir et al., 2005).

Among recent approaches used to treat cancer, therapeutic agents were developed considering the approach of inhibition of tyrosine kinases, key regulators in critical cellular processes such as proliferation, differentiation, survival and metabolism (Blume-Jensen and Hunter, 2001). These tyrosine kinase inhibitors (TKI), often designed as targeted therapies, act by reversible competition against ATP binding to the intracellular catalytic domain of oncogenic tyrosine kinases, such as EGFR (Epidermal Growth Factor Receptor), VEGFR 2 (Vascular Endothelial Growth Factor Receptor 2), FGRF (Fibroblast Growth Factor Receptor) and RET kinases (Rearranged during Transfection), thereby inhibiting cell growth. TKI have been seen to be effective in a large variety of cancers. Recently, a number of studies suggest that several TKI, such as imatinib, gefitinib, and erlotinib, can inhibit the activity of some ABC

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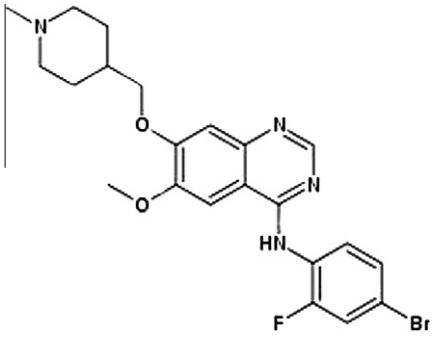

Fig. 1. Chemical structure of vandetanib.

transporters, including P-gp, MRP1 (Multidrug resistance Related Protein 1) and BCRP (Breast Cancer Resistance Protein) (Hamada et al., 2003; Hegedus et al., 2002; Shi et al., 2007). Consequently, they are able to reverse resistance to chemotherapy in multidrug-resistant cancer cells expressing ABC transporters. Among TKIs, the *N*-(4-bromo-2-fluorophenyl)-6-methoxy-7-[(1-methylpiperidin-4-yl)methoxy]quinazolin-4-amine (Fig. 1), named vandetanib (or ZD6474, Caprelsa®-Astra-Zeneca) is a small-molecule tyrosine kinase inhibitor that inhibits EGFR, VEGFR2 and RET kinases (Ryan and Wedge, 2005). Vandetanib (300 mg/d) is the only effective therapeutic option, approved by FDA, in patients with locally advanced or metastatic medullary thyroid carcinoma (Wells et al., 2010). Results from a phase III trial showed the efficiency of the association docetaxel-vandetanib for the treatment of non small-cell lung cancers (Herbst et al., 2010).

In this study, we investigated whether vandetanib could increase the cytotoxicity of two widely used chemotherapeutic agents, namely doxorubicin and cisplatin, in multidrug-resistant cancer cell lines. We first characterized the pharmacological properties of the established resistant cells and, then, we demonstrated that, after a 24 h-exposure, vandetanib is clearly effective in reversing of resistance to doxorubicin, by inhibiting the functionality of P-gp.

2. Material and methods

2.1. Material

Dulbecco's modified Eagle's medium (DMEM), bovine serum and penicillin/streptomycin were purchased from Fischer Scientific (Illkirch, France). MTT, pepstatin, aprotinin, leupeptine, Triton 100×, Bicinchoninic acid protein Assay Reagent Kit, anti-βactin antibody (AC74), rhodamine 123 and probenecid were from Sigma–Aldrich Chemical Co (St Quentin, France). Western Lightning®-ECL was from Perkin Elmer (Courtaboeuf, France). Trizol, superscript II Reverse Transcriptase, oligo-dT and Calcein AM were from Invitrogen (Courtaboeuf, France). 1× PCR SSO Evagreen reagent was purchased from Biorad (Marnes-la-coquette, France). Anti-P-gp antibody (C219) and the secondary anti horseradish peroxidase-conjugated antibodies were from Dako Corporation (Glostrup, Denmark). The anti-MRP1 antibody (A23) was from Enzo Life Sciences (Lyon, france) and the anti-BCRP antibody (BXP21) from Chemicon international (Hampshire, UK) Elacridar was from GlaxoSmithKline (Marly-le-roy, France), cyclosporine A from Novartis (Basel, Switzerland)) and vandetanib from Astra-Zeneca (England). Doxorubicin was purchased from Teva (La defense, France) and cisplatin was obtained from Mylan (St Priest, France).

2.2. Establishment of sensitive and resistant cell lines

Two resistant cell lines, namely the doxorubicin-resistant cell line (IGROV1-DXR) and the cisplatin-resistant cell line (IGROV1-CDDP), were derived from the parental drug-sensitive IGROV1 cells. IGROV1 cell line was derived from a stage III human ovarian carcinoma. The characterization of the parental sensitive IGROV1 has been reported previously (Benard et al., 1985).

The IGROV1-DXR cells and IGROV1-CDDP cells were derived from IGROV1 cells by continuous exposure of these cells to increasing concentrations of either doxorubicin (DXR) or cisplatin (CDDP) in a gradual step-wise manner, up to 0.1 μg/mL for DXR and up to 1 μg/mL for CDDP. Cell lines were grown as adherent monolayers in DMEM supplemented with 10% bovine serum, 100 units/mL penicillin, 100 μg/mL streptomycin under standard cell culture conditions in a humidified incubator containing 5% CO_2 at 37 °C. IGROV1-DXR cells and IGROV1-CDDP cells were maintained in media supplemented with DXR (0.1 μg/mL) and CDDP (1 μg/mL), respectively.

2.3. Growth-inhibition assay

The tetrazolium dye MTT (bromure de 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium) assay was used to evaluate the cytotoxicity of the different drug concentrations, according to the procedure described by (Mosmann, 1983). IGROV1, IGROV1-DXR and IGROV1-CDDP cells, grown in 24-well dishes, were treated with DXR (varying concentrations, up to 20 μg/mL), CDDP (varying concentrations, up to 20 μg/mL), vandetanib (1 or 2 μM), elacridar (Elac 10 μM), a specific inhibitor of P-gp and BCRP (Szakacs et al., 2006), probenecid (prob 1 mM), an MRP inhibitor (Gollapudi et al., 1997), or cyclosporine A (CsA 10 μM), an inhibitor of P-gp and MRP (Qadir et al., 2005; Szakacs et al., 2006), for 24 h (prepared in culture medium) and then were replaced in fresh culture medium for 48 h. Then, medium was removed, 500 μL of MTT solution (0.5 g/L in PBS) were added to each well and the plates were incubated for a further 4 h at 37 °C. The MTT solution was aspirated from each well and 250 μL of isopropanol-HCl were added and mixed thoroughly to completely dissolve the dark blue formazan crystals. Optical density values (OD) were measured at 570 nm using a multiwell-scanning spectrofluometer (MRX II microplate reader, Dynex Technologies). The IC_{50} value was calculated from survival curves. We used inhibitory effect Emax model. Kinetic analyses were carried out using nonlinear regression with the WinNonLin® software (Pharsight, California, USA). The resistance ratio was estimated by dividing the IC_{50} for the MDR cells by that of parental cells. The fold-reversal factor was calculated by dividing the IC_{50} of DXR or CDDP in the absence of vandetanib by that obtained in the presence of vandetanib.

2.4. RNA extraction and reverse transcription-real time polymerase chain reaction (RT-qPCR)

Total mRNA from the parental (IGROV1) cell line, the doxorubicin-resistant cell line (IGROV1-DXR) and the cisplatin-resistant cell line (IGROV1-CDDP) was extracted using Trizol reagent, according to the protocol provided by the manufacturer. The RNA was quantified in ultrapure water by spectrophotometry (wavelength 260 nm), using a Biophotometer (Eppendorf). Integrity of RNA after extraction was checked with the Bioanalyser 2100 and the RNA 6000 Nano LabChip Kit (Agilent technologies). One microgram of total RNA was reverse transcribed (RT) to cDNA by Superscript II reverse transcriptase using oligo-dT as a primer. For PCR amplification of cDNA, 4 μL of the 1:50 diluted RT products was added to 6 μL of solution containing 0.5 μM of each forward and reverse primer and 1× PCR SSO Evagreen reagent. PCRs were carried out using