European Journal of Pharmaceutical Sciences 48 (2013) 644–649

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmaceutical Sciences

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

PXR-mediated P-glycoprotein induction by small molecule tyrosine kinase inhibitors

S. Harmsen [a], I. Meijerman [a,*], R.F. Maas-Bakker [a], J.H. Beijnen [a,c], J.H.M. Schellens [a,b,d]

[a] Utrecht University, Faculty of Science, Department of Pharmaceutical Sciences, Universiteitsweg 99, 3584 CG Utrecht, The Netherlands
[b] The Netherlands Cancer Institute, Division of Experimental Therapy, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands
[c] Slotervaart Hospital, Department of Pharmacy & Pharmacology, Louwesweg 6, 1066 EC Amsterdam, The Netherlands
[d] The Netherlands Cancer Institute, Department of Medical Oncology, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands

ARTICLE INFO

Article history:
Received 10 September 2012
Received in revised form 10 December 2012
Accepted 10 December 2012
Available online 28 December 2012

Keywords:
PXR
Tyrosine kinase inhibitors
Multidrug resistance
P-glycoprotein

ABSTRACT

The rapid development of drug resistance as a result of exposure to small molecule tyrosine kinase inhibitors (TKIs) is an important drawback to the successful use of these agents in the clinic. Although one of the most established mechanisms by which cells acquire drug resistance to anticancer drugs is the up regulation of drug efflux transporters such as P-glycoprotein (PGP), it is currently still unknown whether TKIs have the propensity to induce PGP. The effect of TKIs on the protein expression and activity of PGP was assessed after treatment of LS180 cells with clinically relevant concentrations of the TKIs. In addition, the involvement of the nuclear pregnane X receptor (PXR), a known regulator of PGP expression, was determined. At least five out of the nine tested TKIs (erlotinib, gefitinib, nilotinib, sorafenib, vandetanib) were able to induce the expression of PGP within 48 h in LS180 cells. Accordingly, these TKIs were also shown to affect the accumulation of a P-glycoprotein specific probe substrate. Furthermore, we showed that the pregnane X receptor (PXR), which is an important regulator of PGP induction, is involved in the upregulation of PGP protein expression following exposure to these TKIs. Our data show that PXR-mediated upregulation of PGP expression by TKIs might be a possible mechanism underlying acquired drug resistance in cancer cells.

1. Introduction

Small molecule tyrosine kinase inhibitors (TKIs) have become an important class of anticancer drugs. Their targets, protein tyrosine kinases, are crucial mediators in signaling pathways controlling cell proliferation, differentiation and apoptosis. Deregulation of protein tyrosine kinases as a result of mutations or altered expression is a major underlying cause of cancer and often potentiates the malignant phenotype. Generally, small molecule TKIs have been designed to compete with the ATP-binding site of the intracellular catalytic domains of the oncogenic protein tyrosine kinases, thereby preventing (auto)phosphorylation and subsequent propagation of downstream signals, which ultimately initiates cancer cell death.

The clinical efficacy of TKIs, however, is compromised by acquired drug resistance of cancer cells. Mechanisms that underlie the development of anticancer drug-induced resistance include target protein mutations, altered target protein expression, alternative pathway activation, or constitutive activation of downstream signaling effectors (Zhang et al., 2009). One of the most established mechanisms behind acquired drug resistance in response to anticancer drugs is the upregulation of ATP-binding cassette (ABC) drug efflux transporters (Gottesman, 2002). An important member of this family that has been implicated in (multi)drug resistance is P-glycoprotein (PGP, ABCB1, MDR1; Aszalos, 2007). P-glycoprotein has a pivotal role in the uptake and extrusion of a wide variety of substrates including clinically relevant anticancer drugs such as the taxanes, anthracyclines, epipodophyllotoxins, and campthothecins. Recently, it was also shown that several TKIs such as dasatinib, erlotinib, gefitinib, imatinib, and lapatinib are also transported by PGP (Hegedus et al., 2009; de Vries et al., 2012). Since the clinical efficacy of TKIs is highly dependent on the accessibility of these agents to their intracellular targets, increased PGP-mediated extrusion of TKIs will result in decreased intracellular accumulation, which in turn reduces the clinical efficacy of TKIs.

Abbreviations: ABC, ATP-binding cassette; ATP, adenosine triphosphate; DMSO, dimethylsulfoxide; MDCKII, Madin Darby canine kidney II – MDCKII; MDR1, multidrug resistance protein 1; NR, nuclear receptor; PGP, P-glycoprotein; PXR, pregnane X receptor; siRNA, short inhibitory rinonucleic acid; TKI, tyrosine kinase inhibitor.

* Corresponding author. Tel.: +31 6 20290976.
E-mail address: i.meijerman@uu.nl (I. Meijerman).

0928-0987/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ejps.2012.12.019

Although anticancer drugs, such as paclitaxel and several other widely used agents, were already shown to induce PGP expression (Harmsen et al., 2010), it is still unknown whether also TKIs have the propensity to upregulate PGP expression. Therefore, we determined whether TKIs are able to induce PGP in cancer cells and if TKI-mediated upregulation of PGP affects the accumulation of a PGP probe-substrate. In addition, since the pregnane X receptor (PXR; NR1I2) is an important regulator of xenobiotic-mediated upregulation of PGP (Geick et al., 2001), we examined if this ligand-activated nuclear receptor is involved in the regulation of PGP induction by TKIs.

2. Materials and methods

2.1. Materials

The human colon carcinoma-derived cell line LS180 was obtained from American Type Culture Collection (ATCC-LGC Standards GmbH, Germany). Madin-Darby canine kidney PGP-overexpressing (MDCK)II-MDR1 cells were provided by the Netherlands Cancer Institute (Amsterdam, the Netherlands). All human cell culture media and reagents were purchased from PAA Laboratories (Colbe, Germany) unless otherwise indicated. Fetal bovine serum (FBS) was obtained from Invitrogen (Breda, The Netherlands). Dasatinib, erlotinib, gefitinib, imatinib mesylate, lapatinib tosylate, nilotinib, sorafenib tosylate, sunitinib maleate and vandetanib (Sequoia Research Products Ltd., Pangbourne, UK) were dissolved in DMSO. Zosuquidar (LY335979) was provided by the Netherlands Cancer Institute (Amsterdam, the Netherlands). All other chemicals were of the highest available grade and purchased from Sigma Aldrich (Zwijndrecht, The Netherlands).

2.2. Cell culture

LS180 cells were cultured in RPMI 1640 containing 10% FBS, 2 mM L-glutamine and 25 mM HEPES, supplemented with antibiotics. MDCKII-MDR1 were cultured in DMEM containing 10% FBS, 2 mM L-glutamine, supplemented with antibiotics. All cells were maintained in an incubator with a humidified atmosphere of 5% CO_2 at 37 °C.

2.3. siRNA transfection and TKI treatment

LS180 cells were subjected to transfections with siRNA targeting PXR (sense: cguuuguucgcuuccugagtt; antisense: cucaggaagcgaacaaacgtg), and a scrambled siRNA control, with Lipofectamine RNAiMAX in parallel in 96 well plates. Briefly, LS180 cells (2.5×10^4 cells/well) were reversely transfected with 10 nM siRNA Silencer duplexes (Ambion, Foster City, USA) according to the manufacturer's protocol for Lipofectamine RNAiMAX (Invitrogen). Following 48 h transfection, the TKI treatments were initiated. Cells were treated with clinically relevant concentrations of dasatinib (0.1 μM), erlotinib (10 μM), gefitinib (10 μM), imatinib (10 μM), lapatinib (10 μM), nilotinib (10 μM), sorafenib (1 μM), sunitinib (10 μM), and vandetanib (10 μM). At the end of the 48 h TKI exposure, cells were processed for immunoblotting assays.

2.4. Immunodetection of PGP and PXR

Cells were lysed in RIPA buffer (150 mM NaCl, 10 mM Tris, pH 7.2, 0.1% SDS, 1% Triton X-100, 1% sodiumdeoxycholate, 5 mM EDTA and protease inhibitors (Roche, Basal, Switzerland)). Protein content was determined using a bicinchoninic acid protein assay (Pierce, Rockford, IL, USA). Proteins were reduced in Nupage LDS sample buffer (Invitrogen) containing a final concentration of 30 mM dithiothreitol (DTT). Proteins (10 μg) were separated by SDS–polyacrylamide gel electrophoresis on NuPage Novex Bis-Tris precast 4–12% gradient gels (Invitrogen) and transferred to Immobilon-P PVDF membrane (Millipore, Bedford, MA, USA). The membrane was blocked with 3% bovine serum albumine (BSA) in Tween-20 (0.5%)/tris buffered saline (TBS-T), pH 7.4 for 1 h at RT and then incubated overnight with primary anti-PGP (C219; 1:1000) or anti-PXR (H4417; 1:1000) antibody (Abcam, Cambridge, UK) followed by a 1 h incubation with horseradish peroxidase (HRP)-conjugated anti-mouse secondary antibody (1:1000; Thermo Scientific, Germany). The protein bands were visualized using an enhanced chemiluminescence-based detection reagent (West Femto; Thermo Scientific, Germany) and the intensities of the PGP and PXR bands were determined on a ChemiDoc XRS Imaging system and analyzed with Quantity One analysis software (Bio-Rad, Hercules, CA, USA).

2.5. Rhodamine 123 accumulation assay

The efflux activity of PGP was determined by measuring the accumulation of the fluorescent PGP probe rhodamine 123 as described by Collett et al.Collett et al. (2004). In brief, LS180 cells were pretreated with the TKIs. After 48 h, the cells were washed with Hank's Balanced Salt Solution (HBSS) and incubated at 37 °C for 1 h with rhodamine 123 (10 μM) in the presence or absence of the PGP-specific inhibitor zosuquidar (LY335979; 10 μM; Sheppard et al., 2003) in phenol red-free RPMI medium. After washing thrice with ice-cold HBSS, the cells were lysed with 0.1% (v/v) triton X-100. Fluorescence of rhodamine 123 in the cell lysates was measured with a Mithras LB940 plate reader (Berthold Technologies GmbH & Co. KG, Bad Wildbad, Germany). The ratio of intracellular rhodamine 123 concentrations in the absence and presence of 10 μM zosuquidar is indicative for the efflux activity of PGP.

2.6. P-glycoprotein inhibition assay

MDCKII-MDR1 cells were plated at a concentration of 2.5×10^5 cells/well in 24-well plates. After 24 h, cells were pre-incubated with 0.1% DMSO, dasatinib (0.1 μM), erlotinib (10 μM), gefitinib (10 μM), imatinib (10 μM), lapatinib (10 μM), nilotinib (10 μM), sorafenib (1 μM), sunitinib (10 μM), and vandetanib (10 μM) and 10 μM zosuquidar in phenol red-free DMEM medium. After 30 min, 10 μM rhodamine 123 was added. Following a 1-h incubation with rhodamine 123, cells were washed with ice-cold PBS thrice and lyzed with 0.1% (v/v) triton X-100. Fluorescence of rhodamine 123 in the MDCKII-MDR1 cell lysates was measured with a Mithras LB940 plate reader (Berthold Technologies GmbH & Co. KG, Bad Wildbad, Germany).

2.7. Statistical analysis

The Student's *t* test (two tails) was used to analyze the difference between two groups. Multiple group comparisons were analyzed using ANOVA with Bonferroni post hoc testing and were considered significant when $P < 0.05$. The statistical tests were done using SPSS version 14 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Induction of P-glycoprotein by small molecule tyrosine kinase inhibitors

The effect of TKIs on the protein expression of PGP was determined in LS180 cells that were treated for 48 h with nine TKIs;