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P-glycoprotein (MDR1/ABCB1) and breast cancer resistance protein (BCRP/ABCG2) restrict brain accumulation of the JAK1/2 inhibitor, CYT387

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ABSTRACT

CYT387 is an orally bioavailable, small molecule inhibitor of Janus family of tyrosine kinases (JAK) 1 and 2. It is currently undergoing Phase I/II clinical trials for the treatment of myelofibrosis and myeloproliferative neoplasms. We aimed to establish whether the multidrug efflux transporters P-glycoprotein (P-gp; MDR1; ABCB1) and breast cancer resistance protein (BCRP;ABCG2) restrict oral availability and brain penetration of CYT387. In vitro, CYT387 was efficiently transported by both human MDR1 and BCRP, and very efficiently by mouse Bcrp1 and its transport could be inhibited by specific MDR1 inhibitor, zosuquidar and/or specific BCRP inhibitor, Ko143. CYT387 (10 mg/kg) was orally administered to wild-type (WT), Bcrp1$^{-/-}$, Mdr1a/1b$^{-/-}$ and Bcrp1;Mdr1a/1b$^{-/-}$ mice and plasma and brain concentrations were analyzed. Over 8 h, systemic exposure of CYT387 was similar between all the strains, indicating that these transporters do not substantially limit oral availability of CYT387. Despite the similar systemic exposure, brain accumulation of CYT387 was increased 10.5- and 56-fold in the Bcrp1;Mdr1a/1b$^{-/-}$ mice compared to the WT strain at 2 and 8 h after CYT387 administration, respectively. In single Bcrp1$^{-/-}$ mice, brain accumulation of CYT387 was more substantially increased than in Mdr1a/1b$^{-/-}$ mice, suggesting that CYT387 is a slightly better substrate of Bcrp1 than of Mdr1a at the blood–brain barrier. These results indicate a marked and additive role of Bcrp1 and Mdr1a/1b in restricting brain penetration of CYT387, potentially limiting efficacy of this compound against brain (micro) metastases positioned behind a functional blood–brain barrier.

1. Introduction

ATP-binding cassette (ABC) drug efflux transporters, such as P-glycoprotein (P-gp;MDR1;ABCB1) and breast cancer resistance protein (BCRP;ABCG2) are widely expressed in different tissues (e.g. small intestine, liver, blood–brain barrier) and play important roles in the absorption, distribution, excretion and toxicity of xenobiotics. Many anti-cancer drugs, including several tyrosine kinase inhibitors (TKIs), are substrates of both MDR1 and BCRP, and their interaction with ABC transporters may affect pharmacokinetics, therapeutic efficacy, and toxicity of these drugs in patients. Indeed, several chemotherapeutic agents that are MDR1 and BCRP substrates have restricted brain penetration [1–9]. Improving brain penetration of drugs is of long-standing interest in the clinic, because current systemic therapies are often inefficient in eradicating brain metastases or tumor parts or rims that are behind an intact blood–brain barrier (BBB) [10,11].

Janus kinases (JAK) 1 and 2 are well-characterized signaling kinases, implicated in various signaling pathways that are exploited by malignant cells. They contribute to the pathogenesis of myeloproliferative neoplasms (MPNs), blood disorders that result from an excess production of hematological cells [12–15]. Therefore, recently several small molecule inhibitors for JAK1 and 2 family kinases have been developed, one of which was an ATP-competitive small molecule inhibitor, CYT387 (Fig. 1), with a broad therapeutic activity [16–20].

The inhibitory effect of CYT387 alone or in combination with other conventional drugs on multiple myeloma

Abbreviations: AUC, area under the plasma concentration–time curve; Cmax, maximum drug concentration in plasma; Tmax, the time after administration of a drug when the maximum plasma concentration is reached; BBB, blood–brain barrier; BCRP, breast cancer resistance protein; MDR1, multidrug resistance protein; SD, standard deviation.

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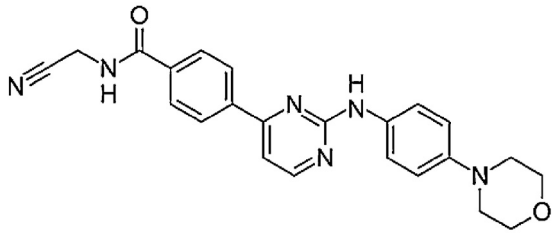

Fig. 1. Chemical structure of CYT387.

proliferation has been demonstrated in vitro using human myeloma cell lines and in vivo using a murine MPN model [16,21]. This drug is currently undergoing Phase I/II clinical trials for the targeted treatment of myelofibrosis, a frequently fatal myeloproliferative neoplasm. With the preliminary data showing significant and durable anemia responses and favorable toxicity profile, CYT387 is so far the best candidate among JAK inhibitors for the management of myelofibrosis in patients (http://www.ymbiosciences.net/upload_files/CYT387_poster_ASCO2011.pdf). Besides involvement in MPN pathogenesis, JAKs are implicated in other disorders including inflammatory and immune-mediated diseases. Therefore, several JAK inhibitors are being investigated for therapeutic activity in other neoplastic and rheumatological disorders, and allograft rejection [17,22]. CYT387 is one of these inhibitors, regarded to have potential for treating other myeloproliferative neoplasms, solid and hematological malignancies, and inflammatory conditions.

CYT387 is administered orally in the clinic. Thus, depending on its interactions with ABC transporters, these might have a significant impact on oral bioavailability and tissue or tumor distribution of CYT387 and thus determine the therapeutic efficacy on both primary tumors and metastases. In this study, we investigated the effect of MDR1 and BCRP on the in vitro transport and in vivo disposition of CYT387.

2. Materials and methods

2.1. Chemicals

CYT387 (H_2SO_4, sulfuric acid salt) was obtained from Sequoia Research Products (Pangbourne, UK). Zosuquidar (Eli Lilly; Indianapolis, USA) was a kind gift from Dr. O. van Tellingen (The Netherlands Cancer Institute, Amsterdam, NL) and Ko143 was obtained from Tocris Bioscience (Bristol, UK). All chemicals used in the chromatographic CYT387 assay were described before [23].

2.2. Transport assays

Polarized canine kidney MDCKII cell lines and subclones transduced with hMDR1, hBCRP, and mBcrp1 cDNA were used and cultured as described previously [7]. Transport assays were performed using 12-well Transwell® plates (Corning Inc., USA). The parental cells and variant subclones were seeded at a density of 3.5 and 2.5×10^5 cells per well, respectively, and cultured for 3 days to form an intact monolayer. Membrane tightness was assessed by measurement of transepithelial electrical resistance (TEER). Preceding the transport experiment, cells were washed twice with PBS and pre-incubated with fresh DMEM medium (Invitrogen, USA) including 10% FBS (Sigma–Aldrich, USA), and with relevant inhibitors for 1 h, if required. The transepithelial transport experiment was started ($t = 0$) by replacing the incubation medium with medium containing 5 μM CYT387 in the donor compartment. In the inhibition experiments, 5 μM zosuquidar (MDR1 inhibitor), 5 μM Ko143 (BCRP/Bcrp1 inhibitor) or 1 μM elacridar (dual MDR1 and BCRP/Bcrp1 inhibitor) were added to both apical and basolateral compartments. Plates were kept at 37 °C in 5% CO_2 during the experiment, and 50 μl aliquots were taken from the acceptor compartment at 2, 4, 8 and 24 h. CYT387 concentrations were measured by LC–MS/MS. Total amount of drug transported to the acceptor compartment was calculated after correction for volume loss for each time point. Experiments were performed in triplicate and the mean amount of transport is shown in the graphs. Active transport was expressed by the relative transport ratio (r), defined as r = apically directed amount of transport divided by basolaterally directed amount of translocation, at a defined time point.

2.3. Animals

Mice were housed and handled according to institutional guidelines complying with Dutch legislation. Animals used were female WT, Mdr1a/1b$^{-/-}$, Bcrp1$^{-/-}$ and Mdr1a/1b$^{-/-}$;Bcrp1$^{-/-}$ mice of a >99% FVB genetic background, between 8 and 10 weeks of age. Animals were kept in a temperature-controlled environment with a 12 h light/12 h dark cycle and received a standard diet (AM-II, Hope Farms) and acidified water ad libitum.

2.4. Drug solutions

1 mg/ml CYT387 solution was obtained by dissolving the drug in dimethylsulfoxide (20 mg/ml), followed by 20-fold dilution in 20% Polysorbate 80, 13% ethanol and 67% H2O vehicle mix and 5% glucose in a ratio of 1:1 (v/v). Mice received a bolus injection, using a blunt-ended needle, of 10 mg/kg CYT387 orally, using a volume of 10 ml/kg body weight. All working solutions were prepared freshly on the day of experiment.

2.5. Plasma and brain pharmacokinetics

Mice were fasted about 2 h before CYT387 was orally administered in order to minimize the variation in absorption. For plasma pharmacokinetic studies, multiple blood samples (60 μl) were collected from the tail vein at 15 and 30 min and 1, 2 and 4 h using heparinized capillary tubes (Sarstedt, Germany). At 2 (in a separate experiment) or 8 h, mice were anesthetized with isoflurane and blood was collected by cardiac puncture. Immediately thereafter, mice were sacrificed by cervical dislocation and brains and a set of organs were rapidly removed. Organs were homogenized on ice in 1% (w/v) bovine serum albumin, and stored at −20 °C until analysis. Blood samples were centrifuged at $2100 \times g$ for 6 min at 4 °C immediately after collection; the plasma fraction was collected and stored at −20 °C until analysis.

2.6. Relative brain accumulation

Relative brain accumulation after oral administration of CYT387 was calculated by determining the CYT387 brain concentration relative to the plasma AUC from 0 to 2 or 0 to 8 h. Brain concentrations at 2 and 8 h were corrected for the amount of drug present in plasma volume (1.4%) in the brain vasculature.

2.7. Drug analysis

CYT387 concentrations in cell culture medium, plasma samples and brain homogenates were determined using liquid chromatography-electrospray–tandem mass spectrometry (LC–MS/MS) based on an assay reported for human plasma [23]. Shortly, samples were pre-treated using protein precipitation with acetonitrile containing cediranib as internal standard. Water diluted extracts were injected onto a sub-2 μm particle, trifunctional bonded octadecyl silica column;a gradient using 0.005% (v/v)

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