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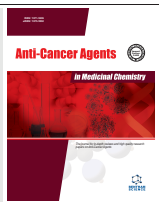
Anti-Cancer Agents in Medicinal Chemistry, 2016, *16*, 810-815

Blood-Brain Barrier and Breast Cancer Resistance Protein: A Limit to the Therapy of CNS Tumors and Neurodegenerative Diseases



Anna Lisa Iorio[1], Martina da Ros[1], Ornella Fantappiè[2], Maurizio Lucchesi[1], Ludovica Facchini[1], Alessia Stival[1], Sabrina Becciani[1], Milena Guidi[1], Claudio Favre[3], Maurizio de Martino[1] Lorenzo Genitori[4] and Iacopo Sardi[1,*]

[1]*Neuro-oncology Unit, Department of Pediatric Medicine, Meyer Children's University Hospital and Department of Health Sciences, University of Florence, Florence, Italy;* [2]*Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy;* [3]*Department of Onco-hematology, Meyer Children's University Hospital, Florence, Italy;* [4]*Neurosurgery Unit, Meyer Children's University Hospital, Florence, Italy*

I. Sardi

Abstract: The treatment of brain tumors and neurodegenerative diseases, represents an ongoing challenge. In Central Nervous System (CNS) the achievement of therapeutic concentration of chemical agents is complicated by the presence of distinct set of efflux proteins, such as ATP-Binding Cassette (ABC) transporters localized on the Blood-Brain Barrier (BBB). The activity of ABC transporters seems to be a common mechanism that underlies the poor response of CNS diseases to therapies.

The molecular characterization of Breast Cancer Resistance Protein (BCRP/ABCG2), as an ABC transporter conferring multidrug resistance (MDR), has stimulated many studies to investigate its activity on the BBB, its involvement in physiology and CNS diseases and its role in limiting the delivery of drugs in CNS.

In this review, we highlight the activity and localization of BCRP on the BBB and the action that this efflux pump has on many conventional drugs or latest generation molecules used for the treatment of CNS tumors and other neurodegenerative diseases.

Keywords: BCRP, Blood-Brain Barrier, brain tumors, multidrug resistance, neurodegenerative diseases.

Received: June 26, 2014 **Revised: October 22, 2014** **Accepted: November 16, 2015**

INTRODUCTION

An important role in the treatment of CNS diseases is played by structure and activity of the BBB, a dynamic interface that separates the brain from the circulatory system, protecting it from potentially harmful chemicals, regulating the transport of essential molecules and maintaining a stable microenvironment [1].

The BBB is made of capillary endothelial cells that are not fenestrated, closely sealed by tight junctions and characterized by the presence of distinct set of transporter proteins, such as human solute carrier, ABC transporters and specific receptors mediating the transcytosis.

Forty-eight types of ABC transporter proteins have currently been identified in humans [2]; among these BCRP has recently gained relevance due to its role in the MDR phenotype [3,4], where this transport protein, linked with P-glycoprotein (P-gp) and Multidrug Resistance-Associated Proteins (MRPs), influences absorption, distribution, metabolism, excretion, and toxicity profile of many drugs.

BCRP was found in many different human tissues including intestine, liver, kidney, testis, placenta, and mammary gland, where it plays its physiological function. Moreover its expression has also been reported in many tumors such as breast, colorectal, head and neck and also in glioblastoma (GBM) cell lines and tumor tissues [5-8]. It is also noteworthy that BCRP seems to be a stem cell marker, whose expression in cancer cells, could be a metabolic event and part of signaling pathways that confer multiple mechanisms of MDR, self-renewal and invasiveness [9].

In this review, we will focus on the role played by BCRP in the transport of chemical agents, such as conventional antineoplastic agents and new molecules, which makes this efflux protein a limiting factor for the treatment of CNS tumors and other neurodegenerative diseases.

BCRP Works in Concert to other MDR Efflux Transporters

In humans, the major efflux pumps responsible for the MDR phenotype are P-gp, BCRP and MRPs [10]; all these transporters belong to the evolutionarily conserved family of the ABC proteins and their structure and function survived to all pressures during evolution [11]. These large plasma membrane proteins, which binds and hydrolyzes ATP, are considered an essential parts of a defense system; their coordinated action is the major contributor against the accumulation of hydrophobic or amphipathic compounds and toxin inside tissues.

The ABC efflux pumps, predominantly located in tissue barriers, recognize a wide range of substrates that remove from the inner to the outer side of cell plasma membrane, or directly into the extracellular space [12].

The best known MDR protein is P-gp. Similar to BCRP, P-gp is principally expressed at the luminal membrane of brain capillary of endothelial cells [13]; moreover, this protein has been identified also in intracellular compartments [14], probably localized in cytoplasmic vesicles, where removes drugs away from their subcellular targets [15].

Tissue distribution of BCRP shows an extensive overlap with P-gp, suggesting that both transporters similarly confer protection from potentially harmful xenobiotics.

Interestingly, as P-gp, also BCRP has been identified in subcellular districts, like mitochondria [16] and perinuclear region [17], where it probably acts as a secondary efflux site of its substrates. Recently, the presence of BCRP has been demonstrated also in the nuclear extracts of human-derived GBM and astrocytoma cell lines. The nuclear membrane expression of BCRP could represents a

*Address correspondence to this author at the Neuro-oncology Unit, Department of Pediatric Medicine, Meyer Children's Hospital. Viale G.Pieraccini 24, 50139 Florence, Italy; Tel: +39 055 5662631; Fax: +39 055 5662746; E-mail: iacopo.sardi@meyer.it

1875-5992/16 $58.00+.00

potentially new mechanism of MDR, regulating the transport and the efficacy of many antineoplastic agents [18].

The combined effect of BCRP and other ABC transporters actually has also been found in a clinical study, in which children with acute lymphoblastic leukemia who were treated with chemotherapy had a significantly increased chance of encephalopathy when they had a specific combination of BCRP and MARP1 polymorphisms compared to when they only had a predisposing genotype for one of the transporters [19].

BCRP Influence CNS Distribution of Conventional Drugs

Knockout models of BCRP have shown the ability of this transporter to reduce the penetration of many substances into the brain [20, 21]; including conventional compounds such as Methotrexate (MTX), Mitoxantrone (MX) and Phenytoin (PHT).

The brain distribution of MTX, a classic antifolate agent commonly used in chemotherapy of primary CNS lymphoma, is severely limited, and only 5% of the free drug in plasma reaches the brain parenchyma crossing BBB [22]; moreover, low cerebral penetration of MTX was observed, in patients with recurrent high-grade gliomas, also by using a microdialysis technique [23]. Recently, *in vivo* studies have confirmed the direct involvement of BCRP in MTX transport across the BBB, showing that the extrusion of this drug is significantly decreased in BCRP knockout model compared with the wild type [24].

MX is an anthracenedione antineoplastic agent with potent *in vitro* activity against malignant brain tumor cell lines; however, its effectiveness as chemotherapeutic drug is hampered by its poor CNS penetration.

In order to evaluate the role of BCRP in limiting the distribution of MX, Cisternino *et al.* have used *in situ* brain perfusion to measure the cerebral uptake of this substrate in *in vivo* model.

P-gp-deficient mutant mice were used to demonstrate that MX transport across the BBB mainly depends on the presence of BCRP, on the luminal membrane of the mouse brain microvessels. This study results indicated that the brain uptake of MX was increased 3.0-fold in P-gp-deficient mice when the drugs were perfused together with the BCRP inhibitor GF120918, showing that this efflux protein represents an important limiting factor of MX distribution in the CNS [25].

In addition to the CNS tumors, BCRP appears to play a key role in MDR phenotype of other neurodegenerative disorders such as Amyotrophic Lateral Sclerosis (ALS), epilepsy, Alzheimer's disease, Parkinson and Human Immunodeficiency Virus (HIV) [26].

ALS is the commonest form of motor neuron disease characterized by extensive inflammation. Mouse models of ALS showed a selective increase in BCRP and P-gp expression, specifically in CNS lesions. Recently, a correlation was found between the transport activity of P-gp/BCRP and disease progression in spinal cord and cerebral cortex capillaries of ALS mouse models (mutant SOD1-G93A and mutant TDP43-A315T mice). This selective increase in expression and activity, of both this two transporters, suggests a highly regulated ALS-driven pharmaco-resistance, and indicates the need to identify strategies to overcome the failures in ALS therapies [27].

Actually, the limited progress in identifying successful therapies in ALS has only resulted in one moderately effective pharmacological agent, riluzole.

Riluzole brain disposition is limited in the ALS mouse model (mutant SOD1-G93A) through interaction with the drug efflux transporters at the BBB [28] and loses effectiveness as disease progresses in this model. Similarly, in patients with ALS, riluzole loses effectiveness in the later stages of disease [29].

Recently, a study by Jablonski *et al.* clearly demonstrate that by blocking P-gp and BCRP, it is possible to enhance riluzole CNS penetration in mice, ultimately restoring its efficacy even when administration begins at onset.

Therefore, revisiting riluzole therapy by blocking P-gp and BCRP with elacridar or similar transporters' inhibitors could be improved quality of life of ALS patients until a more efficacious therapeutic strategy will be identified [30].

Several studies have also revealed a new association of MDR transporters in epileptogenesis and pharmaco-resistant epilepsy [31].

Because of their physicochemical properties, most antiepileptic drugs (AEDs) penetrate through the BBB into the brain by passive diffusion [32]. However, efflux transporters may limit the brain penetration of certain anti-epileptic drugs (AEDs) by actively extruding them back to blood [32, 33].

Indeed, several major AEDs, including PTH, phenobarbital, topiramate, levetiracetam, oxcarbazepine, and lamotrigine, are substrates of human P-gp [32, 33]. Conversely, the role of BCRP and MRPs in AED-resistant epilepsy is less clear.

More recently, by using genetically modified mice that lack either Pgp or BCRP, Nakanishi *et al.* [34] reported that both efflux pumps restrict brain access of several AEDs. This data were confirmed by Romerman *et al.*, who have demonstrated that the major AED lamotrigine is a BCRP substrate by using MDCKII cells, transfected with murine and human efflux transporters. MDCKII cells were used as a well-accepted surrogate BBB model, because these epithelial kidney cells are easy to culture and to transfect, form tight monolayers with restrictive paracellular pathway and BBB-like selective passive permeability, can be used for qualitative predictions of brain distribution, and to distinguish between compounds that pass the BBB by passive diffusion and those that are substrates for active efflux transporters [35, 36].

By using this model, they firstly reported a BCRP-mediated transport of lamotrigine; this observation has important consequence for epilepsy treatment because in AED resistant patients the expression of both transporters is markedly increased. Since lamotrigine is a substrate of both human Pgp and BCRP, overexpression of these transporters may synergistically decrease its brain concentrations, thus causing or contributing to pharmacoresistance, which is the major problem of epilepsy therapy [37].

The role of the ABC proteins has been recently recognized also in the pathology of Alzheimer's disease (AD). In addition to their physiological role as gatekeepers, the role of ABC transporters in neurodegenerative disorders such as AD is also intriguing because they may have a key role to understanding the pathogenesis of this disease.

Recent studies have suggested ABC efflux pumps to play an essential role in controlling the levels of beta amyloid peptides (Aβ) in the brain and that alteration of expression and functional activity of these transporters may contribute to the aggregation of Aβ in the brain, leading to increased risk for developing AD.

The role of BCRP in Aβ extrusion at the BBB was investigated in BCRP knockout and wild type mice after intravenous injection of labeled Aβ. Optical imaging analyses of live animal brains showed that BCRP knockout mice significantly accumulated more Aβ in their brains than wild type mice, suggesting that BCRP may act as a gatekeeper at the BBB to prevent blood Aβ from entering into brain [38].

However, the data that are available are conflicting and, at this point, the role of BCRP in AD is inconsistent and incomplete. While some studies show that BCRP transports Aβ [39, 40] other studies demonstrate that Aβ is not transported by BCRP [41].

Data from AD patient brain samples are also conflicting: BCRP protein levels at the BBB have been reported to be unchanged [42], but another study shows that BCRP expression is increased [43].

These results clearly indicate the need for further investigations about the role of BCRP in the pathology of AD and confirm the validity of its therapeutic targeting in the treatment of AD.

BCRP and new Antineoplastic Approaches

Imatinib mesylate (Gleevec®) is a chemical agent, belongs to the class of tyrosine kinase inhibitors (TKI), commonly used in the treatment of chronic myeloid leukemia (CML) and Gastrintestinal Stromal Tumors (GIST).

Preclinical *in vitro* and *in vivo* studies have shown that imatinib effectively inhibits platelet-derived growth factor, that induces GBM cell growth [44]. However, it has been reported that brain concentration of this TKI is conditioned by the action of BCRP. [^{14}C]imatinib mesylate (12.5 mg/kg) was intravenous (i.v). administered to wild-type, BCRP and P-gp knockout mice; thus, the clearance was determined after measurement of imatinib plasma concentrations by total radioactivity over a 120-minute time period. The clearance of i.v. imatinib resulted 1.6- and 1.25-fold decreased in BCRP and P-gp knockout compared with control mice, showing that BCRP plays an important, and maybe even a more prominent role than P-gp, in the distribution of this drug in *in vivo* model [45].

Dasatinib, a second-generation TKI approved for use in imatinib-resistant CML patients [46], is an extremely strong BCR-ABL inhibitor [47] and also hinders the Src tyrosine kinase, which was identified as a potential target for GBM therapy [48]. Analyzing the intracellular accumulation of [^{14}C]dasatinib, in Mardin Darby canine kidney (MDCKII) wild type, BCRP or P-gp overexpressing cells, Chen *et al.* have observed that cellular delivery of this TKI was significantly limited by active efflux due to both BCRP and P-gp. *In vivo* experiments have also demonstrated dasatinib low brain penetration, in FVB wild-type mice; with brain-to-plasma (B/P) concentration ratios lower than 0.12. Contrariwise, in P-gp/BCRP knockout mice this B/P ratio increased by greater than 10-fold, confirming the central role of these two transporters in limiting brain distribution of this TKI [49].

Recently, it was suggested that sildenafil, a cyclic guanosine monophosphate (cGMP) inhibitor, could strongly inhibit BCRP activity [50] and might be useful to enhance the distribution and potentially the efficacy of anticancer drugs [51]. However, it seems that sildenafil does not allow the BBB penetration of BCRP substrates, and it is not a strong and/or selective inhibitor of ABC-transporters. To determine the effect of this cGMP inhibitor in allow the brain penetration of anticancer agents, P-gp and BCRP knockout mice received 50 mg/kg of sildenafil *per os* (p.o.), followed 30 minutes later by an i.v. dose of docetaxel (33 mg/kg) or topotecan (2 mg/kg); data showed that the brains of knockout mice accumulated significantly more of each drug, indicating that the effect of sildenafil on the brain penetration of both agents was insignificant. Moreover, the brain penetration of sildenafil itself was more than 20-fold higher in knockout *versus* control mice demonstrating that it is an excellent BCRP and/or P-gp substrate [52].

An alternative strategy, developed in order to improve pharmacological availability and effectiveness of drugs into the brain, is to modify the structure of conventional molecules. Pemetrexed (PMX), the last antifolate agent developed from MTX, had shown a strong antitumor activity against a variety of tumors, including CNS malignancies [53]. Unfortunately, similarly to its forerunner, PMX distribution through the BBB is very limited, probably due to MDR efflux pumps cooperation [54].

Molecular Size doesn't Interfere with BBB Permeability

Small molecules such as oxygen, carbon dioxide, glucose, and nucleotide can cross the BBB through a mechanism of passive diffusion.

An interesting unproven theory suggested that passive diffusion could be used to deliver drugs across physiological barrier, in pharmacologically significant amounts, if their molecular mass was between 400-500 Da and their surface area was not greater than 52 Angstroms [55, 56]; but, it has been reported that histamine, an amine of only 100 Da produced as part of a local immune response, readily crosses the capillaries of all peripheral tissues but does not enter into the brain or spinal cord by the BBB [57].

Several small molecules such as gefitinib and erlotinib, have been recently introduced in clinical practice for their ability to inhibit the tyrosine kinase activity of growth factor receptors, such as Epidermal Growth Factor Receptor (EGFR), known to be deregulated in malignant gliomas through overexpression, amplification, and activating mutations, associated with a poor prognosis especially when occurring in younger patients [58].

Consequently, EGFR inhibitors have received a wide interest for implementation in clinical trials for GBM thetapy; unfortunately, a phase II trial shows that patients with recurrent or progressive high-grade glioma, treated with gefitinib, have no objective response to treatment [59].

In the drug development process, it is therefore important to investigate whether candidate agents interact with the major efflux pumps.

For a wide range of drugs that are already used in the clinic, interactions with BCRP and P-gp at the BBB have been shown, especially using knockout mice. In humans, however, it is not straightforward to assess drug transporter interactions at the BBB. To determine whether, as was previously shown for murine P-gp and BCRP [60], gefitinib is also a substrate for human efflux transporters, Vlaming and collaborators have performed cellular accumulation experiments with LLCPK1 cells overexpressing human or murine P-gp, as well as with human MDCKII-cells overexpressing human or murine BCRP. Their results showed that accumulation of [14C]-gefitinib was significantly reduced in LLC-PK1-Pgp cells compared to control. Moreover, like observed for P-gp, they have demonstrated that [14C]-gefitinib was also transported by human and murine BCRP.

Overall, those results indicated that gefitinib is a substrate of both human and murine efflux transporters, albeit with potential species specific differences in their affinities for the drug [61].

An interesting study by de Vries *et al.* used knockout mice models to further investigate the role of ABC trasporters on the brain penetration and efficacy of erlotinib, that seems to be a substrate of BCRP, which actively throws out this small molecule through the BBB limiting its CNS penetration. Wild-type, P-gp and BCRP deficient mice received the drug (50 mg/kg) by intraperitoneal (i.p.) injection; erlotinib concentration were determined in plasma and brain homogenates, by using HPLC assay. The brain Area Under the concentration time Curve (AUC) of erlotinib was highest in P-gp/BCRP knockout (49.6±3.95μg/g*h) and significantly reduced to 31.1±1.7,μg/g*h in P-gp knockout mice; in BCRP knockout, a decrease in brain AUC to 13.0±0.70, μg/g*h was found [62]. Once again, data showed that BCRP, and other efflux proteins, significantly reduce the brain penetration of drugs in wild-type mice compared with P-gp and/or BCRP knockout ones A novel small molecule, vemurafenib, has been recently approved by the FDA for the treatment of patients with metastatic melanoma with a BRAF (V600E) mutation, that results in signaling pathways that promote tumor cell proliferation, invasion, and resistance.

In vitro results showed that vemurafenib intracellular accumulation was 77% lower in MDCKII-BCRP overexpressing cells compared with the parental control line. The difference in accumulation was abolished when a specific BCRP inhibitor, Ko143, was added. Likewise, the accumulation of this drug was 20% lower in MDCKII-P-gp overexpressing line compared with control and, also in this case, the difference in accumulation was abolished when a specific P-gp inhibitor, LY335979, was added; indicating that vemurafenib is a substrate for both P-gp and BCRP.

In agreement with this *in vitro* result, *in vivo* studies using FVB wild-type mice demonstrated that the brain concentrations of vemurafenib were approximately 8- to 30-fold higher in P-gp/BCRP knockout mice than in the wild-type, highlighting the significant impact of P-gp and BCRP on CNS penetration of this drug [63].

Ongoing clinical trials are also evaluating the therapeutic activity of rucaparib, another small molecule, potent inhibitor of poly ADP-ribose polymerase PARP-1 and -2. Rucaparib efficacy, alone or in association with other cytotoxic drugs, was assessed mainly in breast and ovarian cancer patients with mutations in the BRCA gene and in patients with brain micro-metastases located behind a functional BBB.

The *in vivo* disposition of orally administered rucaparib, 10 mg/kg, was evaluated in wild-type, single or combined BCRP and P-gp knockout mice. The plasma availability of rucaparib, over 24 hours, was increased by the absence of BCRP or P-gp 1.7- and 2.5-fold respectively, and by the absence of both by 4-fold compared to control. Moreover the brain concentration of rucaparib, 24 hours after oral administration of the same dose, was significantly increased in BCRP/P-gp knockout by 9.1-fold compared to control mice; suggesting that the availability and brain concentration of this inhibitor are markedly and additively limited by BCRP and P-gp cooperation [64].

CONCLUSION

The treatment of CNS diseases is particularly challenging because the delivery of drug molecules to the brain is often precluded by BBB.

This inability, of many drugs, to reach brain parenchyma can be ascribed to the activity of the ABC efflux transporters, among which BCRP has recently gained relevance due to its role in the MDR phenotype.

Many approaches have been studied to overcome this hurdle, such as the development of new molecules or alternative strategies of administration.

Our group has recently attempted to improve chemotherapy efficacy of CNS tumors by pharmacological modulation of BBB permeabilization. We have demonstrated that morphine (Morph), ondansetron (Ond) and dexamethasone (Dexa), all substrates of BCRP and P-gp commonly used in the management of patients with CNS tumors, allow the accumulation of the chemotherapeutic drug doxorubicin (DOX) within the rat brain.

Our *in vivo* study showed that pretreatment with Morph (10 mg/kg, i.p.) facilitates the delivery of the anticancer agent into the brain, in absence of signs of increased acute systemic toxicity. A quantitative analysis of DOX was performed by LC-MS/MS mass spectrometry; the mean concentration of DOX in the cerebral hemispheres of rodents receiving DOX alone was 5.88±0.34 ng/g fresh tissue *versus* 18.8±1.01 ng/g fresh tissue in those treated with DOX *plus* Morph [65, 66]. Thus, it is conceivable that Morph competing with the efflux transporters localized on BBB could increase the access of DOX to the brain [67].

Similarly, we have reported the ability of Ond and Dexa to allow the brain accumulation of DOX within the rat brain. Ond is a selective 5-hydroxytryptamine(3) (5-HT(3)) receptor antagonist, mainly used in clinical practice as an antiemetic for prophylaxis and treatment of nausea and vomiting related to chemotherapy and anesthesia; while Dexa is routinely used in the management of patients with intracranial tumors to reduce brain edema.

The effect of pretreatment with Dexa (2 mg/kg, i.p., three times in 24 hours) on DOX penetration into the brain of the rat, was assessed administering the chemotherapeutic agent 1 or 2 hours after the last injection of Dexa. The quantitative analysis of the anticancer agent showed that DOX concentration was higher in all the different brain areas of animals pretreated with Dexa, both at 1 and 2 hours. The mean concentration of DOX in the cerebral hemispheres of rats treated with DOX alone resulted 0.14±0.03 ng/mg fresh tissue *versus* 0.30±0.11 ng/mg fresh tissue in those treated with DOX *plus* Dexa at 1 hour (+114%), and 0.30±0.03 ng/mg fresh tissue in those treated with DOX *plus* Dexa hours (+114%).

Likewise, the effect of pretreatment with Ond (2 mg/kg, i.p., three times in 24 hours) on DOX penetration into the brain of the rat, was assessed administering the anticancer agent 1 or 2 hours after the last injection of Ond. DOX concentration resulted higher in all the different brain areas of animals pretreated with Ond; the mean concentration of DOX in the cerebral hemispheres of rats treated with DOX alone was 0.14±0.03 ng/mg fresh tissue *versus* 0.21±0.06 ng/mg fresh tissue in those treated with DOX *plus* Ond at 1 hour (+50%), and 0.39±0.06 ng/mg fresh tissue in those treated with DOX *plus* Ond at 2 hours (+179%) [68,69].

Moreover, pretreatment with any of these drugs did not increase lactate dehydrogenase activity or lipid peroxidation compared to controls, suggesting that Morph, Dexa and Ond pretreatment is able to allow DOX penetration inside the brain within acute cardiac or renal toxicity. Our *in vivo* data hypothesized that competition of these drugs for BCRP and P-gp of BBB increases significantly DOX penetration into the rat brain. These results indicated that the use of competitor molecules can represent valid alternatives to overcome the MDR phenotype, and could enable us to novel therapeutic approaches for refractory or recurrent brain tumors where anticancer drugs are usually hampered by the BBB. Notably, these strategies would offer a non-invasive way of access for many drugs in the CNS, ensuring the physiological activities of transporters in normal tissues and thereby reducing systemic toxicity and side effects by consequent dose adjustment. Further studies are necessary to understand and overcome the therapeutical limitations mainly caused by BBB, BCRP or other ABC transporters, in order to identify the most appropriate pharmacological strategies for the treatment of CNS tumors and other neurodegenerative disorders.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

Supported by: Associazione Italiana per la Ricerca sul Cancro (AIRC), grant RG-6232; “Amicodivalerio” Onlus, “Noi per Voi” Onlus, Fondazione Tommasino Bacciotti.

REFERENCES

[1] Abbott, N.J. Blood-brain barrier structure and function and the challenges for CNS drug delivery. *J. Inherit. Metab. Dis*., **2013**, 36, 437-449.

[2] Doshi, R.; Gutmann, D.A.; Khoo, Y.S.; Fagg, L.A.; Van Veen, H.W. The choreography of multidrug export. *Biochem. Soc. Trans*., **2011**, *39*, 807-811.

[3] Jani, M.; Ambrus, C.; Magnan, R.; Jakab, K.T.; Beéry, E.; Zolnerciks, J.K.; Krajcsi, P. Structure and function of BCRP, a

broad specificity transporter of xenobiotics and endobiotics. *Arch. Toxicol*., **2014**, *88*, 1205-1248.

[4] Nies, A.T. The role of membrane transporters in drug delivery to brain tumors. *Cancer. Lett*., **2007**, 254, 11-29.

[5] Shen, B.; Dong, P.; Li, D.; Gao, S. Expression and function of ABCG2 in head and neck squamous cell carcinoma and cell lines. *Exp. Ther. Med*., **2011**, *2*, 1151-1157.

[6] Salphati, L.; Plise, E.G.; Li, G. Expression and activity of the efflux transporters ABCB1, ABCC2 and ABCG2 in the human colorectal carcinoma cell line LS513. *Eur. J. Pharm. Sci*., **2009**, *37*, 463-468.

[7] Diestra, J.E.; Scheffer, G.L.; Català, I.; Maliepaard, M.; Schellens, J.H.; Scheper, R.J.; Germà-Lluch, J.R.; Izquierdo, M.A. Frequent expression of the multi-drug resistance-associated protein BCRP/MXR/ABCP/ABCG2 in human tumors detected by the BXP-21 monoclonal antibody in paraffin-embedded material. *J. Pathol*., **2002**, *198*, 213-219.

[8] Bleau, A.M.; Huse, J.T.; Holland, E.C. The ABCG2 resistance network of glioblastoma. *Cell Cycle*, **2009**, *8*, 2936-2944.

[9] Nakanishi, T.; Ross, D.D. Breast cancer resistance protein (BCRP/ABCG2): Its role in multidrug resistance and regulation of its gene expression. *Chin. J. Cancer*., **2012**, *31*, 73-99.

[10] Qosa, H.; Miller, D.S.; Pasinelli, P.; Trotti, D. Regulation of ABC efflux transporters at blood-brain barrier in health and neurological disorders. *Brain Res*., **2015**, *1628*, 298-316.

[11] Sheps, J.A.; Ralph, S.; Zhao, Z.; Baillie, D.L.; Ling, V. The ABC transporter gene family of Caenorhabditis elegans has implications for the evolutionary dynamics of multidrug resistance ineukaryotes. *Genome Biol*., **2004**, 5, R15.

[12] Litman, T.; Skovsgaard, T.; Stein, W.D. Pumping of drugs by P-glycoprotein: a two-step process? *J. Pharmacol. Exp. Ther*., **2003**, *307*, 846-853.

[13] Hartz, A.M.; Bauer, B. ABC transporters in the CNS - An inventory. *Curr. Pharm. Biotechnol*., **2011**, *12*, 656-673.

[14] Solazzo, M.; Fantappiè, O.; Lasagna, N.; Sassoli, C.; Nosi, D.; Mazzanti, R. P-gp localization in mitochondria and its functional characterization in multiple drug-resistant cell lines. *Exp. Cell Res*., **2006**, *312*, 4070-4078.

[15] Rajagopal, A.; Simon, S.M. Subcellular localization and activity of multidrug resistance proteins. *Mol. Biol. Cell*, **2003**, *14*, 3389-3399.

[16] Solazzo, M.; Fantappiè, O.; D'Amico, M.; Sassoli, C.; Tani, A.; Cipriani, G.; Bogani, C.; Formigli, L.; Mazzanti, R. Mitochondrial expression and functional activity of breast cancer resistance protein in different multiple drug-resistant cell lines. *Cancer. Res*., **2009**, *69*, 7235-7242.

[17] Lemos, C.; Kathmann, I.; Giovannetti, E.; Calhau, C.; Jansen, G.; Peters, G.J. Impact of cellular folate status and epidermal growth factor receptor expression on BCRP/ABCG2-mediated resistance to gefitinib and erlotinib. *Br. J. Cancer*, **2009**, *100*, 1120-1127.

[18] Bhatia, P.; Bernier, M.; Sanghvi, M.; Moaddel, R.; Schwarting, R.; Ramamoorthy, A.; Wainer, I.W. Breast cancer resistance protein (BCRP/ABCG2) localises to the nucleus in glioblastoma multiforme cells. *Xenobiotica*, **2012**, *42*, 748-755.

[19] Erdilyi, D.J.; Kamory, E.; Csokay, B.; Andrikovics, H.; Tordai, A.; Kiss, C.; Filni-Semsei, A.; Janszky, I.; Zalka, A.; Fekete, G.; Falus, A.; Kovács, G.T.; Szalai, C. Synergistic interaction of ABCB1 and ABCG2 polymorphisms predicts the prevalence of toxic encephalopathy during anticancer chemotherapy. *Pharmacogenomics J*., 2008, 8, 321-327.

[20] Marchetti, S.; Pluim, D.; Van Eijndhoven, M.; Van Tellingen, O.; Mazzanti, R.; Beijnen, J.H.; Schellens, J.H. Effect of the drug transporters ABCG2, Abcg2, ABCB1 and ABCC2 on the disposition, brain accumulation and myelotoxicity of the aurora kinase B inhibitor barasertib and its more active form barasertib-hydroxy-QPA. *Invest. New Drugs*, **2013**, *31*, 1125-1135.

[21] Marchetti, S.; De Vries, N.A.; Buckle, T.; Bolijn, M.J.; Van Eijndhoven, M.A.; Beijnen, J.H.; Mazzanti, R.; Van Tellingen, O.; Schellens, J.H. Effect of the ATP-binding cassette drug transporters ABCB1, ABCG2, and ABCC2 on erlotinib hydrochloride (Tarceva) disposition in *in vitro* and *in vivo* pharmacokinetic studies employing Bcrp1-/-/Mdr1a/1b-/- (triple-knockout) and wild-type mice. *Mol. Cancer Ther*., **2008**, *7*, 2280-2287.

[22] Zhu, J.J.; Gerstner, E.R.; Engler, D.A.; Mrugala, M.M.; Nugent, W.; Nierenberg. K.; Hochberg, F.H.; Betensky, R.A.; Batchelor, T.T. High-dose methotrexate for elderly patients with primary CNS lymphoma. *Neuro Oncol*., **2009**, *11*, 211-215.

[23] Blakeley, J.O.; Olson, J.; Grossman, S.A.; He, X.; Weingart, J.; Supko, J.G. Effect of blood brain barrier permeability in recurrent high grade gliomas on the intratumoral pharmacokinetics of methotrexate: A microdialysis study. *J. Neurooncol*., **2009**, *91*, 51-58.

[24] Li, L.; Agarwal, S.; Elmquist, W.F. Brain efflux index to investigate the influence of active efflux on brain distribution of pemetrexed and methotrexate. *Drug Metab. Dispos*., **2013**, *41*, 659-667.

[25] Cisternino, S.; Mercier, C.; Bourasset, F.; Roux, F.; Scherrmann, J.M. Expression, up-regulation, and transport activity of the multidrug-resistance protein Abcg2 at the mouse blood-brain barrier. *Cancer Res*., **2004**, *64*, 3296-3301.

[26] Robillard, K.R.; Chan, G.N.; Zhang, G.; La Porte, C.; Cameron, W.; Bendayan, R. Role of P-glycoprotein in the distribution of the HIV protease inhibitor atazanavir in the brain and male genital tract. *Antimicrob. Agents Chemother*., **2014**, *58*, 1713-1722.

[27] Jablonski, M.R.; Jacob, D.A.; Campos, C.; Miller, D.S.; Maragakis, N.J.; Pasinelli, P.; Trotti, D. Selective increase of two ABC drug efflux transporters at the blood-spinal cord barrier suggests induced pharmacoresistance in ALS. *Neurobiol. Dis*., **2012**, *47*, 194-200.

[28] Milane, A.; Vautier, S.; Chacun, H.; Meininger, V.; Bensimon, G.; Farinotti, R.; Fernandez, C. Interactions between riluzole and ABCG2/BCRP transporter. *Neurosci. Lett*., **2009**, *452*, 12-16.

[29] Zoccolella, S.; Beghi, E.; Palagano, G.; Fraddosio, A.; Guerra, V.; Samarelli, V.; Lepore, V.; Simone, I.L.; Lamberti, P.; Serlenga, L.; Logroscino, G. SLAP registry. Riluzole and amyotrophic lateralsclerosis survival: A population-based study in southern Italy. *Eur. J. Neurol*., **2007**, *14*, 262-268.

[30] Jablonski, M.R.; Markandaiah, S.S.; Jacob, D.; Meng, N.J.; Li, K.; Gennaro, V.; Lepore, A.C.; Trotti, D.; Pasinelli, P. Inhibiting drug efflux transporters improves efficacy of ALS therapeutics. *Ann. Clin. Trans. Neuro*., **2014**, *1*, 996-1005.

[31] Rizzi, M.; Caccia, S.; Guiso, G.; Richichi, C.; Gorter, J.; Aronica, E.; Alipradi, M.; Bagnati, R.; Fanelli, R.; D'Incalci, M.; Samanin, R.; Vezzani, A. Limbic seizures induce P-glycoprotein in rodent brain: functional implications or pharmacoresistance. *J. Neurosci*., **2002**, *22*, 5833-5839.

[32] Löscher, W.; Luna-Tortós, C.; Römermann, K.; Fedrowitz, M. Do ATP-binding cassette transporters cause pharmacoresistance in epilepsy? Problems and approaches in determining which antiepileptic drugs are affected. *Curr. Pharm. Des*., **2011**, *17*, 2808-2828.

[33] Zhang, C.; Kwan, P.; Zuo, Z.; Baum, L. The transport of antiepileptic drugs by Pglycoprotein. *Adv. Drug Deliv. Rev*., **2012**, *64*, 930-942.

[34] Nakanishi, H.; Yonezawa, A.; Matsubara, K.; Yano, I. Impact of P-glycoprotein and breast cancer resistance protein on the brain distribution of antiepileptic drugs in knockout mouse models. *Eur. J. Pharmacol.*, **2013**, *710*, 20-28.

[35] Hellinger, E.; Veszelka, S.; Toth, A.E.; Walter, F.; Kittel, A.; Bakk, M.L.; Tihanyi, K.; Hada, V.; Nakagawa, S.; Duy, T.D.; Niwa, M.; Deli, M.A.; Vastag, M. Comparison of brain capillary endothelial cell-based and epithelial (MDCK-MDR1, Caco-2, and VB-Caco-2) cell-based surrogate blood-brain barrier penetration models. *Eur. J. Pharm. Biopharm*., **2012**, *82*, 340-351.

[36] Palmer, A.M.; Alavijeh, M.S. Overview of experimental models of the blood brain barrier in CNS drug discovery. *Curr. Protoc. Pharmacol*., **2013**, *62*, 7.15.1-7.15.30.

[37] Römermann, K.; Helmer, R.; Löscher, W. The antiepileptic drug lamotrigine is a substrate of mouse and human breast cancer resistance protein (ABCG2). *Neuropharmacology*, **2015**, *93*, 7-14.

[38] Do, T.M.; Noel-Hudson, M.S.; Ribes, S.; Besengez, C.; Smirnova, M.; Cisternino, S.; Buyse, M.; Calon, F.; Chimini, G.; Chacun, H.; Scherrmann, J.M.; Farinotti, R.; Bourasset, F. ABCG2- and ABCG4-mediated efflux of amyloid-beta peptide 1−40 at the mouse blood-brain barrier. *J. Alzheimer's Dis*., **2012**, *30*, 155-166.

[39] Tai, L.M.; Loughlin, A.J.; Male, D.K.; Romero, I.A. P-glycoprotein and breast cancer resistance protein restrict apical-to- basolateral permeability of human brain endothelium to amyloid-beta. *J. Cereb. Blood Flow Metab*., **2009**, *29*, 1079-1083.

[40] Xiong, H.; Callaghan, D.; Jones, A.; Bai, J.; Rasquinha, I.; Smith, C.; Pei, K.; Walker, D.; Lue, L.F.; Stanimirovic, D.; Zhang, W. ABCG2 is up regulated in Alzheimer's brain with cerebral amyloid angiopathy and may actas a gatekeeper at the blood-brain barrier for Abeta (1-40) peptides. *J. Neurosci*., **2009**, *29*, 5463-5475.

[41] Hartz, A.M.; Bauer, B. ABC transporters in the CNS–aninventory. *Curr. Pharm. Biotechnol*., **2011**, *12*, 656-673.

[42] Wijesuriya, H.C.; Bullock, J.Y.; Faull, R.L.; Hladky, S.B.; Barrand, M.A. ABC efflux transporters in brain vasculature of Alzheimer's subjects. *Brain Res*., **2010**, *1358*, 228-238.

[43] Xiong, H.; Callaghan, D.; Jones, A.; Bai, J.; Rasquinha, I.; Smith, C.; Pei, K.; Walker, D.; Lue, L.F.; Stanimirovic, D.; Zhang, W. ABCG2 is upregulated in Alzheimer's brain with cerebral amyloid angiopathy and may act as a gatekeeper at the blood-brain barrier for Abeta(1−40) peptides. *J. Neurosci*., **2009**, *29*, 5463-5475.

[44] Uhrbom, L.; Hesselager, G.; Ostman, A.; Nister, M.; Westermark, B. Dependence of autocrine growth factor stimulation in platelet-derived growth factor-B-induced mouse brain tumor cells. *Int. J. Cancer*., **2000**, *85*, 398-406.

[45] Breedveld, P.; Pluim, D.; Cipriani, G.; Wielinga, P.; Van Tellingen, O.; Schinkel, A.H.; Schellens, J.H. The effect of Bcrp1 (Abcg2) on the *In vivo* pharmacokinetics and brain penetration of imatinib mesylate (Gleevec): Implications for the use of breast cancer resistance protein and p-glycoprotein inhibitors to enable the brain penetration of imatinib in patients. Cancer. Res., 2005, 65, 2577-2582.

[46] Shah, N.P. Dasatinib. *Drugs Today* (Barc), **2007**, *43*, 5-12.

[47] Cortes, J.; Jabbour, E.; Kantarjian, H.; Yin, C.C.; Shan, J.; O'Brien, S.; Garcia-Manero, G.; Giles, F.; Breeden, M.; Reeves, N.; Wierda, W.G.; Jones, D. Dynamics of BCR-ABL kinase domain mutations in chronic myeloid leukemia after sequential treatment with multiple tyrosine kinase inhibitors. *Blood*, **2007**, *110*, 4005-4011.

[48] Du, J.; Bernasconi, P.; Clauser, K.R.; Mani, D.R.; Finn, S.P.; Beroukhim, R.; Burns, M.; Julian, B.; Peng, X.P.; Hieronymus, H.; Maglathlin, R.L.; Lewis, T.A.; Liau, L.M.; Nghiemphu, P.; Mellinghoff, I.K.; Louis, D.N.; Loda, M.; Carr, S.A.; Kung, A.L.; Golub, T.R. Bead-based profiling of tyrosine kinase phosphorylation identifies SRC as a potential target for glioblastoma therapy. *Nat. Biotechnol*., **2009**, *27*, 77-83.

[49] Chen, Y.; Agarwal, S.; Shaik, N.M.; Chen, C.; Yang, Z.; Elmquist, W.F. P-glycoprotein and breast cancer resistance protein influence brain distribution of dasatinib. *J. Pharmacol. Exp. Ther*., **2009**, *330*, 956-963.

[50] Shi, Z.; Tiwari, A.K.; Shukla, S.; Robey, R.W.; Singh, S.; Kim, I.W.; Bates, S.E.; Peng, X.; Abraham, I.; Ambudkar, S.V.; Talele, T.T.; Fu, L.W.; Chen, Z.S. Sildenafil reverses ABCB1- and ABCG2-mediated chemotherapeutic drug resistance. *Cancer Res*., **2011**, *71*, 3029-3041.

[51] Black, K.L.; Yin, D.; Ong, J.M; Hu, J.; Konda, B.M.; Wang, X.; Ko, M.K.; Bayan, J.A.; Sacapano, M.R.; Espinoza, A.; Irvin, D.K.; Shu, Y. PDE5 inhibitors enhance tumor permeability and efficacy of chemotherapy in a rat brain tumor model. *Brain Res*., **2008**, *1230*, 290-302.

[52] Lin, F.; Hoogendijk, L.; Buil, L.; Beijnen, J.H.; Van Tellingen, O. Sildenafil is not a useful modulator of ABCB1 and ABCG2 mediated drug resistance *in vivo*. *Eur. J. Cancer*, **2013**, *49*, 2059-2064.

[53] Kuo, T.; Recht, L. Optimizing therapy for patients with brain metastases. *Semin. Oncol*., **2006**, *33*, 299-306.

[54] Dai, H.; Chen, Y.; Elmquist, W.F. Distribution of the Novel Antifolate Pemetrexed to the Brain. *J. Pharmacol. Exp. Ther*., **2005**, *315*, 222-229.

[55] Pardridge, W.M. Blood-brain barrier drug targeting: the future of brain drug development. *Mol. Interv*., **2003**, *3*, 90-105.

[56] Fischer, H.; Gottschlich, R.; Seelig, A. Blood-brain barrier permeation molecular parameters governing passive diffusion. *J. Membr. Biol*., **1998**, *165*, 201-211.

[57] Pardridge, W.M. The blood-brain barrier: bottleneck in brain drug development. *Neuro. Rx*., **2005**, *2*, 3-14.

[58] Rich, J.N.; Reardon, D.A.; Peery, T.; Dowell, J.M.; Quinn, J.A.; Penne, K.L.; Wikstrand, C.J.; Van Duyn, L.B.; Dancey, J.E.; McLendon, R.E.; Kao, J.C.; Stenzel, T.T.; Ahmed Rasheed, B.K.; Tourt-Uhlig, S.E.; Herndon, J.E. 2nd; Vredenburgh, J.J.; Sampson, J.H.; Friedman, A.H.; Bigner, D.D.; Friedman, H.S. Phase II trial of gefitinib in recurrent glioblastoma. *J. Clin. Oncol*., **2004**, *22*, 133-142.

[59] Franceschi, E.; Cavallo, G.; Lonardi, S.; Magrini, E.; Tosoni, A.; Grosso, D.; Scopece, L.; Blatt, V.; Urbini, B.; Pession, A.; Tallini, G.; Crinò, L.; Brandes, A.A. Gefitinib in patients with progressive high-grade gliomas: a multicentre phase II study by Gruppo Italiano Cooperativo di Neuro-Oncologia (GICNO). *Br. J. Cancer*, **2007**, *96*, 1047-1051.

[60] Agarwal, S.; Sane, R.; Gallardo, J.L.; Ohlfest, J.R.; Elmquist, W.F. Distribution of gefitinib to the brain is limited by P-glycoprotein (ABCB1) and breast cancer resistance protein (ABCG2)-mediated active efflux. *J. Pharmacol. Exp. Ther*., **2010**, *334*, 147-155.

[61] Vlaming, M.L.; Läppchen, T.; Jansen, H.T.; Kivits, S.; Van Driel, A.; Van de Steeg, E.; VAN der Hoorn, J.W.; Sio, C.F.; Steinbach, O.C.; DeGroot, J. PET-CT imaging with [18F]-gefitinib to measure Abcb1a/1b (P-gp) and Abcg2 (Bcrp1) mediated drug–drug interactions at the murine blood–brain barrier. *Nucl. Med. Biol*., **2015**, *42*, 833-841.

[62] De Vries, N.A.; Buckle, T.; Zhao, J.; Beijnen, J.H.; Schellens, J.H.; van Tellingen, O. Restricted brain penetration of the tyrosine kinase inhibitor erlotinib due to the drug transporters P-gp and BCRP. *Invest. New Drugs*, **2012**, *30*, 443-449.

[63] Mittapalli, R.K.; Vaidhyanathan, S.; Sane, R.; Elmquist, W.F. Impact of P-glycoprotein (ABCB1) and breast cancer resistance protein (ABCG2) on the brain distribution of a novel BRAF inhibitor: Vemurafenib (PLX4032). *J. Pharmacol. Exp. Ther*., **2012**, *342*, 33-40.

[64] Durmus, S.; Sparidans, R.W.; van Esch, A.; Wagenaar, E.; Beijnen, J.H.; Schinkel, A.H. Breast cancer resistance protein (BCRP/ABCG2) and P-glycoprotein (P-GP/ABCB1) restrict oral availability and brain accumulation of the PARP inhibitor rucaparib (AG-014699). *Pharm. Res*., *2015*, *32*, 37-46.

[65] Sardi, I. Morphine facilitates doxorubicin penetration in the central nervous system: A new prospect for therapy of brain tumors. *J. Neurooncol*., **2011**, *104*, 619-620.

[66] Sardi, I.; la Marca, G.; Giovannini, M.G.; Malvagia, S.; Guerrini, R.; Genitori, L.; Massimino, M.; Aricò, M. Detection of doxorubicin hydrochloride accumulation in the rat brain after morphine treatment by mass spectrometry. *Cancer Chemother. Pharmacol*., **2011**, *67*, 1333-1340.

[67] Löscher, W.; Potschka, H. Drug resistance in brain diseases and the role of drug efflux transporters. *Nat. Rev. Neurosci*., **2005**, *6*, 591-602.

[68] Sardi, I.; la Marca, G.; Cardellicchio, S.; Giunti, L.; Malvagia, S.; Genitori, L.; Massimino, M.; De Martino, M.; Giovannini, M.G. Pharmacological modulation of blood-brain barrier increases permeability of doxorubicin into the rat brain. *Am. J. Cancer. Res*., **2013**, *3*, 424-432.

[69] Sardi, I.; Fantappiè, O.; la Marca, G.; Giovannini, M.G.; Iorio, A.L.; da Ros, M.; Malvagia, S.; Cardellicchio, S.; Giunti, L.; De Martino, M.; Mazzanti, R. Delivery of doxorubicin across the blood-brain barrier by ondansetron pretreatment: A study *in vitro* and *in vivo*. *Cancer. Lett*., **2014**, *353*, 242-247.