

Breast Cancer Resistance Protein and P-Glycoprotein in Brain Cancer: Two Gatekeepers Team Up

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Abstract: Brain cancer is a devastating disease. Despite extensive research, treatment of brain tumors has been largely ineffective and the diagnosis of brain cancer remains uniformly fatal. Failure of brain cancer treatment may be in part due to limitations in drug delivery, influenced by the ABC drug efflux transporters P-gp and BCRP at the blood-brain and blood-tumor barriers, in brain tumor cells, as well as in brain tumor stem-like cells. P-gp and BCRP limit various anti-cancer drugs from entering the brain and tumor tissues, thus rendering chemotherapy ineffective. To overcome this obstacle, two strategies – targeting transporter regulation and direct transporter inhibition – have been proposed. In this review, we focus on these strategies. We first introduce the latest findings on signaling pathways that could potentially be targeted to down-regulate P-gp and BCRP expression and/or transport activity. We then highlight in detail the new paradigm of P-gp and BCRP working as a “cooperative team of gatekeepers” at the blood-brain barrier, discuss its ramifications for brain cancer therapy, and summarize the latest findings on dual P-gp/BCRP inhibitors. Finally, we provide a brief summary with conclusions and outline the perspectives for future research endeavors in this field.

Keywords: BCRP, P-gp, brain cancer, glioblastoma, multidrug resistance, blood-brain barrier, regulation, inhibition.

1. INTRODUCTION

The number of new cases of malignant brain cancers has significantly increased over the last two decades [1, 2]. In 2010, an estimated 22,000 new patients were expected to be diagnosed and 13,000 patients were expected to die of brain cancer in the United States [1]. Brain cancers can be divided into two categories: primary brain tumors and metastatic brain tumors. *Primary brain tumors* originate in the brain and usually do not metastasize. These tumors represent only 2% of all cancers but account for a disproportionate rate of morbidity and mortality [3]. *Metastatic brain tumors* originate outside of the central nervous system (CNS) elsewhere in the body and spread to the brain as metastases [4]. Metastatic brain tumors develop in 10–30% of cancer patients [5], and are the most common type of brain tumors with a four times greater annual incidence compared to primary brain tumors [6]. The incidence of brain metastases has increased over the last decade mainly due to improved treatment of primary peripheral cancers resulting in increased patient survival, as well as due to the development of newer tools to image and detect tumors of the CNS. Despite extensive research, treatment of metastatic brain tumors has been largely ineffective and the diagnosis of brain cancer remains uniformly fatal [6].

One major challenge researchers face today is effective delivery of anti-cancer drugs to primary and metastatic cancers in the CNS. The primary impediment to successful drug delivery into the CNS is the blood-brain barrier (BBB). The BBB is an endothelial interface that separates the brain from the blood and shields the CNS from exposure to circulating toxins and potentially harmful chemicals [7]. At the same time, this protective barrier excludes therapeutic drugs from entering the brain, and thus, becomes an obstacle for drugs intended to treat CNS diseases, such as brain cancers.

At the molecular level, brain capillary endothelial cells that form the BBB are joined together by tight junctions that limit paracellular passage of solutes into the brain. Circulating solutes

can therefore only gain access to the brain by passive diffusion, or uptake transport [8]. The BBB is further fortified by ATP-binding cassette (ABC) efflux transporters that limit xenobiotics, including a large number of therapeutic drugs, from entering the brain. P-glycoprotein (P-gp, *ABCB1* or *MDR1*) and breast cancer resistance protein (BCRP, *ABCG2*) are two prominent members of the ABC transporter superfamily. Both have broad and partly overlapping substrate specificities that include a variety of structurally diverse drugs currently used in the clinic [9–11]. These two “gatekeeper” transporters constitute a vital part of the protective defense mechanism at the BBB by limiting drugs from accessing the brain and thereby rendering them ineffective. Moreover, recent literature suggests that P-gp and BCRP team up and work together at the BBB to restrict brain penetration of drugs [12–16].

The present review is focused on this phenomenon and the challenge that these two transporters pose to chemotherapeutic delivery into the brain. We review P-gp and BCRP with respect to their roles and regulation at the BBB, and summarize recent findings on the P-gp/BCRP teamwork in restricting brain penetration of anti-cancer drugs.

2. P-GLYCOPROTEIN IN BRAIN CANCER

2.1. History

In 1976, Rudy Juliano and Victor Ling discovered a high molecular weight membrane glycoprotein in mutant cancer cells that appeared to alter membrane permeability for chemotherapeutics, and consequently named it P-glycoprotein (“permeability glycoprotein”; [17]). Shortly afterwards it became clear that P-glycoprotein (P-gp) is a highly potent ATP-driven efflux transporter that actively pumps its substrates out of cells, even against a concentration gradient [18]. This discovery was groundbreaking because it provided the first explanation for treatment failure due to resistance to multiple chemotherapeutics, which is a frequently observed phenomenon in cancer.

Several years later, in 1989, P-gp protein expression was detected at the human BBB [19, 20] and subsequent studies confirmed the presence of P-gp in the luminal (apical) membrane at the BBB of dogfish, killifish, mouse, rat, cat, dog, monkey, pig, and cow [20–

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30]. In addition, P-gp was found in primary brain tumors and is now recognized to be a critical transporter that conveys resistance to a large number of anti-cancer drugs, for example taxanes, vinca-alkaloids, etoposide and analogues, anthracyclines, lanafarnib, imatinib, and topotecan [31, 32]. Thus, P-gp has been a focus of BBB, brain tumor, and drug delivery research for almost two decades.

2.2. P-glycoprotein Inhibition in Brain Cancer

One strategy to improve brain delivery of anti-cancer drugs is to directly block P-gp transport function at the BBB by using transporter inhibitors. The first P-gp inhibitor was found by serendipity in 1981 by Tsuruo *et al.*, who showed that verapamil, a calcium channel blocker, inhibits P-gp-mediated drug efflux in resistant tumor cells, thereby overcoming drug resistance [33]. As a result, over the years numerous chemicals have been screened for their potential to inhibit P-gp, and a variety of inhibitors were developed that differ in potency, selectivity, and side effects [34, 35]. However, only a few compounds have been tested for their potential to enhance drug delivery to the brain. The first proof-of-principle that P-gp inhibition can be used to treat brain cancer came from a study in nude mice with intracerebrally implanted human U-118 MG glioblastoma [36]. In this study, Fellner *et al.* identified P-gp as the major factor in limiting the anti-cancer therapeutic paclitaxel from crossing the BBB and permeating into the CNS [36]. Consistent with this, treating glioblastoma-bearing mice with paclitaxel had no effect on tumor size but pretreating mice with the P-gp inhibitor PSC833 (valsopodar) increased paclitaxel brain levels and reduced tumor size by 90% [36]. Subsequent studies using the P-gp inhibitors cyclosporine A, elacridar (GF120918), tariquidar (XR9576), and zosuquidar (LY335979) confirmed these findings and demonstrated that P-gp inhibition increases paclitaxel brain levels [37-40]. It has now been shown that elacridar and tariquidar are not P-gp-specific inhibitors, but at higher concentrations also inhibit BCRP [41-43]. A recent study also demonstrated that oral, bi-weekly co-administration of the new P-gp inhibitor HM30181A with paclitaxel decreased tumor volume of K1735 melanoma brain metastases and U-87 MG glioblastoma in cancer animal models [44].

Together, direct P-gp inhibition improves brain drug delivery of some anti-cancer drugs and treatment of brain tumors in animal models. Currently, no reports are available on the use of the above mentioned transporter inhibitors in brain cancer patients. However, tariquidar is being tested in an ongoing phase I clinical trial to treat brain cancer among other cancer types in children (www.clinicaltrials.gov; NCT00020514v). Thus, it remains to be demonstrated if the strategy of transporter inhibition can be translated from animal model to patient. Hence, the search for more potent, efficacious, and selective P-gp inhibitors continues.

2.3. Targeting P-gp Regulation

In this review, we will also comment on signaling pathways that affect P-gp and BCRP at the blood-brain and blood-tumor barriers, in brain tumors and brain cancer stem cells and that could potentially be used to improve delivery of chemotherapeutics. In this context, the goal of targeting transporter regulation is to down-regulate transporter expression and/or functional activity, thereby reducing drug efflux and overcoming drug resistance.

The field of BBB transporter regulation is relatively new and only few studies have been conducted. The first study demonstrating P-gp down-regulation at the BBB was published in 2004. This study focused on ET-1 signaling through the ETB receptor, NOS, and PKC, which rapidly decreased P-gp activity in isolated rat brain capillaries [45]. Another report showed that BBB P-gp is regulated by the inflammatory mediators LPS, TNF- α , and ET-1, which activated TLR4, TNFR1, ETB receptor, NOS, and PKC, leading to reduced P-gp activity [46]. In a follow-up study, Rigor *et al.* identified PKC β (I) as the responsible PKC isoform for down-

regulation of P-gp activity in this pathway [47]. Importantly, this study provides proof-of-principle that targeting PKC β (I) increases brain uptake of the P-gp substrate verapamil.

In another study, Hawkins *et al.* made a similar observation using vascular endothelial growth factor (VEGF; [48]). It was shown that VEGF decreased P-gp activity in rat brain capillaries via activation of flk-1 and Src, likely through Src-mediated phosphorylation of caveolin-1. This finding implies that P-gp activity could be acutely diminished in pathological conditions associated with increased brain VEGF expression and that VEGF/Src signaling at the BBB could be targeted to decrease P-gp activity [48].

Together, two signaling pathways have been identified that could potentially be used to down-regulate P-gp transport activity at the BBB; one involves signaling of inflammatory mediators through PKC β (I), the other one involves VEGF signaling through flk-1 and Src. It remains to be demonstrated if targeting these pathways improves delivery of chemotherapeutics across the BBB and into brain tumors. For more details on signaling pathways that regulate P-gp at the BBB we refer the reader to [49].

3. BCRP IN BRAIN CANCER

3.1. History

In 1998, more than 20 years after the discovery of P-gp, Doyle *et al.* cloned the ABC transporter breast cancer resistance protein (BCRP, MXR: mitoxantrone resistance protein, ABCP1) from a multidrug-resistant human breast cancer cell line [50]. Four years later, in 2002, two groups found BCRP to be physiologically expressed in brain capillary endothelial cell cultures and that BCRP was localized at the luminal membrane of rat and human brain capillaries [51-53]. BCRP has also been detected at the human, cow, rat, and mouse BBB [53-56]. In addition, BCRP is highly expressed at the plasma membrane of tumor stem cells [57, 58], where it could be involved in stem cell differentiation, protection against xenobiotics, and cancer cell survival under hypoxic conditions [59]. Little is known about BCRP expression in brain cancer. In primary CNS lymphoma, BCRP protein expression and transport activity have been shown to be down-regulated [60]. In contrast, in neuroepithelial tumors such as ependymomas and in glioma tumor stem-like cells BCRP protein and activity are highly up-regulated, causing multidrug resistance [61-63].

At the functional level, BCRP is a half transporter that works as a homodimer and possibly as a heterodimer with other ABCG half transporter isoforms [52]. A significant overlap in substrate specificity between P-gp and BCRP has been demonstrated [64, 65], and anti-cancer drugs handled by BCRP include tyrosine kinase inhibitors (imatinib, nilotinib, gefitinib, erlotinib, dasatinib, sorafenib, lapatinib, apatinib, and tandutinib; [12, 13, 66-69]), topotecan, irinotecan, epirubicin, doxorubicin, daunorubicin, and mitoxantrone [70, 71]. Studies show that BCRP restricts these chemotherapeutics from permeating across the BBB and penetrating into brain tumors. As with P-gp, BCRP-mediated drug resistance in brain tumors is in part due to transporter up-regulation at the blood-tumor barrier contributing to reduced delivery and efficacy of anti-cancer drugs [63].

3.2. BCRP Inhibition in Brain Cancer

Few compounds have been identified that specifically inhibit BCRP. Fumitremorgin C (FTC), a fungal toxin, was the first reported BCRP inhibitor [72], but is not suitable for *in vivo* studies due to severe neurotoxic side effects. This led to the development of the FTC-derivatives Ko132, Ko134, and Ko143 that are 2-3-fold more potent, less toxic, and designed for use *in vivo* [73]. Recent efforts focused on tyrosine kinase inhibitors such as imatinib, nilotinib, gefitinib, and erlotinib that directly interact with BCRP at the substrate binding site and that block ATPase activity of the transporter [68]. These compounds have a unique pharmacologic profile in that they are effective chemotherapeutics, as well as

potent BCRP inhibitors and transporter substrates. In this regard, *in vivo* studies showed that BCRP, together with P-gp, limited brain uptake of imatinib and that BCRP inhibition significantly increased imatinib brain penetration [74]. In a similar study, Breedveld *et al.* demonstrated that inhibition of BCRP with pantoprazole increased imatinib brain levels 1.8-fold [75]. However, co-administration of the P-gp and BCRP inhibitor elacridar improved brain penetration of imatinib by 4.2-fold [75]. The same group also showed that dual BCRP/P-gp inhibition using elacridar improved oral bioavailability and CNS penetration of anti-cancer drugs [76]. To what extent elacridar inhibits P-gp and/or BCRP depends on the local inhibitor concentration. Consequently, the elacridar dose determines what drug permeates at which amount through the BBB.

While these studies demonstrate the importance of BBB BCRP for brain uptake of anti-cancer drugs, they also show that for some compounds inhibition of either BCRP or P-gp alone is not sufficient to increase delivery into the brain.

3.3. Targeting BCRP Regulation

Various signaling pathways have been shown to down-regulate BCRP, which is expected to improve anti-cancer drug delivery into brain tumors. In this regard, it was demonstrated that estrogens play a role in BCRP regulation. Imai *et al.* showed that estrone and 17 β -estradiol (E2) reverse BCRP-mediated drug resistance [77] and that E2 triggers post-transcriptional down-regulation of BCRP in human breast cancer cell lines [77]. Ee *et al.* identified an estrogen response element (ERE) in the BCRP promoter region and showed ERE activation by binding of the E2/estrogen receptor α complex, which up-regulated BCRP mRNA expression [78]. From this study, however, it is unclear if BCRP protein expression and/or transport activity were also affected by ERE activation.

Other studies that were conducted in various tissues reported BCRP regulation through the PI3K/Akt signaling pathway [79, 80]. In these studies, PI3K/Akt signaling triggered BCRP internalization and translocation from the plasma membrane to the cytoplasmic compartment in stem cells and renal epithelial cells and was involved in regulating BCRP expression [79, 80].

With regard to brain cancer, Bleau *et al.* published a study showing PTEN/PI3K/Akt regulation of BCRP activity in glioma tumor stem-like cells [61, 62]. In these cells, activation of Akt lead to BCRP translocation from the cytoplasm to the plasma membrane and increased BCRP-mediated efflux of anti-cancer drugs, which contributed to drug resistance and tumorigenicity. These findings are interesting considering recent studies where we demonstrated E2-mediated BCRP regulation in isolated brain capillaries and established a link between E2 and PTEN/PI3K/Akt signaling [54, 81]. We showed that E2 signaling through ER β , PTEN/PI3K/Akt and GSK3 triggered BCRP internalization from the brain capillary plasma membrane, which was followed by proteasomal degradation of the transporter and reduced BCRP functional activity and protein expression [54, 81]. These findings suggest that PTEN/PI3K/Akt-mediated up-regulation of BCRP activity in glioma tumor stem-like cells that Bleau *et al.* observed [61, 62] could potentially be blocked, which may be one possibility to reduce BCRP-mediated resistance to chemotherapeutics at the level of the blood-brain and blood-tumor barriers. However, despite these studies it remains to be demonstrated if targeting BCRP regulation at the BBB, in brain tumors, and/or in brain tumor stem cells is a valid strategy to improve drug delivery of chemotherapeutics into the CNS. For more details on signaling pathways that regulate BCRP at the BBB we refer the reader to [49].

4. P-GP AND BCRP IN BRAIN CANCER

4.1. Two Gatekeepers Team Up at the Blood-Brain Barrier

The discovery of BCRP in brain endothelial cells changed the long standing opinion that P-gp is the sole important transporter responsible for efflux of drugs at the BBB. However, BCRP ex-

pression at the BBB has not been unequivocally correlated to low brain penetration of all BCRP substrates. For example, Lee *et al.* conducted *in situ* brain perfusion studies using the BCRP substrates dehydroepiandrosterone sulfate and mitoxantrone and reported that brain penetration of the two compounds was not increased in *Bcrp1*^(-/-) (BCRP knockout) mice [82]. Similarly, Giri and coworkers showed that BCRP mediated efflux of the antiretroviral drugs abacavir and zidovudine *in vitro* [83]. However, despite the absence of BCRP, brain uptake of these two compounds was not elevated in *Bcrp1*^(-/-) mice [84]. One conclusion drawn from these studies was that BCRP played a minor role in drug efflux at the BBB and another study showed that interaction of BCRP with substrates *in vitro* rarely translates to visible effects at the BBB *in vivo* [85].

In contrast, other studies demonstrated BCRP transport activity at the BBB. Cisternino and colleagues showed that BCRP limits prazosin and mitoxantrone, two prototypical BCRP substrates, from penetrating into the brain [86]. Likewise, Enokizono *et al.* and Breedveld *et al.* reported that brain distribution of drugs increased significantly in *Bcrp1*^(-/-) mice [75, 87]. Moreover, we recently reported that sorafenib transport into the brain was significantly increased in *Bcrp1*^(-/-) mice [13].

These conflicting results on BCRP-mediated drug efflux from the brain initiated a controversy on the role of this transporter at the BBB that lead to further studies. With the development of the P-gp/BCRP knockout mouse (*Mdr1a/1b*^(-/-) *Bcrp1*^(-/-); [88]), researchers have been provided with the opportunity to study the combined impact of these two efflux transporters on the delivery of drugs across the BBB. de Vries *et al.* showed that brain uptake of topotecan, a substrate for both P-gp and BCRP, was not increased in mice lacking BCRP (*Bcrp1*^(-/-)) [15]. In P-gp knockout mice (*Mdr1a/1b*^(-/-)) topotecan brain levels increased slightly by 1.5-fold. In contrast, in mice lacking both P-gp and BCRP (*Mdr1a/1b*^(-/-) *Bcrp1*^(-/-)), topotecan brain uptake was increased by more than 12-fold. Thus, absence of both P-gp and BCRP resulted in an effect that was significantly larger than the combined effects from the single transporter knockout mice. This finding was confirmed by Polli *et al.* using lapatinib in P-gp/BCRP knockout mice [16]. We have shown the same with dasatinib [14], gefitinib [12] and sorafenib [13]. Even though these drugs are substrates for both P-gp and BCRP, absence of only one of the transporters did not significantly increase delivery of either drug to the brain, but the greatest enhancement in brain penetration was seen when both transporters were absent or inhibited at the BBB. Several studies now show that this is true for other dual P-gp and BCRP substrates as well (Table 1, [69, 89, 90]). Fig. 1 summarizes recent data by Kawamura *et al.* [91] that demonstrate this phenomenon. These findings suggest that inhibition of either P-gp or BCRP can be compensated by the respective other transporter, and that both transporters “cooperate” with each other in preventing chemotherapeutic drugs from entering the brain.

P-gp and BCRP cooperation implies that absence of either P-gp or BCRP alone does not result in an appreciable increase in brain penetration of dual substrates. In BCRP knockout mice (where P-gp is present), P-gp alone is sufficient to prevent drugs from penetrating into the brain. Likewise, in P-gp knockout mice (where BCRP is present) BCRP alone is sufficient to limit drug uptake into the brain. The greatest enhancement in brain penetration of dual substrates is always seen when both P-gp and BCRP are absent in the combined P-gp/BCRP knockout mice (Figs. 1 and 2).

An insight into the mechanism of P-gp/BCRP cooperation can be gained by looking at relative transporter affinities of substrate drugs, and relative transporter expression levels at the BBB (assuming protein expression correlates with transport capacity for both transporters). In this regard, Kamiie *et al.* used LC-MS to quantify membrane transporter expression at the mouse BBB and found approximately 5-fold higher P-gp protein levels compared to those of BCRP [92]. Significantly higher protein expression levels at the BBB make P-gp appear to be the dominant efflux transporter for

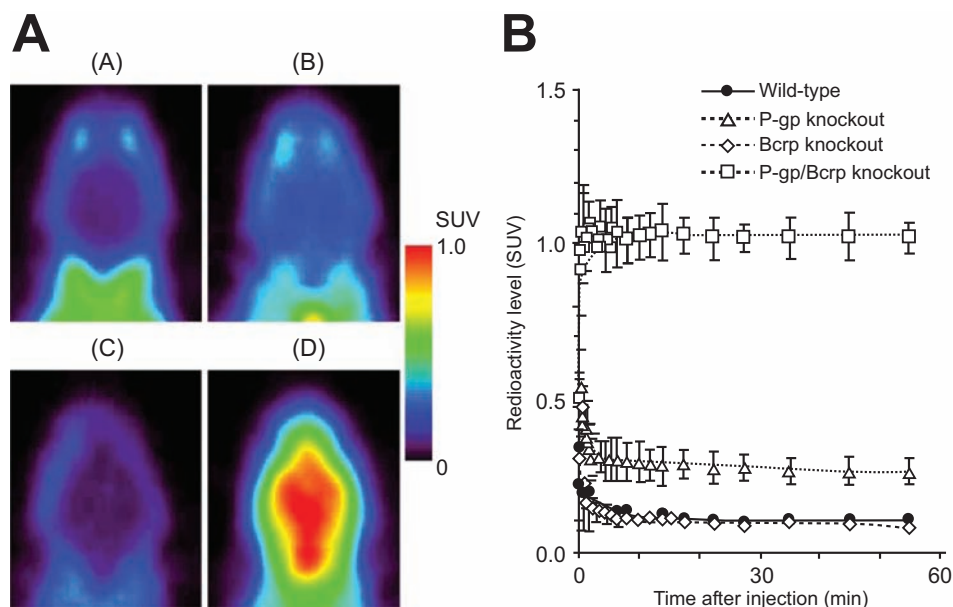


Fig. (1a). Transaxial PET images showing [11C] GF120918 in the brain of a (A) wild-type, (B) P-gp knockout, (C) Bcrp knockout and (D) P-gp/Bcrp knockout mouse. GF120918 is a substrate for and inhibits both P-gp and BCRP. The radioactivity level is low in the wild-type and BCRP knockout mouse, and increases slightly in the P-gp knockout mouse. The greatest increase in brain radioactivity is seen in the P-gp/Bcrp knockout mouse where both P-gp and BCRP are absent.

Fig. (1b). shows the quantification of brain radioactivity in the four mouse types.

Figures reproduced with permission from Kawamura *et al.*, *Mol Imaging Biol* (2010), DOI: 10.1007/s11307-010-0313-1

Table 1. Brain Distribution of Dual P-gp and BCRP Substrates

Drug	Fold Increase in Brain/Plasma Ratios Relative to Wild-Type Mice			Reference
	P-gp KO	BCRP KO	P-gp/BCRP KO	
Topotecan	1.5	1.5	12	de Vries <i>et al.</i> [15]
Dasatinib	4	1	9	Chen <i>et al.</i> [14]
Gefitinib	31.1	13.7	108	Agarwal <i>et al.</i> [12]
Sorafenib	1	4	10	Agarwal <i>et al.</i> [13]
Erlotinib	2.9	1.2	8.5	Kodaira <i>et al.</i> [90]
Imatinib	3.6	2.5	12.6	Breedveld <i>et al.</i> [75]
Tandutinib	2	1	13	Yang <i>et al.</i> [69]
Lapatinib	4	1	42.5	Polli <i>et al.</i> [16]
Flavoperidol	3.4	1.2	14.2	Kodaira <i>et al.</i> [90]
Mitoxantrone	1.7	1.4	8	Kodaira <i>et al.</i> [90]

The brain-to-plasma ratio does not significantly increase in single P-gp or BCRP knockout mice, where the other transporter is still present and restricts brain penetration. In combined P-gp/BCRP knockout mice where both P-gp and BCRP are absent, brain penetration of all compounds is dramatically increased.

many dual substrates that have similar affinities to both P-gp and BCRP. In comparison, due to lower protein expression levels, BCRP-mediated efflux appears to be minor and becomes apparent only when P-gp or both transporters are absent. For example, for a compound with moderate P-gp affinity, higher P-gp expression levels (higher P-gp transport capacity) will compensate for lower transporter affinity, resulting in a pronounced P-gp effect on the efflux of this compound at the BBB. This is true for almost all anti-cancer drugs mentioned above (Table 1), with the exception of sorafenib and dantrolene. Both these compounds have a significantly higher affinity for BCRP than for P-gp [13, 90]. Therefore,

BCRP is the dominant transporter in keeping these drugs out of the brain and an effect of P-gp on drug penetration is only noticeable in BCRP and P-gp/BCRP knockout mice. Kodaira *et al.* explained P-gp/BCRP cooperation by determining the net contribution of each transporter to the overall efflux of various drugs at the BBB [90]. The authors showed that for many dual substrates, P-gp-mediated efflux out of the brain was greater than that by BCRP. On the other hand, P-gp-mediated efflux of dantrolene (high affinity BCRP substrate) was 10-fold lower than BCRP-mediated dantrolene efflux.

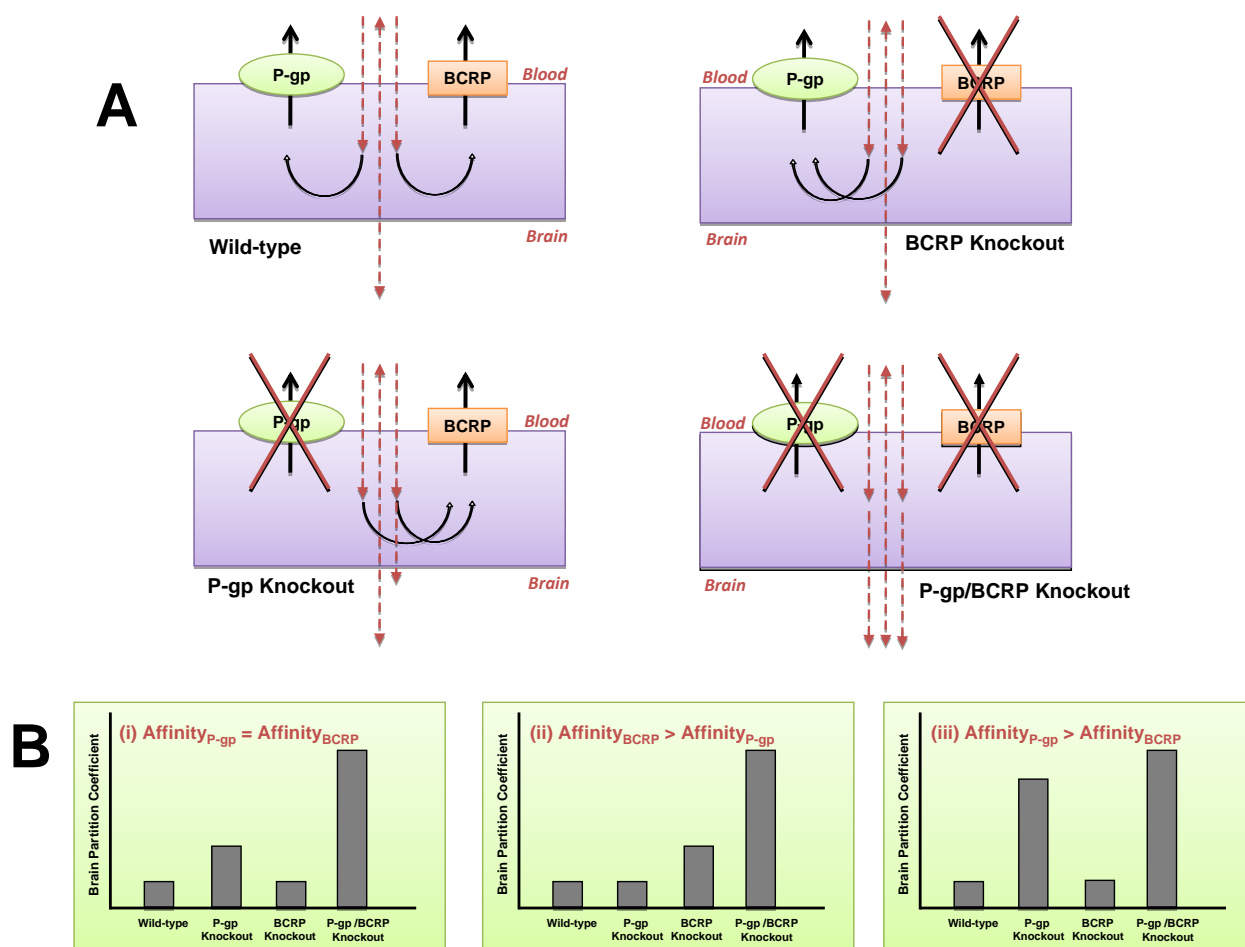


Fig. (2A). Cooperation of P-gp and BCRP at the Blood-Brain Barrier.

P-gp and BCRP work together at the BBB and restrict brain penetration of dual substrates. Absence of either P-gp or BCRP alone may not result in a significant increase in brain penetration of dual substrates. In BCRP knockout mice (where P-gp is present), P-gp alone is sufficient to prevent drugs from penetrating into the brain. Likewise, in P-gp knockout mice (where BCRP is present), BCRP also is sufficient to limit drug uptake into the brain. The greatest enhancement in brain penetration of dual substrates is always seen when both P-gp and BCRP are absent in the combined P-gp/BCRP knockout mice.

Fig. (2B). Impact of P-gp/BCRP Cooperation on Brain Distribution of Three Hypothetical Dual Substrates.

Note: for both transporters it is assumed that protein expression correlates with transport capacity.

- I. For drugs with similar affinity for both P-gp and BCRP, P-gp is the dominant transporter due to higher P-gp protein expression levels at the BBB (higher transport capacity). Thus, P-gp-mediated drug efflux out of the brain is larger compared to BCRP-mediated efflux. As a result, the drug brain-to-plasma ratio is slightly increased in P-gp knockout mice, but unchanged in BCRP knockout mice. Absence of both P-gp and BCRP in P-gp/BCRP knockout mice results in drug brain levels that are significantly larger than the combined levels from the single transporter knockout mice. This phenomenon has been reported for drugs like dasatinib [14], gefitinib [12], topotecan [15], and lapatinib [16].
- II. For drugs with significantly higher affinity for BCRP than P-gp, BCRP is the dominant transporter in keeping these drugs out of the brain. The P-gp effect is only noticeable in BCRP knockout and combined P-gp/BCRP knockout mice. This has been observed for drugs like sorafenib [13] and dantrolene [90].
- III. For drugs with higher affinity for P-gp than BCRP, larger P-gp expression combined with high transporter affinity results in substantial P-gp-mediated efflux. In BCRP knockout mice, where P-gp is still present, drug brain-to-plasma ratio is unchanged. This has been reported for quinidine [90].

In all three scenarios the largest increase in drug brain-to-plasma ratio is seen when both P-gp and BCRP are absent at the BBB.

While the above studies have been conducted in animal models, it is now clear that BBB P-gp and BCRP expression is species-dependent. In this regard, Uchida *et al.* recently reported that at the human BBB, BCRP protein levels are higher compared to P-gp protein levels [93]. Using LC-MS, the authors determined 8 fmol/ μg total protein for BCRP vs. 6 fmol/ μg total protein for P-gp in human brain capillaries. However, to draw a clear conclusion from these absolute transporter protein levels on the importance of each transporter for brain drug delivery is difficult. LC-MS measures total transporter protein and does not distinguish between transporter protein that is functionally active in the luminal membrane of the brain capillary endothelium and transporter protein that

is inactive in intracellular vesicle membranes. For example, LC-MS measures both BCRP monomer and dimer, but only BCRP dimer is the functionally active form [94]. From what we know today only functionally active transporter protein in the luminal membrane of the brain capillary endothelium affects drug delivery across the BBB. Thus, although total BCRP protein expression at the human BBB is higher compared to P-gp, it is impossible to say at this point in time which transporter is more important for brain drug delivery in patients. To make such a statement we will need information on the functional expression of each transporter at the BBB, the local drug concentration, and the drug-transporter affinity.

P-gp/BCRP cooperation at the BBB suggests two fundamental realities. First, these two transporters can significantly affect drug delivery to the brain, thereby influencing drug efficacy. Second, combined inhibition of both P-gp and BCRP is potentially an attractive therapeutic strategy to improve delivery and thus efficacy of substrate drugs in the CNS. Many of the chemotherapeutic drugs mentioned above have been clinically unsuccessful in treating brain cancers. Even though P-gp- and BCRP-mediated cooperative efflux transport is not limited to anti-cancer agents, combined inhibition of both transporters might have the biggest impact in the treatment of brain cancers, where a small increase in drug brain uptake might dramatically improve anti-cancer efficacy.

In summary, absence of either P-gp or BCRP alone does not enhance brain distribution of dual substrates, but genetic or chemical knockout of both transporters is required to significantly increase brain uptake of dual P-gp/BCRP substrate anti-cancer drugs. Thus, current research indicates that P-gp and BCRP team up at the BBB and “cooperate” in preventing dual substrates from entering the brain. This finding has led to a paradigm shift in the field of BBB transporter research.

4.2. Dual Inhibition of P-gp and BCRP at the BBB

Given the cooperation of P-gp and BCRP at the BBB, developing compounds that are potent inhibitors of both transporters may prove beneficial. Elacridar (GF120918) is a dual P-gp/BCRP inhibitor that has undergone extensive preclinical and clinical evaluation [95]. Elacridar has been used in several preclinical studies to inhibit P-gp and BCRP at the BBB with the purpose of enhancing brain distribution of simultaneously administered compounds [40, 96-99]. These studies demonstrated that the greater than additive increase in brain penetration is not restricted to P-gp/BCRP knockout animals, but can also be observed with dual P-gp/BCRP inhibitors. For example, Chen *et al.* showed that brain penetration of dasatinib increased dramatically with co-administration of elacridar [14]. Likewise, we showed that elacridar significantly enhanced gefitinib and sorafenib brain uptake [12, 13], and de Vries *et al.* published similar findings for topotecan [15]. Thus, in preclinical studies, elacridar significantly increased brain penetration of drugs that are dual P-gp/BCRP substrates.

Apart from these compounds that were developed for use as multi-drug resistance reversal agents, several studies examined drugs that are dual P-gp/BCRP substrates to competitively inhibit both transporters. These include several anti-cancer tyrosine kinase inhibitors that have been shown to be substrates for both P-gp and BCRP. *In vitro* studies show that tyrosine kinase inhibitors like erlotinib [100], gefitinib [101], lapatinib [102] and sunitinib [103] inhibit ABC transporters, mainly P-gp and BCRP, and suggest the potential use of these agents as combination therapy to improve drug pharmacokinetics. In 2006, Zhuang *et al.* showed that concurrent administration of gefitinib results in a significant increase in brain penetration of topotecan [104]. The same group showed that gefitinib also increased intracellular tumor exposure to topotecan in a mouse model of glioma [105]. In a recent clinical trial, Furman and coworkers used gefitinib to inhibit intestinal P-gp and BCRP and showed that it increased oral bioavailability of irinotecan [106]. An interesting study by Nakanishi *et al.* in 2006 showed that imatinib attenuated its BCRP-mediated resistance by suppressing BCRP expression [107]. The underlying mechanism for these differential responses involved downstream effects of imatinib leading to decreased phosphorylation of Akt, subsequently leading to reduced BCRP expression [107]. Many tyrosine kinase inhibitors have an inhibitory effect on the PTEN/PI3K/Akt signaling pathway. These drugs can thus reduce functional activity and protein expression of ABC transporters, especially BCRP, by blockade of PI3K/Akt signaling. Combination of tyrosine kinase inhibitors with other anti-cancer drugs can therefore have a bimodal effect on ABC transporters, wherein decreased transporter expression/function

coupled with competitive inhibition can result in significantly increased drug penetration across the BBB and potentially substantially increased drug levels in brain tumors.

In summary, concurrent treatment with dual P-gp/BCRP inhibitors can improve delivery and thus efficacy of substrate drugs in the CNS. Recent data imply that the use of tyrosine kinase inhibitors to inhibit P-gp/BCRP could have multiple benefits, especially if the anti-cancer agent enhances its own delivery to the brain.

5. THE BLOOD-BRAIN BARRIER IN BRAIN TUMORS

Recent studies indicate that the integrity of the BBB in brain tumors (“blood-brain tumor barrier”) is compromised, questioning its role in limiting delivery of chemotherapeutics into brain tumors. Indeed, reports show that anticancer drug concentrations in resected tumor tissue are remarkably high. In this regard, Pitz *et al.* provided a summary of anticancer drug concentrations in brain tumors and showed that drug concentrations are high in contrast enhancing tumor areas [108]. Hofer and coworkers demonstrated that gefitinib concentrations in brain tumors were about 10-fold higher compared to gefitinib plasma levels [109]. Likewise, Blakeley *et al.* showed that local methotrexate levels in brain tumors were significantly greater than methotrexate plasma levels [110]. All these reports suggest that the BBB is disrupted in brain tumor tissue and does not restrict drug delivery to the tumor.

Glioblastomas are an example of a highly invasive brain tumor, with a central core that is a necrotic mass, where the BBB is most likely disrupted. Chemotherapeutics can easily traverse the impaired barrier and reach the tumor, which explains the high tumor drug concentrations that have been reported. However, tissue at the tumor borders that is immediately adjacent to healthy brain parenchyma may have an intact BBB that restricts drug delivery. In this regard, Pitz *et al.* reported that anti-cancer drug concentrations in non-contrast enhancing brain areas were low compared to drug concentrations in tumor tissue [108]. Blakeley *et al.* also showed that methotrexate brain penetration was significantly lower in areas adjacent to tumors compared to the tumor core [110].

Thus, the BBB is compromised and disrupted in the tumor core (blood-brain tumor barrier), but may be fully intact at the growing tumor border ([111]; Fig. 3). This phenomenon has significant clinical implications for chemotherapeutic treatment after surgical removal of the primary tumor core. Residual tumor cells in the tumor border with intact barrier limit anti-cancer drug uptake; yet, it is these cells that often grow into larger and more aggressive tumors [112]. Therefore, it is important to highlight the need for efficient treatment of residual tumor cells in invasive areas after surgery [113]. A detailed review on this topic has recently been published by Agarwal *et al.* [114].

6. SUMMARY, CONCLUSIONS AND FUTURE PERSPECTIVES

Recent brain cancer research demonstrates that the BBB drug efflux transporter BCRP is, in addition to P-gp, another obstacle for delivering chemotherapeutic drugs into the brain. It is now clear that both BCRP and P-gp are important elements of barrier function and their expression and transport activity are regulated by distinct signaling pathways. For many anti-cancer drugs it was shown that inhibition of one of the two transporters is not sufficient to deliver drugs into the brain because of compensation by the respective other transporter. These findings lead to the currently accepted paradigm that P-gp and BCRP work as a “cooperative team of gatekeepers” at the BBB. Such P-gp/BCRP teamwork efficiently protects the brain, but at the same time prevents effective CNS therapy, which poses a tremendous clinical problem for the treatment of brain cancers. Two strategies have been developed to circumvent P-gp and BCRP at the BBB and improve drug delivery to the brain. One strategy is to target signaling pathways that control P-gp and BCRP with the goal of down-regulating transporter func-

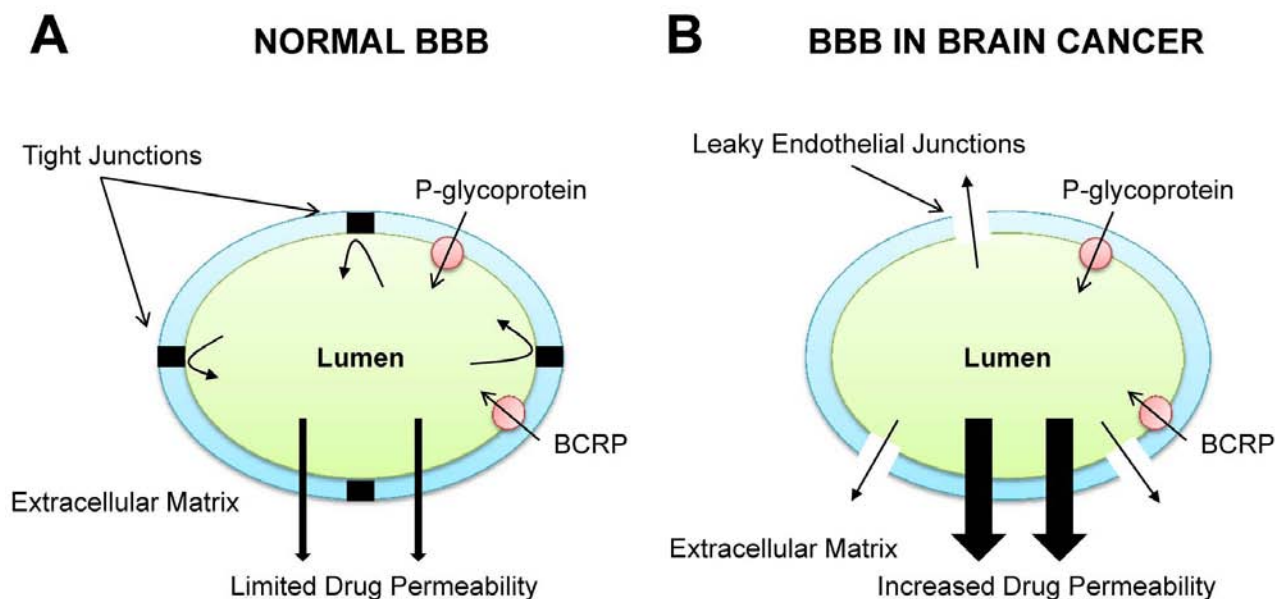


Fig. (3). Comparison of BBB and Blood-Brain Tumor Barrier.

A) Limited BBB permeability due to tight junctions and efflux transporters such as P-gp and BCRP. **B)** In the central core of brain tumors, the “blood-brain tumor barrier” is often disrupted and leaky resulting in increased barrier permeability for drugs.

tion and/or expression. Several pathways have been identified for P-gp and one has recently been found for BCRP. However, a common pathway for both transporters that could potentially be targeted for therapeutic purposes has not been identified yet. A second strategy is combined inhibition of both P-gp and BCRP at the BBB that has been demonstrated to significantly increase brain uptake of chemotherapeutics that are dual P-gp/BCRP substrates. These findings are remarkable and provide a glimpse of hope, but also raise the question: Where do we go from here?

Future research in this field will have to address several points. First, it will be critical to determine if increases in anti-cancer drug brain levels by combined P-gp/BCRP inhibition or down-regulation of transporter function halts brain tumor growth and reduces tumor size. Second, it will also be important to assess if therapeutic effects on brain tumor growth and size translate into prolonged survival. Third, studies will have to demonstrate if inhibiting or down-regulating P-gp/BCRP is a valid therapeutic strategy that can be used chronically over the long term. Fourth, it will have to be tested if P-gp/BCRP inhibition or down-regulation leads to sustained treatment success or if other tumor drug resistance mechanisms will evolve and undo any therapeutic progress made. Lastly, it will have to be shown if an arrest in tumor growth can be treated as a chronic disease or if brain tumors and brain tumor stem-like cells can be completely eradicated. These challenging questions will have to be answered in brain tumor animal models first before translation to patients can occur.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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