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ABCB1 Attenuates the Brain Penetration of the PARP Inhibitor AZD2461

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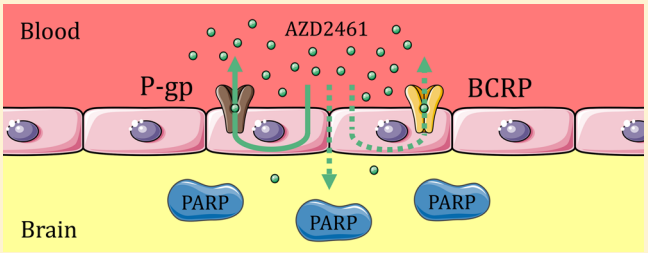
ABSTRACT: Poly (ADP−ribose) polymerase (PARP) inhibitors are a relatively new class of anticancer agents that have attracted attention for treatment of glioblastoma because of their ability to potentiate temozolomide chemotherapy. Previous studies have demonstrated that sufficient brain penetration is a prerequisite for efficacy of PARP inhibitors in glioma mouse models. Unfortunately, however, most of the PARP inhibitors developed to date have a limited brain penetration due to the presence of P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) at the blood−brain barrier. AZD2461 is a novel PARP inhibitor that is unaffected by P-gp mediated resistance in breast cancer models and thus appears to have promising characteristics for brain penetration. We here use a comprehensive set of in vitro and in vivo models to study the brain penetration and oral bioavailability of AZD2461. We report that AZD2461 has a good membrane permeability. However, it is a substrate of P-gp and BCRP, and P-gp in particular limits its brain penetration in vivo. We show that AZD2461 has a low oral bioavailability, although it is not affected by P-gp and BCRP. Together, these findings are not in favor of further development of AZD2461 for treatment of glioblastoma.



KEYWORDS: *glioma, pharmacokinetics, PARP, ABCG2, ABCB1, AZD2461*

1. INTRODUCTION

Poly (ADP−ribose) polymerase (PARP) is an important enzyme in the DNA damage response.[1] It is mainly involved in base excision repair but also plays a role in nonhomologous end-joining. Cancer cells that lost a functional homologous recombination pathway depend more heavily on these DNA damage response pathways, and therefore, PARP inhibitors have gained major interest for cancer treatment. The first breakthrough in the field of PARP inhibition came when olaparib, a first-in-class PARP inhibitor, demonstrated synthetic lethality in BRCA1-deficient triple negative breast cancer.[2] Unfortunately, preclinical studies have demonstrated that olaparib is a substrate for the efflux transporter P-glycoprotein (P-gp) and that upregulation of this transporter in tumor cells led to acquired resistance.[2,3]

P-gp and its relative breast cancer resistance protein (BCRP) have been discovered as factors conferring multidrug resistance to tumor cells, but they are also involved in the protection of healthy tissue throughout the body.[4] First, they are abundantly expressed in the liver and kidney where they are involved in excretion. Second, they are present in the intestinal wall and limit intestinal uptake of potentially harmful substances. Lastly and importantly, they are the dominant efflux transporters at various barrier sites such as the blood−brain barrier (BBB) and blood−testis barrier and reduce the distribution of xenobiotics into these organs. The BBB consists of the brain endothelial cells that are being supported by pericytes and astrocyte foot processes.[5] P-gp (ABCB1/MDR1) and BCRP (ABCG2) are expressed at the apical membrane of the brain endothelial cells and recognize and efflux many xenobiotics, including numerous anticancer agents, back into the bloodstream when attempting to diffuse across the BBB.[6]

The role of P-gp and BCRP in the BBB is of great interest for treatment of intracranial cancers such as glioblastoma (GBM), since these are all at least in part protected by a functional BBB.[7] Previous studies have shown that drugs inhibiting validated targets in GBM, such as Wee1 or CDK4/6, can be efficacious against extracranially transplanted GBM tumors or when BBB functionality is compromised as a result

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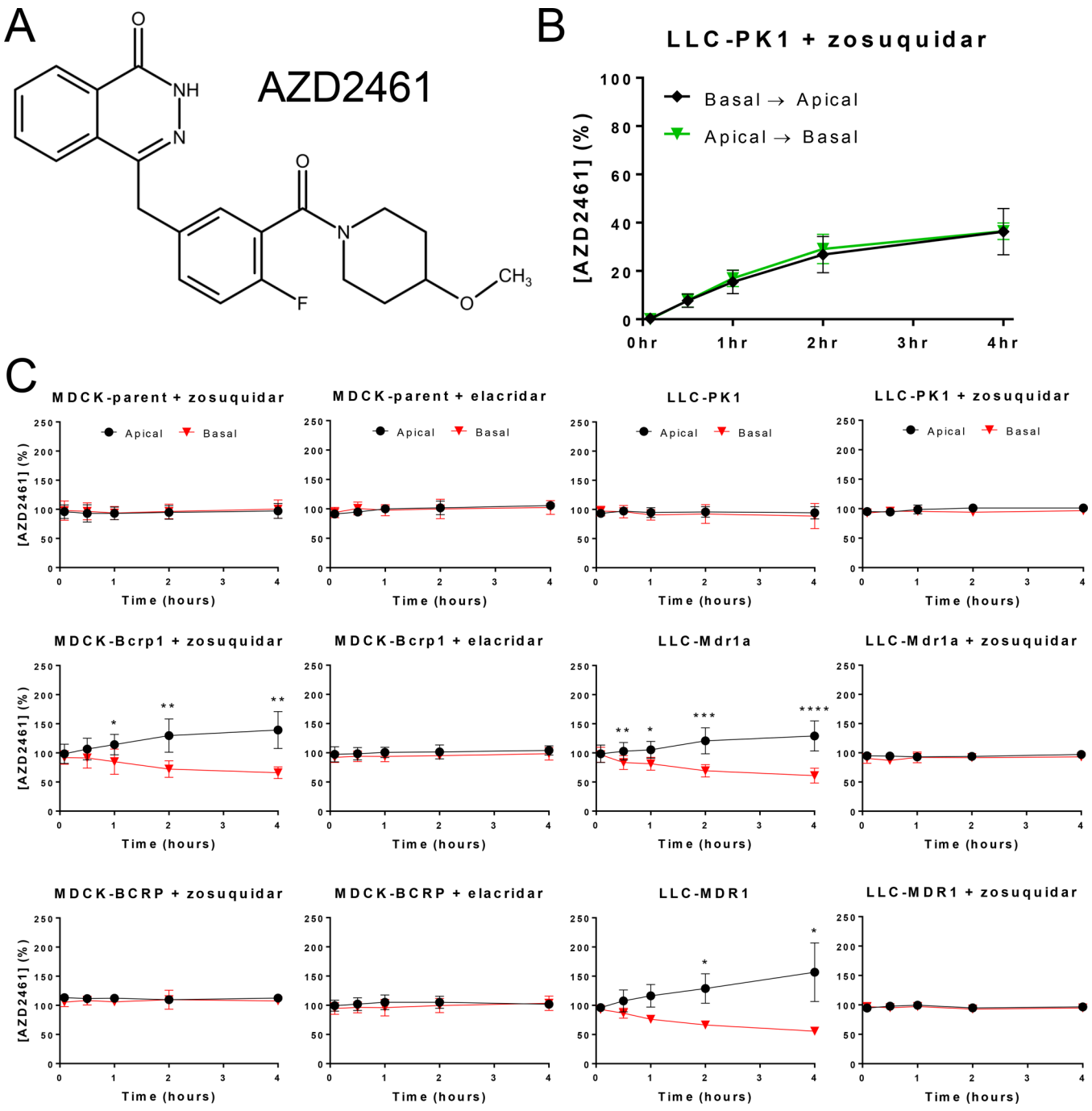

Figure 1. Transport of AZD2461 by P-gp and BCRP in vitro. (A) The chemical structure of AZD2461. (B) A conventional transport assay (CTA) in the presence of zosuquidar to block endogenous P-gp activity. In both directions, AZD2461 efficiently diffuses over an LLC-PK1 monolayer. Within 4 h, the AZD2461 almost reached equilibrium. (C) Concentration equilibrium transport assays (CETAs) using MDCK or LLC cells that overexpress murine BCRP, (Bcrp1), human BCRP, murine P-gp (Mdr1a), or human P-gp (MDR1). Translocation of AZD2461 was observed over MDCK-Bcrp1, LLC-Mdr1a, and LLC-MDR1 monolayers. In all cases, transport selectivity was confirmed by using the P-gp inhibitor zosuquidar or the dual P-gp/BCRP inhibitor elacridar. Zosuquidar was used in all MDCK cell lines to inhibit endogenous P-gp activity. Data are represented as mean ± SD ($n \geq 4$); * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

of genetic loss of ATP-binding cassette (ABC) transporters.[8,9] However, many of these agents are much less efficacious when the tumor is situated behind a functionally intact BBB. PARP inhibitors have demonstrated promising preclinical results in treatment of GBM as a sensitizer to temozolomide chemotherapy, especially in a PTEN-deficient tumor that may consequently have a compromised homologous recombination pathway.[10,11] Unfortunately, however, P-gp and BCRP at the BBB have already been shown to limit brain penetration and prevent antitumor efficacy of earlier generation PARP inhibitors such as rucaparib,[12,13] talazoparib,[14] and veliparib.[10]

Recently, a new PARP inhibitor has been described that is not susceptible to P-gp mediated resistance in BRCA-deficient mammary tumors.[15,16] Tumors that were fully resistant to olaparib due to P-gp upregulation were still responsive to treatment with AZD2461. Based on these data, it was

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postulated that AZD2461 is not a substrate of P-gp, although pharmacokinetic data was not provided. Here, we investigate whether AZD2461 is a substrate of P-gp and BCRP and whether these transporters limit the brain penetration of AZD2461 to assess its potential for further clinical development for treatment of intracranial malignancies. We report that AZD2461 is mainly a P-gp substrate and that its brain penetration in wild-type mice is considerably reduced as a result of P-gp efflux activity at the BBB. As such, we conclude that AZD2461 is not an attractive candidate for further clinical investigation in the context of brain tumors.

2. MATERIALS AND METHODS

2.1. Drugs. AZD2461 was purchased from Syncom B.V. (Groningen, The Netherlands), zosuquidar was purchased from Eli Lilly (Indianapolis, IN), and olaparib was purchased from MedKoo (Research Triangle Park, NC). Elacridar was kindly provided by GlaxoSmithKline (Research Triangle Park, NC).

2.2. Cell Culture. All cell lines used here were previously generated at The Netherlands Cancer Institute by Dr. A.H. Schinkel.[17−19] All cells were cultured under 37 °C and 5% CO_2 conditions in minimal essential medium containing 10% FBS, 1% L-glutamine, 1% sodium pyruvate, 1% MEM vitamins, 1% nonessential amino acids, and 1% penicillin/streptomycin (all from Life Technologies, Carlsbad, CA).

2.3. Concentration Equilibrium Transport Assays. Conventional bidirectional transport assays (CTAs) and concentration equilibrium transport assays (CETAs) were carried out as described previously.[20] AZD2461 was used at a concentration of 100 nM. Zosuquidar (a P-gp inhibitor) and elacridar (a dual P-gp/BCRP inhibitor) were used at a concentration of 5 μM to block transport activity when needed, as this concentration is sufficient to fully inhibit P-gp and BCRP-mediated transport. Transwells exceeding 1.5% carboxyl-[^{14}C]-inulin translocation per hour were excluded from the analysis, as these were considered leaky.

To prepare AZD2461 transport assay samples for LC−MS/MS analysis, 10 μL medium samples were mixed with 30 μL of acetonitrile/formic acid (100:1 v/v). After centrifugation, the supernatant was diluted 5-fold in water. AZD2461 was subsequently measured using an LC−MS/MS system as described below.

2.4. Animals. Mice were housed on a 12 h light/dark cycle. Food and water was provided ad libitum. Room temperature was maintained at 21 °C. All animal housing and studies were approved by the institutional animal experimental committee and conducted according to national law and institutional guidelines.

2.5. Pharmacokinetic Studies. We carried out pharmacokinetic studies with AZD2461 in wild-type (WT), *Abcg2*$^{-/-}$, *Abcb1a/b*$^{-/-}$, and *Abcb1a/b;Abcg2*$^{-/-}$ FVB mice.[21−23] AZD2461 was formulated in DMSO/Cremophor EL/water (1:1:8 v/v/v) for intravenous (i.v.) dosing at both 10 and 50 mg/kg. For oral (100 mg/kg) administration, the drug was suspended in 0.5% hydroxypropyl methyl cellulose and mixed overnight at 4 °C for optimal suspension. Bleeding from the tail vein was used to draw blood at intermediate time points, whereas at the terminal time point, blood was also collected by cardiac puncture. Brain tissue was subsequently collected. Plasma was obtained from whole blood by centrifugation (5 min, 5000 rpm, 4 °C). After weighing, tissues were homogenized in 1% (w/v) bovine serum albumin using a FastPrep-24 (MP-Biomedicals, NY). AZD2461 was extracted from plasma and brain homogenate via *tert*-butyl methyl ether liquid−liquid extraction. Olaparib was used as an internal standard for LC−MS/MS analysis.

2.6. LC−MS/MS Analysis. AZD2461 concentrations were measured using an LC−MS/MS system that consisted of an UltiMate 3000 LC System (Dionex, Sunnyvale, CA) and an API 4000 mass spectrometer (Sciex, Framingham, MA). Samples were analyzed using a ZORBAX Extend-C18 column (Agilent, Santa Clara, CA), preceded by a Securityguard C18 precolumn (Phenomenex, Utrecht, The Netherlands). Elution was done using a mixture of mobile phase A (0.1% formic acid in water (v/v)) and mobile phase B (methanol) in a 5 min gradient from 20 to 95% B, followed by 95% B that was maintained for 3 min, and then re-equilibration at 20% B. The multiple reaction monitoring parameters were 396.2/281.1 (AZD2461) and 435.2/281.2 (olaparib). System control and data analysis was done using Analyst 1.6.2 software (AB Sciex; Foster City, CA). The accuracy and precision of the LC−MS/MS method were within the acceptable range. The detection limit was 0.3 nM, the lower limit of quantification was 2 nM, and the upper limit of quantification was 100 nM AZD2461 in plasma.

2.7. Pharmacokinetic and Statistical Analysis. CETA results were analyzed with the general linear model repeated measures procedure of SPSS (v22; SPSS Inc., Chicago, IL) as described in detail before.[20] All other statistical analyses in this study involved one-way analysis of variance followed by post hoc Bonferroni tests, since more than two groups were compared. For all analyses, differences were considered statistically significant when $p < 0.05$. The pharmacokinetic parameters of AZD2461 were determined using the PK solver plugin in Microsoft Excel.[24] The standard error of the oral bioavailability was calculated using the formula below

$$SD_F = F\sqrt{\left(\frac{SD_{AUC_{p.o.}}}{AUC_{p.o.}}\right)^2 + \left(\frac{SD_{AUC_{i.v.}}}{AUC_{i.v.}}\right)^2}$$

3. RESULTS

3.1. P-gp and BCRP Transport AZD2461 in Vitro. We first established whether AZD2461 (Figure 1A) could be transported by P-gp and BCRP in vitro. To this end, we conducted a set of transport experiments using cell lines that overexpress human or murine P-gp or BCRP. We cultured these cells in a transwell system, essentially creating apical and basolateral compartments being separated by a cellular monolayer. Before analyzing transporter substrate affinity, we first confirmed that AZD2461 is sufficiently permeable over the monolayer using a conventional transport assay (CTA) setup (Figure 1B), In this setup, a given compound is added to one compartment (the donor compartment), and the concentration in the other compartment (the acceptor compartment) is measured over time as a measure of a compound's cellular membrane permeability. The establishment of a compound's membrane permeability is important for the interpretation of the results of the more sensitive concentration equilibrium transport assay (CETA). In a CETA, a compound is added to both compartments of the transwell system at equal concentrations, and the concentration in both compartments is again measured over time. However, without establishing a compound's potential to diffuse over the monolayer, absence

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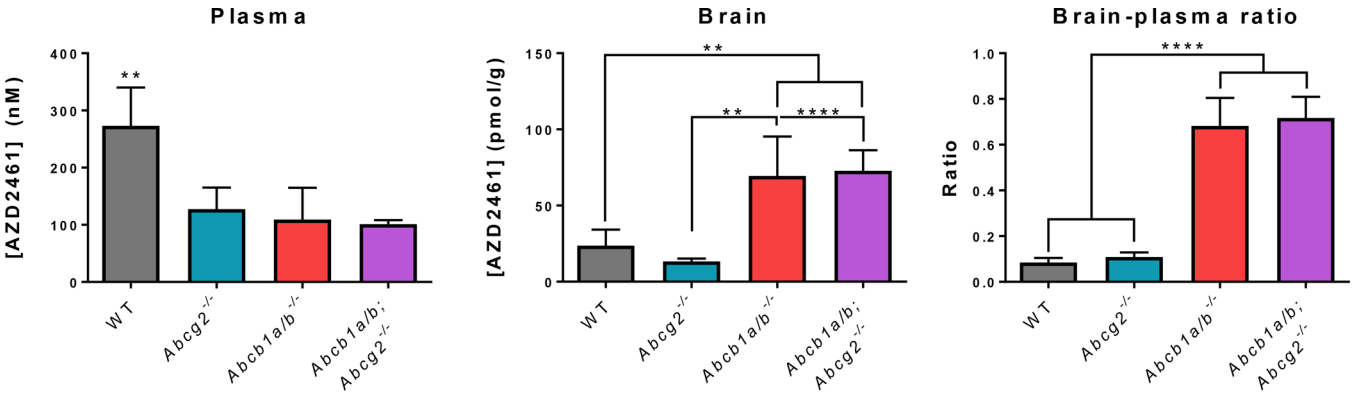

Figure 2. P-glycoprotein restricts the brain penetration of AZD2461 in vivo. AZD2461 (10 mg/kg) was intravenously administered to wild-type, *Abcg2*$^{-/-}$, *Abcb1a/b*$^{-/-}$, and *Abcb1a/b;Abcg2*$^{-/-}$ mice. One hour after injection, blood and brain tissue was collected for LC−MS/MS analysis. A 3-fold increased brain concentration and corresponding brain−plasma ratio was observed in *Abcb1a/b*$^{-/-}$ and *Abcb1a/b;Abcg2*$^{-/-}$ compared to wild-type mice. Interestingly, wild-type mice had an approximately 2.5-fold increased plasma concentration compared to all knockout mouse strains. All data are represented as mean ± SD (n = 4); ** p < 0.01; **** p < 0.0001.

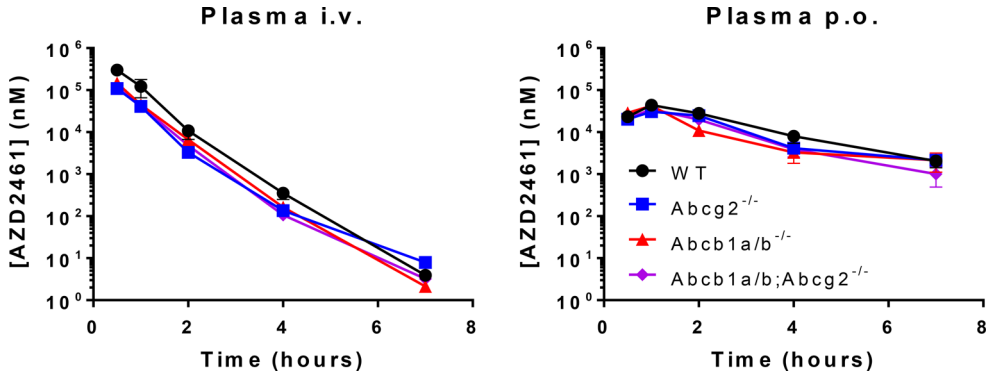

Figure 3. No impact of P-gp and BCRP on the plasma concentrations of AZD2461 following intravenous and oral administration. AZD2461 was administered intravenously (50 mg/kg) and orally (100 mg/kg) to wild-type, *Abcg2*$^{-/-}$, *Abcb1a/b*$^{-/-}$, and *Abcb1a/b;Abcg2*$^{-/-}$ mice. No differences were observed in the plasma concentration time curves among the different mouse strains. The oral bioavailability of AZD2461 is poor, since oral administration to wild-type mice only yielded a dose-adjusted AUC of approximately 10% compared to intravenous administration (see Table 1). All data are represented as mean ± SD ($n \geq 4$).

of translocation in a CETA can also be caused by poor membrane permeability.

After we established that AZD2461 is membrane permeable, we measured AZD2461 translocation over ABC-transporter-expressing cellular monolayers using the CETA setup. No translocation was observed in the parental cell lines, whereas AZD2461 was efficiently translocated by monolayers over-expressing murine Bcrp1, murine Mdr1a (P-gp), and human MDR1 (P-gp) (Figure 1C). These findings clearly indicate that AZD2461 is a substrate for murine BCRP and murine and human P-gp. No translocation occurred in human BCRP-overexpressing monolayers. It is a common finding, however, that compounds are more efficiently translocated by the murine Bcrp1 than the human BCRP-expressing MDCK cells. We expect that this is caused by a lower and/or less polarized expression of BCRP in the MDCK-BCRP cell line. Consequently, it is still possible that AZD2461 can be transported by human BCRP, albeit that it is likely just a weak substrate.

3.2. P-gp Restricts the Brain Penetration of AZD2461 in Vivo. To investigate whether transport affinity for P-gp and BCRP has any impact on the brain penetration of AZD2461, we measured the AZD2461 brain concentration in wild-type (WT) FVB mice following intravenous administration and compared it to the concentrations obtained in mice that were genetically engineered to lack one or both of these ABC transporters. Mice lacking Bcrp1 (*Abcg2*$^{-/-}$) did not have a higher brain concentration than WT mice, indicating that P-gp is able to fully compensate for the loss of Bcrp1 in these mice (Figure 2). In contrast, we could observe a 3-fold increased brain concentration in mice lacking P-gp (*Abcb1a/b*$^{-/-}$), indicating that this transporter restricts the brain penetration of AZD2461. A combined deletion of P-gp and BCRP (*Abc1a/b;Abcg2*$^{-/-}$) did not result in a further elevated AZD2461 brain level compared to *Abcb1a/b*$^{-/-}$ mice, suggesting that Bcrp1 does not efficiently transport AZD2461 at the blood−brain barrier. Since Bcrp1 is able to transport AZD2461 in vitro (Figure 1C), it could be that a difference in the brain−plasma ratio of *Abcb1a/b*$^{-/-}$ and *Abc1a/b;Abcg2*$^{-/-}$ mice does exist. However, in order to reach statistical significance, many more mice will be required with these small differences. The expression of Bcrp1 at the murine BBB is about 5-fold lower compared to Mdr1a.[25] Therefore, the effects of P-gp are much easier to detect in mice than those of Bcrp1, especially when studying weak substrates. Notably, however, the expression of BCRP at the human BBB is higher than that of P-gp.[26] The observation that AZD2461 is a substrate of Bcrp1 therefore

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Table 1. Pharmacokinetic Parameters of AZD2461 after Oral and i.v. Administration to WT, *Abcg2*$^{-/-}$, *Abcb1a/b*$^{-/-}$, and *Abcb1a/b;Abcg2*$^{-/-}$ FVB Mice[a]

route	parameter	time (h)	genotype			
			WT	*Abcg2*$^{-/-}$	*Abcb1a/b*$^{-/-}$	*Abcg2;Abcb1a/b*$^{-/-}$
i.v. 50 mg/kg	AUC_{plasma} (μg/mL·h)	0−∞	200 ± 99	66 ± 18****	98 ± 22**	73 ± 5.2****
	C_{max} (μg/mL)		120 ± 53	43 ± 13*	58 ± 9.4	46 ± 5.8*
	t_{max} (h)		0.5	0.5	0.5	0.5
	$t_{1/2}$ (h)		0.44 ± 0.02	0.59 ± 0.14	0.42 ± 0.03	0.45 ± 0.06
	V_z (L/kg)		0.20 ± 0.11	0.68 ± 0.21	0.33 ± 0.10	0.44 ± 0.07
	CL (L/kg·h)		0.31 ± 0.16	0.79 ± 0.16	0.53 ± 0.13	0.69 ± 0.05
p.o. 100 mg/kg	AUC_{plasma} (μg/mL·h)	0−∞	45 ± 19	35 ± 7.2	32 ± 5.3	32 ± 6.3
	C_{max} (μg/mL)		17 ± 3.5	12 ± 2.9	18 ± 11	14 ± 3.2
	t_{max} (h)		1.0 ± 0.0	1.2 ± 0.4	0.8 ± 0.3	1.0 ± 0.0
	$t_{1/2}$ (h)		1.3 ± 0.3	1.5 ± 0.3	1.6 ± 0.6	1.1 ± 0.5
	F (%)		11 ± 7.5	27 ± 9.1	16 ± 4.5	22 ± 4.6
	V_z/F (L/kg)		4.8 ± 2.0	6.3 ± 2.0	7.9 ± 4.2	5.4 ± 3.3
	CL/F (L/kg·h)		2.5 ± 0.9	2.9 ± 0.5	3.2 ± 0.6	3.2 ± 0.6

[a]AUC, area under the curve; C_{max}, maximum concentration in plasma; t_{max}, time to reach maximum plasma concentration; $t_{1/2}$, elimination half-life; V_z, apparent volume of distribution; CL, apparent clearance; F, oral bioavailability; V_z/F, apparent volume of distribution after oral administration; CL/F, apparent clearance after oral administration. Data are represented as mean ± SD ($n \geq 4$). * $p < 0.05$; ** $p < 0.01$; **** $p < 0.0001$ relative to WT.

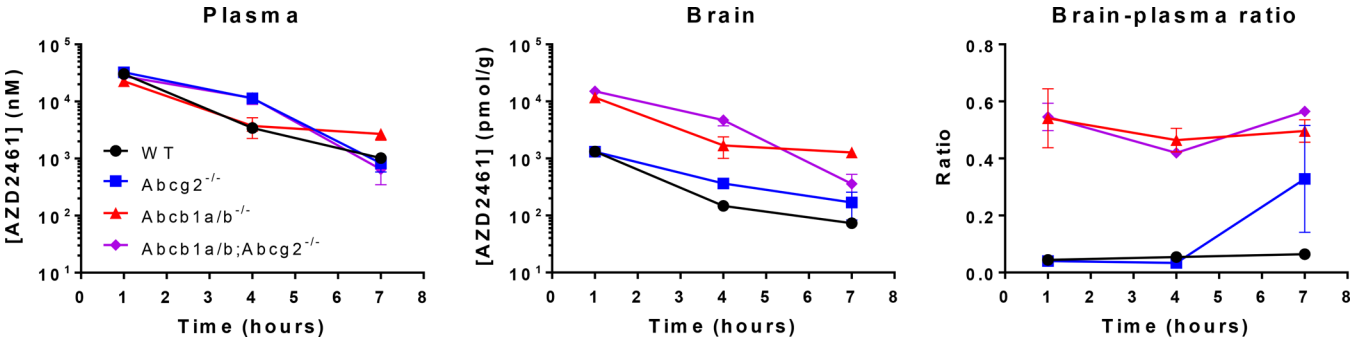

Figure 4. P-glycoprotein restricts the brain penetration of AZD2461 following oral administration. AZD2461 was administered orally to wild-type, *Abcg2*$^{-/-}$, *Abcb1a/b*$^{-/-}$, and *Abcb1a/b;Abcg2*$^{-/-}$ mice at a dose of 100 mg/kg. A markedly increased brain concentration and brain−plasma ratio were observed in *Abcb1a/b*$^{-/-}$ and *Abcb1a/b;Abcg2*$^{-/-}$ mice, in line with the results obtained after intravenous administration (see Figure 2). All data are represented as mean ± SD ($n \geq 4$).

suggests that the effects of BCRP at the human BBB may be more pronounced.

Interestingly, we observed an approximately 2.5-fold increased plasma concentration in WT mice compared to any of the ABC-transporter knockout mice. The reason for this higher plasma concentration is unclear, but if we correct for this higher plasma concentration by calculating the brain−plasma ratio, the difference in AZD2461 brain penetration between WT and *Abcb1a/b*$^{-/-}$ and *Abc1a/b;Abcg2*$^{-/-}$ mice is even more pronounced at roughly 7-fold. Together, these data clearly show that the brain penetration of AZD2461 is restricted by P-gp but not by BCRP.

3.3. AZD2461 Has a Poor Oral Bioavailability That Is Unaffected by P-gp and BCRP. Since PARP inhibitors are often administered orally to patients,[27] and P-gp and BCRP can limit the oral uptake of pharmaceuticals,[4] we also determined the oral bioavailability of AZD2461 in WT and knockout mice. We applied 100 mg/kg of AZD2461 administered as a 10 mg/mL suspension in 0.5% hydroxypropyl methyl cellulose in water, as this dose and dose form were routinely used in preclinical studies.[2,3] The plasma levels ranged between 1 and 10 μM (Figure 3), yielding an oral bioavailability in WT mice of only about 10% (Table 1). Compared to wild-type mice, no significantly increased oral bioavailability was found in any of the transporter knockout mouse strains (Table 1). In line with the previous i.v. results (Figure 2), we again found that wild-type mice had considerably higher plasma levels following intravenous administration of AZD2461, as indicated by an elevated AUC_{iv} and C_{max}. In summary, these data demonstrate that AZD2461 has a poor oral bioavailability, but this is not due to P-gp and BCRP.

3.4. AZD2461 Achieves Similar Brain−Plasma Ratios following Oral and Intravenous Administration. We next studied the brain penetration of AZD2461 at several time points after oral administration. The brain penetration of AZD2461 in WT mice was again low, with a brain−plasma ratio never exceeding 0.1 (Figure 4). Similarly, the brain−plasma ratios were also low in *Abcg2*$^{-/-}$ mice. In contrast, the brain penetration in *Abcb1a/b*$^{-/-}$ and *Abcb1a/b;Abcg2*$^{-/-}$ mice was significantly higher. With steady brain−plasma ratios of approximately 0.5 at all measured time points, the difference in AUC_{brain}:AUC_{plasma} between WT and P-gp knockout mice was 10-fold. Taken together, we conclude that P-gp at the BBB restricts the brain penetration of AZD2461 following oral and intravenous administration.

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4. DISCUSSION

PARP inhibitors are a relatively new class of anticancer agents that attenuate DNA damage repair by inhibiting poly (ADP−ribose) polymerase. AZD2461 is a novel PARP inhibitor, and its efficacy has been unaffected by P-gp in breast cancer models. We reasoned that AZD2461 might be an interesting candidate for treatment of GBM when its brain penetration is indeed not restricted by important efflux transporters expressed at the blood−brain barrier. This study, however, demonstrates that AZD2461 is a substrate of P-gp and BCRP and that P-gp in particular limits AZD2461 brain penetration in vivo. Moreover, we have also found that AZD2461 has a relatively low oral bioavailability. These findings are not in favor of further development of AZD2461 for treatment of GBM.

Glioblastoma is extensively treated, as standard-of-care consists of maximum surgical resection followed by fractionated radiotherapy and concomitant temozolomide chemotherapy.[28] Temozolomide is an alkylating agent that exerts its cytotoxicity by forming methyl adducts on the DNA. Such adducts are often repaired by base excision repair, a process in which PARP plays a pivotal role. Consequently, PARP inhibition may be an attractive strategy to improve standard-of-care therapy for GBM. Encouraging results have been demonstrated using intracranial glioma mouse models.[29,30] Moreover, Halford et al. very recently presented data in the American Society for Clinical Oncology meeting on the use of olaparib in patients with relapsed GBM.[31] However, olaparib is a substrate of P-gp, and several studies have shown that P-gp and/or BCRP in the BBB can restrict the efficacy of treatment with PARP inhibitors.[10,13,14] In order to fully exploit the potential of PARP inhibition in GBM, it is worthwhile to continue investing candidate PARP inhibitors that are not substrates of these ABC transporters. Recently, Chornenkyy et al. demonstrate efficacy of niraparib in orthotopic models of pediatric high-grade glioma and diffuse intrinsic pontine glioma.[32] This is in line with preliminary reports that niraparib displays a high brain−plasma ratio,[33] although it is yet unknown if niraparib is recognized by P-gp or BCRP.

In contrast to previous studies showing that AZD2461 is not amenable to P-gp mediated resistance in breast cancer models, we here report that this PARP inhibitor is a substrate of P-gp. The reason why overexpression of P-gp was insufficient to confer AZD2461 resistance to a breast cancer model is unexplored. None of these studies included pharmacokinetic data in plasma and tumor samples. Considering that AZD2461 is a weaker substrate than olaparib, it might be that just enough AZD2461 is able to accumulate into these breast cancer cells to cause sufficient PARP inhibition, whereas this threshold of required PARP inhibition is not achieved with olaparib. However, at the BBB, the hurdle of P-gp may be greater as P-gp may be more densely expressed in brain endothelial cells than in tumor cells. Furthermore, the AZD2461 influx into tumor cells might be higher than that across the BBB as a result of higher pinocytic activity and the presence of additional uptake mechanisms. Additionally, AZD2461 needs to cross only the cellular membrane to diffuse into a tumor cell but has to diffuse across the apical and basloteral endothelial membranes to enter the brain. Together, these factors might allow more efficient efflux of weaker substrates at the BBB compared to tumor cells, as exemplified by the 10-fold difference in AUC_{brain}:AUC_{plasma} ratio we observed between WT and *Abcb1a/b;Abcg2*$^{-/-}$ mice.

As we demonstrate here, the oral bioavailability of AZD2461 is only approximately 10%. Although not uncommon for small molecular targeted agents, a poor oral bioavailability is generally problematic for clinical development, because it often leads to a high variability in exposure in patients.[34] Large variations in drug exposure increase the risk of over and undertreatment, resulting in either toxicity or ineffective therapy. A similar low oral bioavailability has been found for olaparib in mice receiving olaparib in a microsuspension.[35] Notably, the oral bioavailability increased to 50% when using a soluble dosing form, suggesting that incomplete drug dissolution is the limiting factor. The formal bioavailability of olaparib in patients has not been established due to the absence of an intravenous formulation. However, discrepancies in the relative oral bioavailability of olaparib between capsules and tablet solid dose forms were found,[36] suggesting suboptimal intestinal dissolution and uptake in patients. Based on our results, we expect that AZD2461 will have similar qualities as olaparib with respect to oral dose forms. A phase I study investigating the tolerability and pharmacokinetics of oral AZD2461 in refractory solid cancer patients has been completed, but unfortunately, no data has yet been reported (ClinicalTrials.gov Identifier NCT01247168). Notably, some other clinically advanced PARP inhibitors seem to have substantially higher oral bioavailabilities than AZD2461,[30,37,38] which would be an argument as to why AZD2461 might not be the preferred PARP inhibitor to develop further.

In summary, we have demonstrated that AZD2461 is a substrate of P-gp and BCRP and that P-gp substantially limits the brain penetration of AZD2461 in mice. Moreover, AZD2461 has a low oral bioavailability, indicating that AZD2461 does not offer a pharmaceutical advantage over other PARP inhibitors. Together, these findings are not in favor of further clinical development of AZD2461 for treatment of glioblastoma.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

ABC, ATP-binding cassette; AUC, area under the curve; BBB, blood−brain barrier; BCRP, breast cancer resistance protein; CETA, concentration equilibrium transport assay; CTA, conventional transport assay; GBM, glioblastoma; PARP,

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poly(ADP−ribose) polymerase; P-gp, P-glycoprotein; WT, wild-type

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