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ARTICLE

Is there a possibility that P-glycoprotein reduces reproductive toxicity in males but breast cancer resistance protein does not?

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Abstract

In traditional understanding, P-glycoprotein (P-gp) and Breast Cancer Resistance Protein (BCRP) are regarded as efflux transporters that can decrease the concentration of their substrates in the testis, thereby reducing reproductive toxicity in males (RTM) and protecting spermatogenesis. However, there is currently no direct pharmacological evidence demonstrating that P-gp and BCRP can reduce the occurrence of drug-induced RTM. In this study, we chose small molecule targeted anti-tumor agents as model drugs and systematically retrieved and collected information on the transporters and RTM for these drugs, followed by correlation analysis. The results showed a lower incidence of RTM for P-gp substrate drugs, which aligns with previous knowledge. Surprisingly, BCRP substrate drugs exhibited higher rates of RTM in various dimensions, contradicting previous notions. This discrepancy may be attributed to the differential distribution and transport directions of P-gp and BCRP on the blood–testis barrier (BTB). For the first time, this study may provide clues that BCRP may facilitate the passage of exogenous compounds across the BTB, increasing the occurrence of RTM, rather than protecting spermatogenesis as traditionally believed. Furthermore, this study provides the first direct verification of the role of P-gp in reducing RTM and protecting spermatogenesis.

Preliminary results were presented at the American Association of Pharmaceutical Scientists Annual Meeting, November 5, 2018, Washington DC.

Zhiheng Yu and Wei Liu should be considered joint first authors.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

The efflux transporters P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) have long been regarded as crucial components in reducing the reproductive toxicity of exogenous substances at the blood–testis barrier (BTB). However, there is no direct evidence demonstrating the protective role of P-gp and BCRP on spermatogenesis.

WHAT QUESTION DID THIS STUDY ADDRESS?

This study employed evidence-based medicine approaches, using small molecule targeted anti-tumor drugs as model drugs, to analyze the correlation between P-gp and BCRP and the occurrence of reproductive toxicity in males (RTM).

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

Consistent with prior research, drugs that were solely P-gp substrates exhibited the lowest rate of RTM, suggesting the protective role of P-gp in spermatogenesis. However, contrary to conventional understanding, this study revealed a positive correlation between the drug substrates of BCRP and RTM, suggesting a potential detrimental effect of BCRP on spermatogenesis. The underlying reasons for this phenomenon may be linked to the positional location of BCRP at the BTB.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

This study may provide some clues that drugs which are substrates of BCRP are more likely to exhibit RTM, indicating that not all efflux transporters located at biological barriers necessarily provide protective effects against the physiological toxicity of exogenous substances.

INTRODUCTION

It has been long believed that efflux transporters on the blood–testis barrier (BTB) could limit xenobiotic entrance and accumulation in testis and protect spermatogenesis[1], like the blood–brain barrier, BTB is one of the most effective physiological barriers. P-glycoprotein (P-gp) and Breast Cancer Resistance Protein (BCRP) are the most common efflux transporters involved in the absorption, distribution, metabolism, and excretion (ADME) of drugs,[2] both of which are abundantly expressed in testis.[3,4]

Numerous studies employing gene knock-out mice have shown that P-gp and BCRP can decrease testis drug concentrations, including vinblastine, ivermectin, digoxin, cyclosporine, ondansetron, loperamide, genistein, coumestrol, and daidzein.[5–9] All of the studies measured only drug concentrations in testis homogenate rather than the incidence of reproductive toxicity in males (RTM). In other words, the protective effects of P-gp and BCRP on spermatogenesis were mainly based on indirect pharmacokinetic evidence. However, thus far, no study has directly observed that P-gp and BCRP can reduce the RTM of drugs.

Many small molecule targeted anti-tumor drugs are substrates of P-gp and BCRP[10,11]; however, they could also cause RTM.[12] Thus, here we have examined small molecule targeted anti-tumor drugs as model drugs, systematically collecting transporter and RTM information, and analyzed the influence of P-gp and BCRP on these drugs' RTM.

METHODS

RTM information of drugs

This study included all the small molecule targeted anti-tumor drugs approved by U.S. Food and Drug Administration (FDA) through 2020. RTM was categorized as reducing male fertility by affecting spermatogenesis including reproductive organs damage, alterations in endocrine function, regulation of gamete maturation and release, reduction in sperm count, alterations in sperm motility or morphology as per FDA's guidance.[13] RTM information for all of the drugs was collected from FDA drug labels and clinical pharmacology reviews and divided into "No RTM drugs" and "RTM drugs." The experimental animal species used for reproductive toxicity studies and the relative exposure ratio obtained during RTM studies were summarized. Relative exposure ratio was calculated as the

ratio of a drug's AUC for a RTM dose in an experimental animal to the AUC for the label recommended dose in humans.[14] A smaller relative exposure ratio suggests that RTM could be induced at a lower exposure level and vice versa.

Transporter information of drugs

In analyzing the relationship between a drug's RTM and P-gp and/or BCRP information, transporter profiles were divided into four groups: (1) Not a transporter substrate (None), (2) Solely P-gp substrate (P-gp), (3) Solely BCRP substrate (BCRP), and (4) Substrate of both P-gp and BCRP (Dual). For dual substrates, we further collected the efflux ratio, intrinsic clearance or K_m for both transporters that roughly represent the transporting susceptibility of each drug. The usual designation for these efflux transporters is capitalized BCRP and P-gp in humans and lower case bcrp and p-gp in animals. Here, we make no distinction, using capitalized designations exclusively since although transporter data may be available from either an animal or human cells, relative exposure ratio values are only from human studies.

All the transporter information was obtained from new drug approval (NDA) documents available from the websites of the FDA, European Medicines Agency (EMA), Japanese Pharmaceuticals and Medical Devices Agency (PDMA), Australian Therapeutic Goods Administration (TGA), and the UW Drug Interaction Database (DIDB).

Statistical analyses

Summarized transporter information and the frequency of RTM in each group of drugs were compared. In addition, the correlation between a RTM drug's transporter information and its relative exposure ratio were analyzed. Continuous variables were tested for normality first to determine whether the unpaired *t*-test or the Mann–Whitney *U*-test was appropriate. Fisher's exact or Chi-squared tests were used to compare categorical variables. All statistical analyses were conducted using SPSS 26.0 and GraphPad Prism 9.

RESULTS

We identified 66 small molecule targeted anti-tumor drugs approved by the FDA, but specific data for BCRP was not reported for five of them. Accordingly, 61 small molecule targeted anti-tumor drugs were eventually involved in this study, with approval dates from 2001 to 2020, for various pharmacological targets, as shown in Table 1.

RTM was reported in 33 out of the 61 drugs. Most drugs were substrates of transporters, with only seven drugs being not transporter substrates. In all, 51 drugs were substrates of P-gp, of which 33 were dual substrates. Only three drugs were solely BCRP substrates.

The relationship between RTM and transporter information is shown in Figure 1. Less than 30% of solely P-gp substrates showed RTM, while 100% of solely BCRP substrates reported RTM. More than half of none-substrate or dual-substrate reported RTM, 57.1% and 63.6%, respectively. Solely P-gp substrates had a lower RTM comparing with solely BCRP substrates ($p=0.04$) and dual transporter substrates ($p=0.02$). There was no difference in RTM between solely P-gp substrates and none transporter substrates.

The subgroup of dual P-gp and BCRP substrates was further analyzed. Transporting ability of P-gp and BCRP for 16 of 33 drugs exhibiting RTM was available. The transporting susceptibility of most drugs were assessed by the measured efflux ratio in the same cell experimental system ($n=14$). One drug was assessed by including intrinsic clearance and K_m. Among the 16 drugs, six drugs exhibited stronger transport susceptibility via BCRP, while 10 drugs demonstrated stronger transport susceptibility via P-gp. The results in Figure 2 showed that the drugs with higher transporting susceptibility of P-gp had a lower RTM ($p=0.03$).

Most drugs in Table 1 were tested in rats ($n=57$); other experimental animals included dog ($n=19$), monkey ($n=8$), mouse ($n=2$), and minipig ($n=1$). Of the drugs tested in rats, 33 exhibited RTM, of which 26 drugs also had relative exposure ratio data as presented in Figure 3a. Solely P-gp substrates showed the highest exposure ratio (6.03 ± 5.56) and solely BCRP substrates the lowest (0.75 ± 0.35). To reduce the influence of small sample size on statistics, we conducted further exploratory analysis by combining solely BCRP substrates and dual-substrates into the "BCRP" group, and solely P-gp substrates and non-substrates into the "non-BCRP" group. The results in Figure 3b show that the exposure ratio in the BCRP group was lower than the non-BCRP group (2.54 vs. 5.57, $p=0.049$).

DISCUSSION

P-gp and BCRP have always been identified as two protectors for spermatogenesis since they could transport a broad range of xenobiotics that are detrimental to spermatogenesis out of testis against a concentration gradient.[1] This study showed that only P-gp could provide protection while BCRP showed the opposite. Among the 61 small molecule targeted anti-tumor drugs, solely P-gp substrates rather

TABLE 1 Small molecule targeted anti-tumor drugs analyzed in this study.

Drug name	Pharmacological targets	FDA approval date	P-gp	BCRP	RTM Inform-ation	Experimental animals for RTM
Capmatinib	MET	2020	■	□	●	Rat; Monkey
Pemigatinib	FGFR	2020	■	■	●	Rat; Monkey
Selumetinib	MEK1/2	2020	■	■	●	Mouse
Ripretinib	KIT/PDGFRα	2020	■	■	●	Rat
Avapritinib	PDGFRα	2020	□	□	●	Rat; Dog
Pralsetinib	RET	2020	■	■	●	Rat
Selpercatinib	VEGF and RET	2020	■	■	●	Rat; Minipig
Tucatinib	HER2	2020	■	■	●	Rat
Entrectinib	TRK, ALK, ROS-1	2019	□	□	●	Rat; Dog
Zanubrutinib	BTK	2019	■	□	●	Rat
Alpelisib	PI3K	2019	■	■	●	Rat; Dog
Pexidartinib	CSF-1R	2019	□	□	●	Rat; Dog
Ceritinib	ALK	2019	■	■	●	Rat; Monkey
Baricitinib[22]	JAK1/2	2019	■	■	●	Rat
Fedratinib	JAK2 and FLT3	2019	■	□	●	Rat
Upadacitinib	JAK	2019	■	■	●	Rat
Binimetinib[23]	MEK1, MEK2	2018	■	■	●	Rat; Monkey
Ivosidenib	IDH1	2018	■	□	●	Rat
Encorafenib	BRAF V600E	2018	■	□	●	Rat
Glasdegib	SMO	2018	■	■	●	Rat
Talazoparib	PARP1/2	2018	■	■	●	Rat; Dog
Fostamatinib	FGFR	2018	■	□	●	Rat
Larotrectinib	TRK	2018	■	■	●	Rat; Monkey
Dacomitinib	EGFR	2018	■	■	●	Rat
Lorlatinib	ALK	2018	■	□	●	Rat; Dog
Abemaciclib[24]	CDK4/6	2017	■	■	●	Rat; Dog
Copanlisib	PI3K	2017	■	■	●	Rat; Dog
Enasidenib	IDH2	2017	□	□	●	Rat
Ribociclib	CDK4/6	2017	■	□	●	Rat; Dog
Acalabrutinib	BTK	2017	■	■	●	Rat
Neratinib	HER-2	2017	■	□	●	Rat; Dog
Brigatinib[25]	ALK, ROS-1, IGF-1R	2017	■	■	●	Rat; Monkey
Tofacitinib	JAK	2016	■	□	●	Rat
Sonidegib	Hedgehog pathway inhibitor	2015	□	□	●	Rat
Cobimetinib	MEK	2015	■	□	●	Dog
Alectinib	ALK and RET	2015	□	□	●	Rat; Monkey
Lenvatinib	VEGF	2015	■	■	●	Dog
Palbociclib	CDK4/6	2015	■	■	●	Rat; Dog
Nintedanib	VEGFR/PDGFR/ FGFR	2014	■	□	●	Rat
Idelalisib	PI3K	2014	■	■	●	Rat
Trametinib	MEK 1 / 2	2013	■	□	●	Rat; Dog

TABLE 1 (Continued)

Drug name	Pharmacological targets	FDA approval date	P-gp	BCRP	RTM Inform-ation	Experimental animals for RTM
Dabrafenib[26]	BRAF	2013	■	■	●	Rat; Dog
Ibrutinib[27]	BTK	2013	■	□	●	Rat
Afatinib[28]	EGFR/HER2/ HER4	2013	■	■	●	Rat
Vismodegib	Hedgehog pathway inhibitor	2012	■	□	●	Rat
Axitinib	VEGF inhibitor	2012	■	■	●	Mouse
Bosutinib	BCR-ABL/Src	2012	■	□	●	Rat
Cabozantinib[29,30]	RET, MET, VEGF	2012	□	□	●	Rat; Dog
Ponatinib	BCR-ABL	2012	■	■	●	Rat; Monkey
Regorafenib[31–33]	Multiple Tki	2012	□	■	●	Rat; Dog
Vandetanib	Multiple Tki	2011	□	■	●	Rat
Vemurafenib	BRAF	2011	■	■	●	Rat; Dog
Crizotinib	ALK/ROS-1	2011	■	□	●	Rat
Pazopanib	VEGF	2009	■	■	●	Rat
Lapatinib[34]	EGFR/HER-2	2007	■	■	●	Rat
Sunitinib	Multiple Tki	2006	■	□	●	Rat
Dasatinib	BCR-ABL/Src	2006	■	■	●	Rat
Sorafenib[35]	Multiple Tki	2005	□	■	●	Rat; Dog
Erlotinib	EGFR	2004	■	■	●	Rat
Gefitinib	EGFR	2003	■	■	●	Rat
Imatinib[36]	BCR-ABL	2001	□	□	●	Rat

Note: RTM data for the drugs was obtained from FDA label and clinical pharmacology review.

Transporter information was obtained from NDA documents of FDA, EMA, PDMA, and TGA, and references cited by DIDB.

■ = Substrate of transporter; □ = Not a substrate of transporter.

● = No RTM; ● = Having RTM.

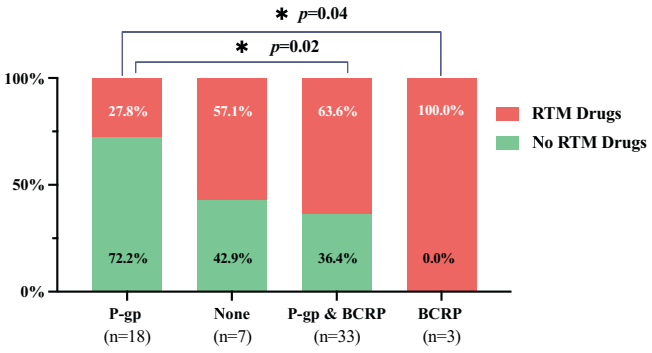

FIGURE 1 The relationship between RTM and transporter information of drugs (*$p < 0.05$).

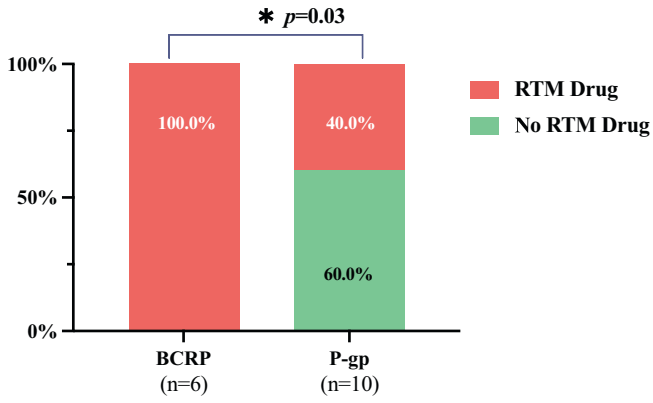

FIGURE 2 The relationship between RTM and transporting ability of P-gp and BCRP for dual-substrate (*$p < 0.05$).

than drugs transported by both P-gp and BCRP showed the lowest RTM. Solely BCRP substrates showed 100% RTM. The apparent relationship between transporter and RTM information suggests that P-gp may reduce a drug's RTM while BCRP may increase the occurrence of RTM. Further analysis of dual substrates showed that drugs with a higher transport extent of BCRP gave a higher incidence of RTM. When we analyzed the relative exposure ratios from drugs' RTM experiments alongside transporter information, similar results were obtained. Solely P-gp substrates showed the highest relative exposure ratio in RTM drugs while

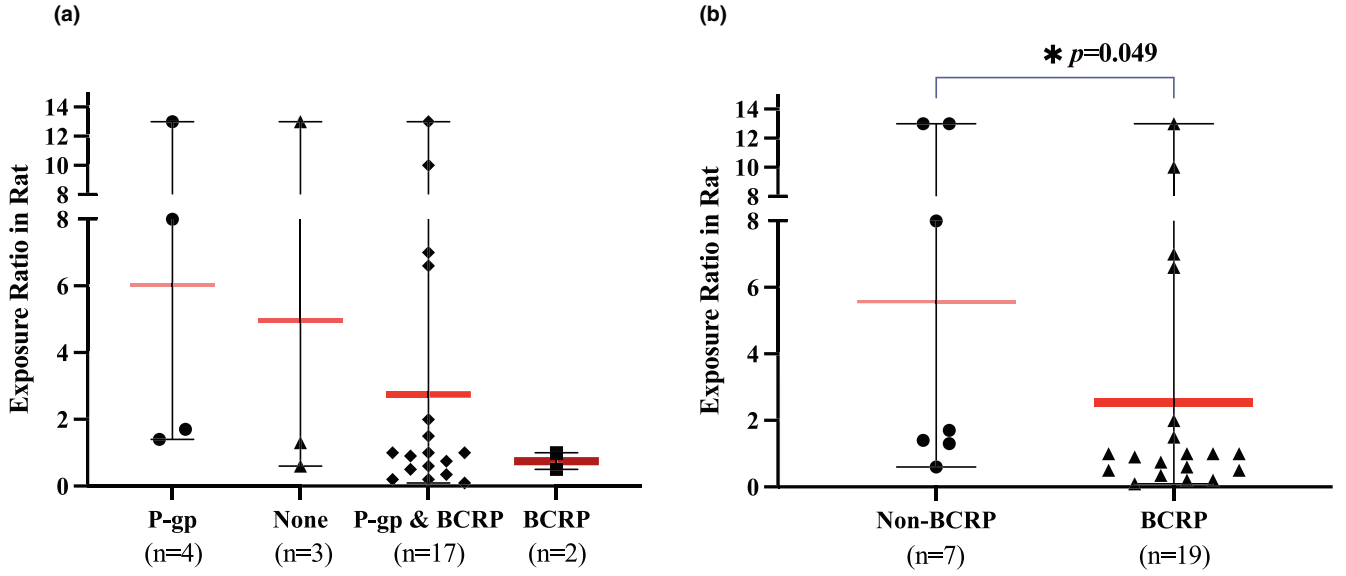

FIGURE 3 Exposure ratios in rats for RTM drugs. (a) Drugs transported by P-gp or/and BCRP and none. (b) Drugs transported by BCRP and non-BCRP.

solely BCRP substrates showed the lowest, suggesting that solely BCRP substrates exhibited a greater possibility for inducing RTM under low exposure, which further supported our hypothesis that the effects of P-gp and BCRP on spermatogenesis may be opposite.

To explore the possible reasons for these results, we collected positional information behind P-gp and BCRP in the testis. Testis cells were categorized into three parts: germ cells, BTB cells, and interstitial cells. The positions of P-gp and BCRP in each part were summarized in Table 2. P-gp was only found on rat's late spermatids[15] in germ cells, but it was abundant in BTB cells on the basal side of Sertoli cells, myoid cells, and both the luminal and basal sides of capillary endothelia.[3,16] P-gp was also found on Leydig cells.[16] BCRP was found in germ cells in rodent's spermatogonia and late spermatids[17] and also on BTB Sertoli cells, myoid cells, and the luminal side of capillary endothelia. But unlike P-gp, BCRP is found expressed only on the luminal side of Sertoli cells.[3,18]

The influence of transporters on drug toxicity appears to be not only dependent on transport direction but also on the specific location of transporters in tissues and organs,[19] and the opposing effects of P-gp and BCRP on spermatogenesis may be explained by their position in testis, as shown in Figure 4. P-gp and BCRP were located on different sides of Sertoli cells, which appears to play the most important role for BTB cells. P-gp is co-localized with basal ectoplasmic specializations and tight junctions at the Sertoli cell surface,[15] which suggests that P-gp could act as a "gatekeeper" for its substrates to efflux them out of the seminiferous tubules. In contrast, BCRP was found on the Sertoli-elongated spermatid interface[18] located on the luminal side of Sertoli cells. Such a location of BCRP on Sertoli cells could allow transport of substrates into the lumen of seminiferous tubules, which might decrease sperm survival. Due to opposite positions on Sertoli cells, P-gp and BCRP appear to show an antagonistic relationship with RTM. RTM occurred less frequently when P-gp showed a stronger susceptibility to drug transport, and the opposite was true as well.

Not all studies detected BCRP on Sertoli cells, which may explain by the stage-specific expression of BCRP at the luminal side of Sertoli cells during the seminiferous epithelial cycle of spermatogenesis.[18] Stage-specific

TABLE 2 Position of P-gp and BCRP in different testis cells.

Classification of cells	P-gp	BCRP
Germ cells		
Spermatogonia	△	▲
Primary spermatocyte	△	△
Secondary spermatocyte	?	△
Early spermatid	△	?
Late spermatid	▲	▲
Spermatozoa	?	?
BTB cells		
Basal capillary endothelia	▲	?
Luminal capillary endothelia	▲	▲
Myoid cells	▲	▲
Basal sertoli cells	▲	△
Luminal sertoli cells	△	▲
Interstitial cells		
Leydig cells	▲	△

▲ = expressed; △ = not expressed; ? = not known.

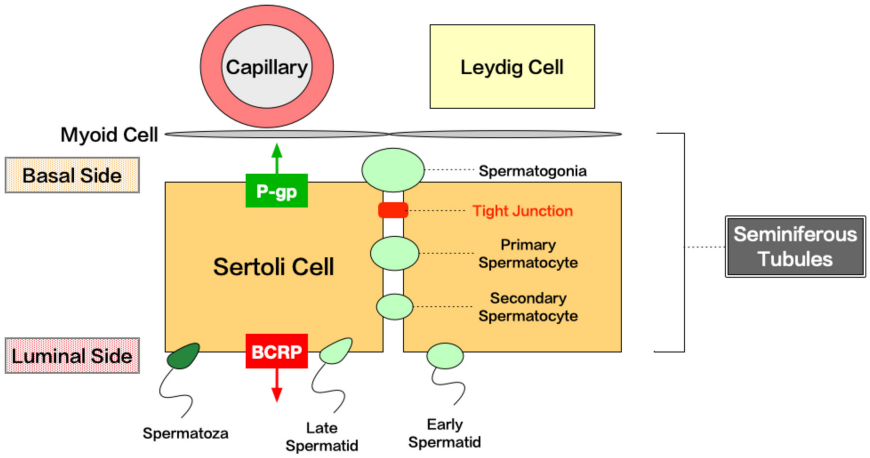

FIGURE 4 The structure of the Blood–Testis Barrier.

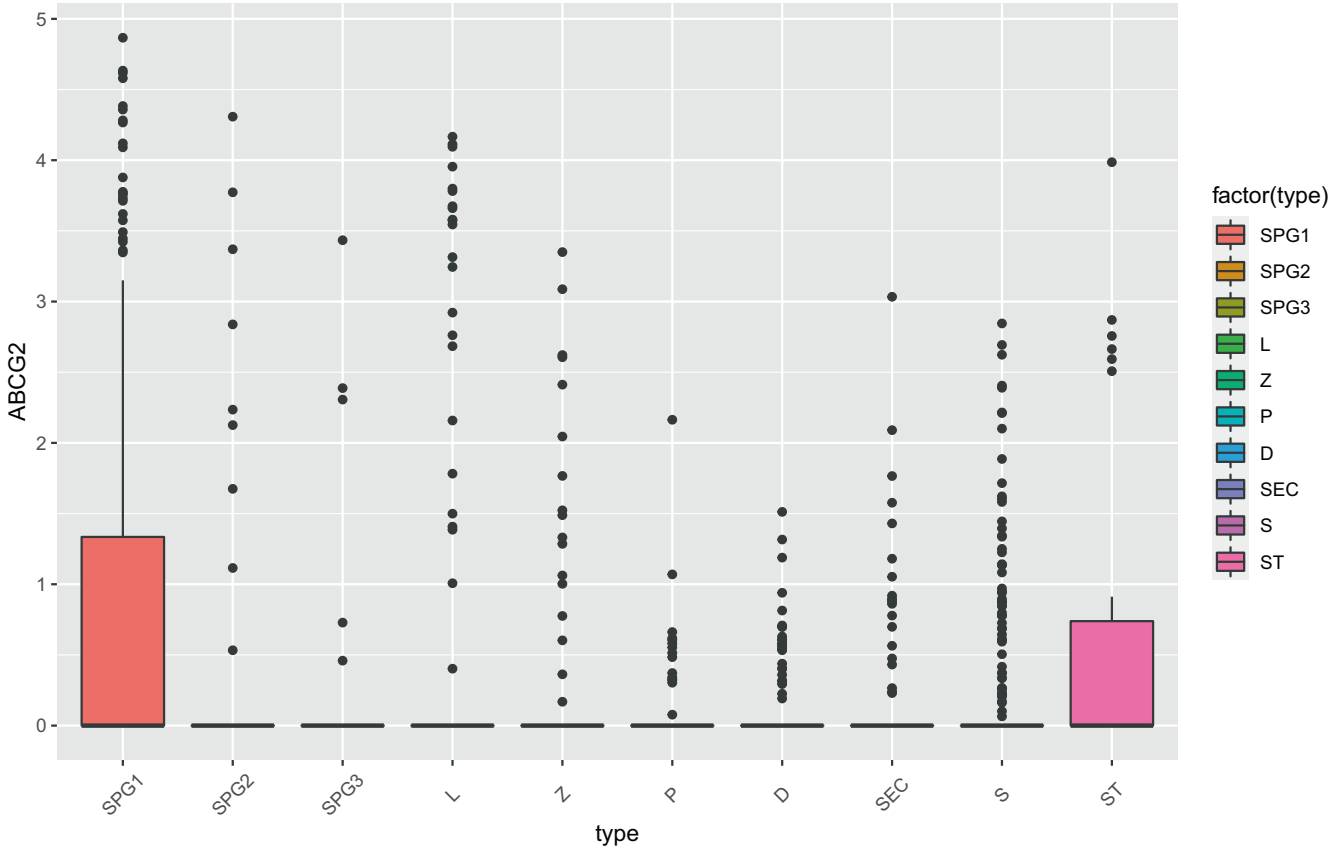

FIGURE 5 The single-cell RNA sequencing of ABCG2 in testis. SPG1-Spermatogonia, SPG2-Spermatogonia in the differentiation, SPG3-differentiated spermatogonia, L-Spermatocyte in the thin line stage, Z-Spermatocyte in the even-line stage, P-Spermatocyte in the thick line stage, D-Spermatocyte in the two-line stage, SEC-Secondary Spermatocyte, S-Spermatozoa, ST-Sertoli cell.

expression of BCRP suggests that BCRP only was expressed on Sertoli cells during stage VI to stage VIII of cycles of seminiferous epithelial and that may be why some studies[9,20] did not detect BCRP on mouse Sertoli cells by immunochemistry, while some studies did detect BCRP on rat Sertoli cells by immunofluorescence analysis using larger sample sizes.[18] During stage VI to stage VIII, BCRP is expressed at the luminal side of Sertoli cells, which could efflux xenobiotics into seminiferous tubules inducing RTM. At present, BCRP in human Sertoli cells has not been detected by immunohistochemistry,[3] but the results of single cell sequencing shows that BCRP was expressed in human Sertoli cells,[21] as shown in Figure 5 which may be related to the stage-specific expression as well.

Since most of small molecule targeted anti-tumor drugs are dual transporters, there are limited data for the

sole transporters to increase the statistical efficiency for our hypothesis. Although there is a tendency in all studies for a protective effect of P-gp, no statistical difference can be seen due to the small sample size. To ensure the comparability of transporting ability between drugs, P-gp and BCRP, efflux ratios, intrinsic clearance and K_m tested in the same cell experiment were included in our analysis. Due to different RTM sensitivity exhibited in various experimental animals, we only examined relative exposure ratios from rats, the most common experimental animal for RTM. It should be noted that the relative exposure ratio is also related to the intrinsic cytotoxicity and target specificity of the drug, rather than directly representing the ratio of drug exposure between plasma and seminiferous tubules. Furthermore, due to limitations in available data, only P-gp and BCRP were examined, despite the possibility that other efflux transporters, such as MRPs, and uptake transporters might also influence RTM. These transporters should be explored in future research.

Considering the comparability of animal experimental data, the RTM information for the model drugs in this study was sourced exclusively from U.S. FDA labels and clinical pharmacology reviews. This choice was based on the belief that the experimental results submitted to the FDA as part of the New Drug Application (NDA) process possess a high level of scientific rigor and accuracy. Regarding transporter information, we conducted a comprehensive search and included data from the DIDB as well as databases of other national regulatory agencies. This decision was primarily motivated by the varying requirements for transporter studies across different countries. Additionally, for some drugs that were approved earlier in the United States, transporter-related research might not have been mandatory, and thus, the inclusion of other databases was necessary to supplement the data. We note, as questioned by one reviewer, that we did not attempt to analyze the physicochemical characteristics of the various antibody substrates based on our belief that transporter measured parameters are more than the physicochemical differences of clinically approved antibody substrates.

Here, the potential effects of P-gp and BCRP on drugs' RTM rather than concentration in testis were studied for the first time. The results may provide some clues that BCRP may hurt spermatogenesis due to its position in testis. These findings may further deepen the clinical understanding of the influence of drug transporters on drug RTM.

AUTHOR CONTRIBUTIONS

Z.Y. and W.L. wrote the manuscript. S.Z., L.Z.B., and J.Y. designed the research. Z.Y. and W.L. performed the research. Z.Y., W.L., Z.W., S.Z., and L.Z.B. analyzed the data. J.Y. and Y.C. contributed analytical tools.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests for this work.

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