

EFFECT OF ATP-BINDING CASSETTE TRANSPORTERS ON ANTIPSYCHOTICS-INDUCED CYTOTOXICITY IN BLOOD BRAIN BARRIER ENDOTHELIAL CELLS

BY

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ABSTRACT

The ATP-binding cassette (ABC) transporters as P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) have been identified in several physiological sites. It has been suggested to play an important role in disposition of many drugs and environmental toxins. This study investigated the effects of antipsychotics (APs), including chlorpromazine (CPZ), risperidone (RIS), clozapine (CLZ), haloperidol (HAL), in their cytotoxic concentrations, on functions of P-gp and BCRP in human micro-vascular endothelial cells (HMVECs) of blood brain barrier (BBB). Firstly, the cytotoxic effects of APs were studied using alamar blue (AB) assay and inhibitory concentrations 50 (IC₅₀) were estimated. Presence of both P-gp and BCRP transporters was confirmed biochemically and functionally by western blotting and rhodamine-123 (Rh123) assays respectively. Verapamil and Ko134 were used as inhibitors for P-gp and BCRP transporters respectively. Lastly, the effect of transporters inhibitors verapamil (100µM) and Ko134 (10nM) on APs –induced cytotoxicity was studied. Results showed that APs were cytotoxic to the HMVECs of BBB. CPZ was the most cytotoxic. Presence of P-gp and BCRP was confirmed biochemically and functionally. In IC₅₀ APs showed inhibitory effect on the transporters, using Rh123 as a substrate. Also, transporters blockade increased APs-induced cytotoxicity with variable degrees. The present results suggest that a potential source of pharmacokinetic interactions exist between ABC transporters substrates and several antipsychotics in toxic conditions.

Keywords: *Antipsychotics, Cytotoxicity, P-glycoprotein transporter, Breast cancer resistance protein, Blood brain barrier.*

INTRODUCTION

ATP-binding cassette (ABC) transport-

ers play a major role for the distribution and elimination of drugs from and to the brain. This large superfamily consists of

membrane proteins which are able to transport a wide variety of substrates across membranes against concentration gradients with ATP hydrolysis as a driving force (Linnet and Ejsing, 2008).

P-glycoprotein (P-gp) is the first identified and best studied ABC transporter of the MDR1 gene product. It is a 170-kDa phosphorylated glycoprotein, which acts as a multi-specific, ATP-driven drug efflux pump (Sharom et al., 1995). Over expression of P-gp in tumour cells causes multidrug resistance in these cells (Juliano and Ling, 1976). Several studies showed a predominant distribution of P-gp at the apical membrane of the endothelial cells of BBB (Sugawara et al., 1990, Beaulieu et al., 1997 and Fricker and Miller, 2004). P-glycoprotein transporters were shown to have a broad substrate specificity and they can handle various classes of drugs including chemotherapeutics, immunosuppressants, antibiotics, anti-HIV drugs, opioids and calcium channel blockers (Fricker and Miller, 2004).

The first P-gp inhibitor described is the calcium channel blocker verapamil. It inhibits the efflux of drugs that are P-gp substrates and restores drug sensitivity in multi-drug resistant leukaemia cell lines (Tsuruo et al., 1981). Another first-generation inhibitor is the immunosuppressive drug cyclosporine A. Both drugs are substrates of P-gp as well, suggesting

that they act as competitive inhibitors. However, due to low binding affinities of these inhibitors, high doses causing toxic effects would be required for the clinical use (Linnet and Ejsing, 2008).

Breast cancer resistance protein (BCRP) transporter was first identified in a highly doxorubicin-resistant breast cancer cell line (MCF-7/AdrVp), and was therefore named breast cancer resistance protein (Doyle et al., 1998). Besides the BBB, BCRP is expressed in placenta, bile canaliculi, colon and small intestine (Doyle and Ross, 2003). Like P-gp, BCRP is localized at the apical surface of the micro-vessel endothelium (Cooray et al., 2002). The substrate specificity of BCRP is broad, comprising a wide variety of drugs (e.g. mitoxantrone, topotecan, and prazosine), carcinogens and dietary toxins (van Herwaarden and Schinkel, 2006).

BCRP has several substrates in common with P-gp, such as doxorubicin, daunorubicin, and rhodamine-123 (Doyle and Ross, 2003). Analysis of the total mRNA pool indicates that the expression of BCRP in the BBB is higher than P-gp, therefore it was concluded that BCRP might play an important role in the exclusion of xenobiotics from the brain (Eisenblatter et al., 2003).

Antipsychotics (APs) are the primary medications used for treatment of various

psychotic and bipolar disorders. They are well known to be cytotoxic to different cell lines (Gil-ad et al., 2001 and Donard et al., 2003). APs are also known to be substrates for p-gp and BCRP transporters (Sharom, 2008). In this study, the effect of four APs [chlorpromazine (CPZ), haloperidol (HAL), risperidone (RIS) and clozapine (CLZ)] on P-gp and BCRP transporters in HMVECs of BBB was studied and at the same time, the effect of transporters on the tested drugs' cytotoxicity was evaluated.

MATERIAL AND METHODS

a. Chemicals and reagents:

Antipsychotics CPZ, HAL and, RIS were purchased from Sigma-Aldrich (St. Louis, MO, USA), while CLZ was purchased from Abcam (Cambridge, MA, USA). Stock solutions of all drugs were made in ethanol (vehicle). Each drug was tested in 5 concentrations (1 mM, 100uM, 10uM, 1uM and 100nM). Drugs were dissolved in ethanol and PRMI-1640 media. The selected APs are commonly prescribed for patients being treated for schizophrenia or bipolar disorder.

Verapamil, Rh123 and Ko143 were bought from Sigma-Aldrich (St. Louis, MO, USA). For the solubilizing solution, 125ml was made of 12.5 ml 10% triton (100X) and 0.1N HCL. Then, the volume is adjusted to 125 ml with isopropanol.

Regarding western blotting, P-gp was probed using the C219 antibody (Signet Laboratories, Dedham, MA, USA) in dilution 1:200 and anti-mouse IgG-HRP antibody (Dako Cytomatics, Glostrup, Denmark) in dilution 1:1000. While for probing of BCRP transporter, the primary antibody Bxp-53 (Enzo Life Sciences, Farmingdale, NY) in dilution 1:5000 and peroxidase conjugated affinipure rabbit anti-rat IgG antibody at 1/10000 dilution (Protein Tech group, Chicago, USA) were used. Western blotting was visualized via enhanced chemi-luminescence detection (ECL kit, Amersham, Buckinghamshire, UK).

According to Elmorsy et al. (2014), cells were maintained in PRMI-1640 medium containing heat inactivated 20% FBS, 2 mM L-glutamine, 1mM sodium pyruvate, 1%MEM vitamins, 1% MEM, non-essential amino acids (Gibco, Paisley, UK), 100 units of penicillin G per ml, and 100 mg of streptomycin sulfate per ml. All media components were purchased from Sigma, unless other source is mentioned.

b. Methods:

1. Alamar blue assay:

Alamar blue (AB) assay is a well-known viability assay. Following the manufacturer protocol, 10×10^3 cells are seeded per well in 96 well plates (Nunclon surface), incubated for 24 hours and treated with the tested concentrations of the anti-

psychotics for 24 hours. Ten μL of AB reagent was added to each well and further incubated for four hours. Then, absorption was read by Dyne MRX micro-plate reader (Dyne technologies, Chantilly, VA, USA). Viability was expressed as a percentage relative to the vehicle control wells readings of the same concentration of ethanol. The experiments were done in triplicates with three wells of each treatment concentration in each experiment.

2. Confirmation of the presence of ABC-transporters in HMVECs of BBB:

Presence of ABC transporters was confirmed in our cell line passage biochemically by western blotting and functionally by Rh123 assay as follows:

2.1 Western blotting for P-gp and BCRP transporters:

The presence of P-gp and BCRP was studied by western blotting, following Papa et al. (2008). Briefly, the samples were loaded onto 4% acrylamide/bisacrylamide gel. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed and proteins were transferred to the nitrocellulose membrane. The membrane was blocked for two hours at 37°C with 5% powdered skimmed milk in PBS containing 0.05% tween 20 (PBS-T). Washed membranes were incubated overnight at 4°C with the primary antibodies. Washed membranes were then incubated for two hours at room temperature with

the secondary antibodies in PBS-T containing 1% milk powder. Membranes were washed in PBS-T and P-gp or BCRP were visualized using enhanced chemiluminescence detection.

2.2 Rhodamine 123 assay:

Rhodamine123 (Rh-123) was shown to be a substrate for both P-gp and ABCG2 transporters (Sharom, 2008). Functionally, the transporters were evaluated by studying the ability of the cells to retain more amount of Rh-123 by using the well-known inhibitors verapamil for P-gp transporter and Ko143 for ABCG2 transporters. The endothelial cells of the BBB were seeded (20×10^3 /well) in 24 wells plates and left till confluence. Wells with media without cells were used as a blank. Cells were incubated with Rh-123 ($10 \mu\text{g}/\text{ml}$ Hanks) for 15 minutes. Rh-123 was removed and wells were washed twice. Verapamil ($1 \mu\text{M}$, $10 \mu\text{M}$, $100 \mu\text{M}$ and $1000 \mu\text{M}$) and Ko143 (1nM , 10nM and 100nM) were incubated with the cells in Hanks solution for 30 minutes. Cells were washed twice again with PBS and destroyed by the solubilizing solution. The retained Rh-123 was assessed by fluorescence plate reader (Dyne technologies, Chantilly, VA, USA) using wave lengths (480nm and 530nm) for excitation and emission respectively. Blanks were subtracted from all readings. Retained Rh123 due to different concentrations of the inhibitors was expressed as a percentage

from the corresponding vehicle control readings.

3. The effect of APs on transporters:

The effect of APs on transporters was evaluated using Rh123 as a substrate by studying the effect of inhibitory concentrations 50 (IC₅₀) of the tested APs on the retention ability of HMVECs of BBB for Rh123. Experiments were designed as previously mentioned for transporters assay. Instead of verapamil, cells were incubated with IC₅₀ of the tested drugs for one hour. Then, media were removed and wells were washed twice with PBS. Then, cells were destroyed by the solubilizing solution and the retained Rh123 was assessed by the fluorescence plate reader. Blanks were subtracted from all readings. Retained Rh-123 due to different concentrations of the inhibitors was expressed as a percentage from the corresponding vehicle control readings.

4. Effect of transporters on the cytotoxic effect of APs on HMVECs of BBB:

To assess the effect of ABC transporters on APs -induced cytotoxicity, the fore-mentioned AB assay was repeated with addition of transporters inhibitors; verapamil (100µM) or Ko134 (10nM) to the APs treated wells in the different APs tested concentrations. Also, the cytotoxic effects of the transporters inhibitors alone were assessed by another AB plates. All experiments were done in triplicates.

Statistical analysis:

Data were given as mean ± standard error of the mean (SEM). Pair wise comparisons were performed by two-tailed unpaired t-test. Mean values were considered to be statistically different at a value of P<0.05. IC₅₀ are quoted with 95% confidence intervals. All statistical calculations were done using PRISM 3 (Graph-Pad Software Inc., San Diego, CA).

RESULTS

Figure (1) demonstrated that the tested APs reduced the viability of HMVECs of BBB in relation to concentrations and durations of exposures. After fitting of curves, the data of the respective IC₅₀ AB showed that CPZ is the most cytotoxic as shown in table (1).

HMVECs of BBB showed an immune-reaction with the C219 in the molecular weight range of 170 kDa, indicating the presence of P-gp in these cells. Also, western blot analysis with the Bxp-53 showed a distinct immune-reaction in the molecular weight range of 70 kDa (Figure 2). This is indicative for the expression of BCRP in HMVECs of BBB.

The function of P-gp transporters and BCRP was assessed using well known transporters inhibitors and substrates. Verapamil 100µl was highly effective as an inhibitor of the transporter to block signifi-

cantly pumping of Rh123. In addition, Ko134 was used as BCRP inhibitor. Ko134 (10nM) was found to be highly effective to block significantly pumping of Rh-123 as illustrated in figure (3).

Antipsychotics, in estimated IC_{50} were found to block pumping of Rh123 and increasing the retained Rh123 and inside the treated cells, as shown in figure (4) with p values: 0.011, 0.0093, 0.0024 and 0.044 for CPZ, HAL, RIS and CLZ, respectively. CPZ was found to be the most effective, while RIS was found to be the least. The cytotoxic effect of both verapamil (100 μ M) and Ko134 (10nM) on HMVEC_s of BBB was excluded via AB assays. So, these concentrations can block the ABC transporters without effect on the cell viability in the subsequent studies regarding the effect of ABC transporters on APs-induced cytotoxicity.

Figure (5) demonstrated that the P-gp transporters inhibitors increased the cytotoxic effect of APs for variable degrees. The effect was more significant with RIS. No significant effects were seen with the highest and lowest APs tested concentrations.

Regarding the effect of BCRP transporter blocking, addition of Ko134 (10nM) increased the cytotoxic effect of APs for variable degrees (Figure 6). No significant effects were seen with the highest and APs tested concentration (1mM).

Comparison of the calculated IC_{50} (Table 1) revealed lower IC_{50} indicating more cytotoxic effect, in response to ABC transporters inhibition. Furthermore, IC_{50} revealed that blocking of BCRP has more significant effect on APs induced cytotoxicity than P-gp transporter blocking.

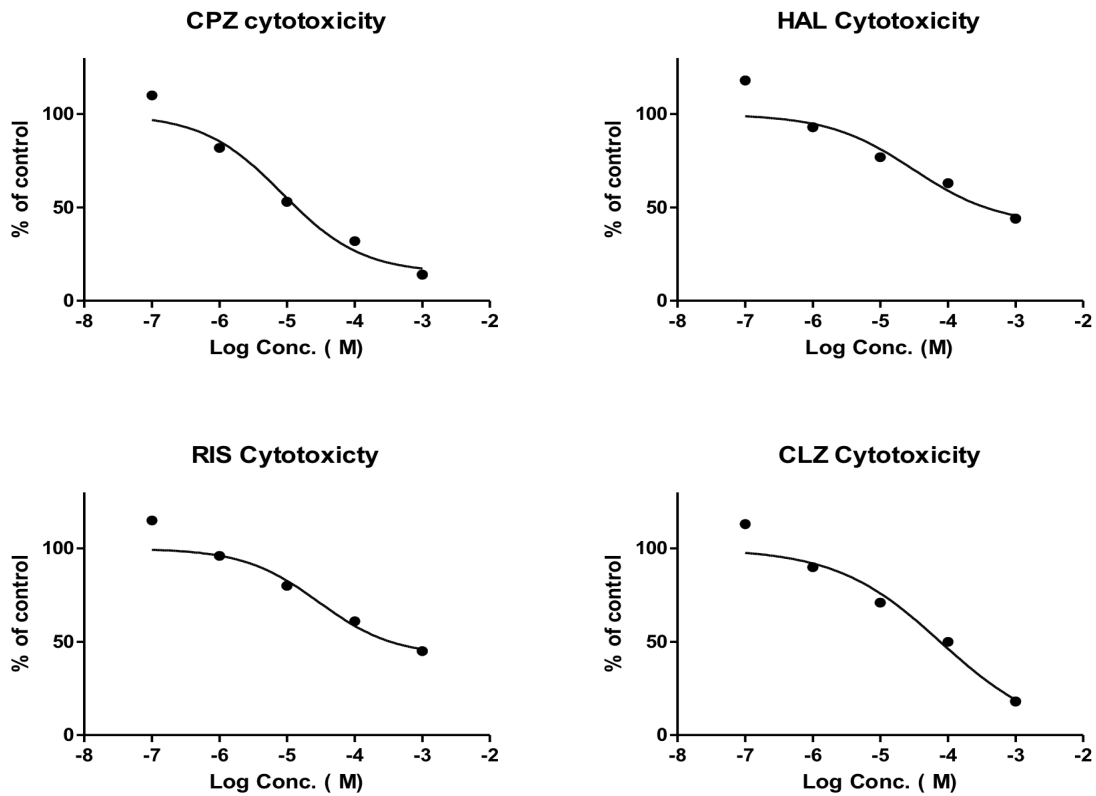


Figure (1) : Alamar blue assay results show that the tested antipsychotics reduced the viability of human micro-vascular endothelial cells (HMVECs) of blood brain barrier after 24hrs drug treatment. The effect was concentration-dependant. N.B. Chlorpromazine (CPZ), haloperidol (HAL), risperidone (RIS), clozapine (CLZ).

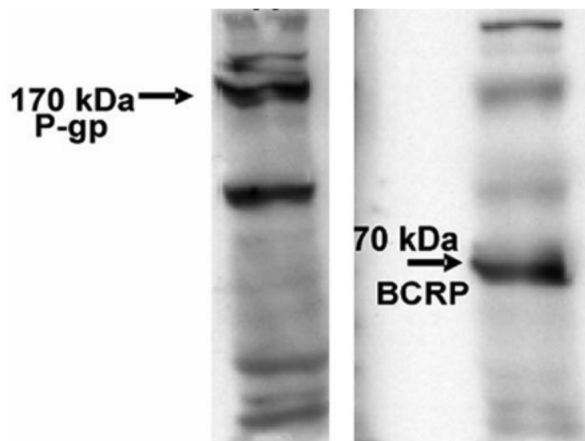


Figure (2) : Western blot probing for P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) transporters in human micro-vascular endothelial cells (HMVECs) of blood brain barrier.

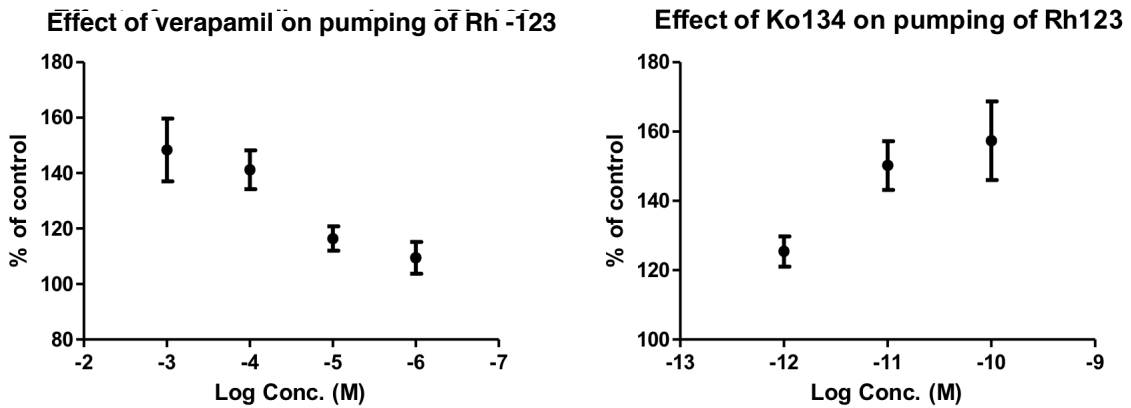


Figure (3) : Inhibitory effects of verapamil and Ko134 on p-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) transporters of human micro-vascular endothelial cells (HMVECs) of BBB respectively. There is no significant difference between the inhibitory effects of verapamil 100 and 1000µM. No significant difference regarding the effect of Ko134 in both concentrations 10 and 100 nM.

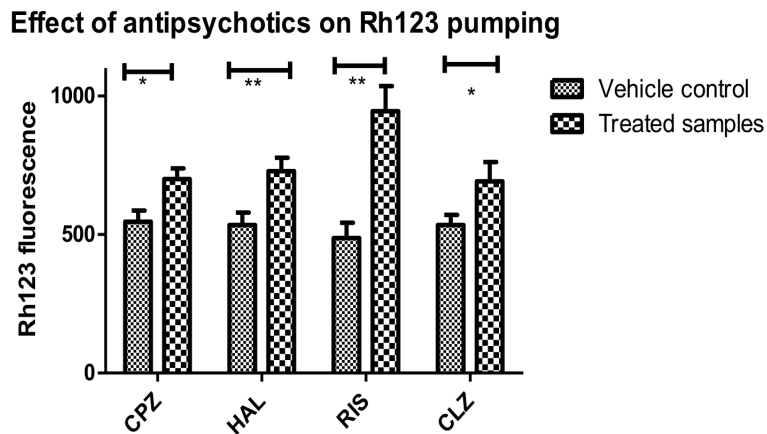


Figure (4) : The effect of antipsychotics on pumping of Rh-123 in human micro-vascular endothelial cells of BBB. All APs significantly reduced pumping of Rh-123 with increased Rh-123 fluorescence.

N.B. Chlorpromazine (CPZ), haloperidol (HAL), risperidone (RIS), clozapine (CLZ).

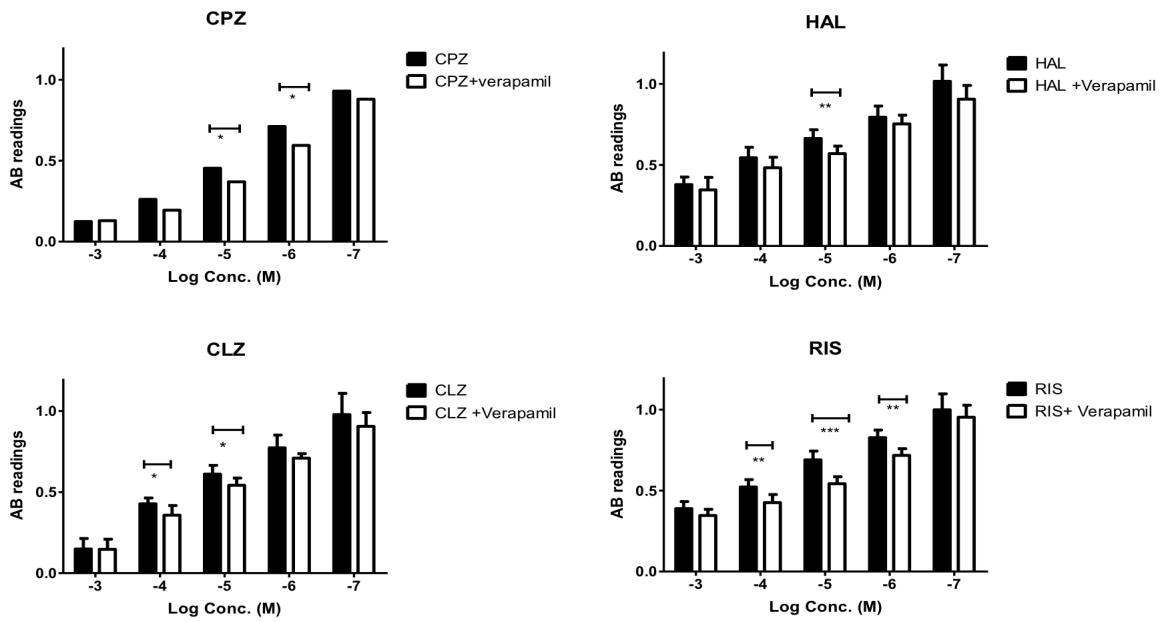


Figure (5) : The effect of P-glycoprotein (P-gp) transporter inhibitor verapamil (100 μM) on the cytotoxicity of antipsychotics in human micro-vascular endothelial cells of BBB. N.B. Chlorpromazine (CPZ), haloperidol (HAL), clozapine (CLZ), risperidone (RIS).

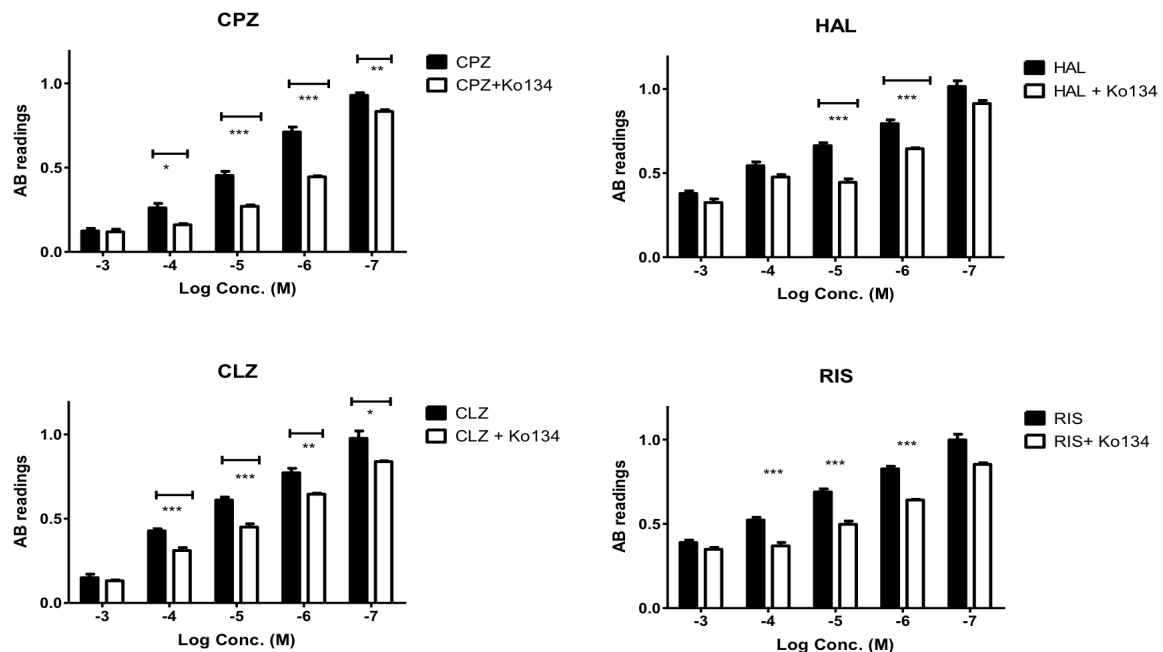


Figure (6) : The effect of breast cancer resistance protein (BCRP) transporter inhibitor Ko134 (10 nM) on the cytotoxicity of antipsychotics in human micro-vascular endothelial cells HMVECs of blood brain barrier.

Table (1) : The effect of breast cancer resistance protein (BCRP) transporter inhibitor Ko134 (10 nM) and P glycoprotein (P-gp) transporter verapamil (100µM) on the cytotoxicity of antipsychotics in human micro-vascular endothelial cells (HMVECs) of BBB.

Antipsychotics	Antipsychotics without ATP-binding cassette (ABC) transporter inhibitors	Antipsychotics in presence of P-gp transporter inhibitor verapamil (100µM)	Antipsychotics in presence of BCRP transporter inhibitor Ko134 (10nM)
Chlorpromazine (CPZ)	75 µM	64 µM	51 µM
Haloperidol (HAL)	301 µM	276 µM	247 µM
Risperidone (RIS)	307 µM	253 µM	212 µM
Clozapine (CLZ)	139 µM	112 µM	91 µM
N.B. IC ₅₀ were calculated by non-line curve fitting.			

DISCUSSION

This study was done to evaluate the effect of ABC transporters in HMVECs of BBB on the APs-induced cytotoxicity. Micro-vascular endothelial cells were isolated for BBB cultures mostly from animal sources (Audus and Borchardt, 1986 and Raub et al., 1992) especially from rats (Abbott et al., 1992 and Rist et al., 1997). As they are primary cultures, there is a need to frequently start a new culture, because freshly isolated brain micro-vascular cells dedifferentiate rapidly (Liebner et al., 2000). Consequently, cultures are different each time. To minimize variations, continuous cell lines, preferably of human origin, are more accepted for establishing in vitro barrier models (Tarja et al., 2004).

Antipsychotics, especially typical preparations, were shown to be cytotoxic to various cell lines (Donard et al., 2003). The cytotoxic effect of the new APs was not studied well except for CLZ as its effect on blood elements was considered in previous work (Hampson, 2000 and Ahn et al., 2004).

In the present study, CPZ was found to be the most cytotoxic followed by CLZ, while RIS and HAL were the safest. The present results are in accordance with the findings of Dwyer et al. (2003) who showed that CPZ and CLZ were also more cytotoxic to PC12 cells than HAL and RIS using MTS assay, 48 hours post-treatment. The same was also stated by Jones-Brando et al. (2003), who used fi-

broblasts after exposure time up to 96 hours.

Presence of P-gp and BCRP was confirmed, in the current work, by western blot in this cell line passage. Inhibitors and substrates for both P-gp transporters and BCRP were studied in many previous publications (Tanaka et al., 1994; Beaulieu et al., 1997; Virginito et al., 2002).

The mycotoxin fumitremorgin C (FTC), isolated from *Aspergillus fumigatus*, is a specific and potent inhibitor of BCRP transporter. Unfortunately, it induces tremors or convulsions in experimental animals. Hence, less toxic and synthetically tractable analogs are used as BCRP inhibitors, from which Ko134 is the most potent (25 nM is sufficient to inhibit 90% of BCRP transporter activity) with low inhibitory effect on P-gp transporters (Sharom, 2008).

In the present study, we used Ko134 as an inhibitor for BCRP transporter. 10nM was found to be an ideal inhibitory concentration for the subsequent experiments as there was no significant difference between Ko134 10nM and 100 nM. So, Ko134 (10nm) can give the best inhibitory effect with less cytotoxicity on the cells.

For P-gp transporters, a lot of inhibitors are well known including calcium channel blockers such as verapamil, calmodulin

antagonists such as phenothiazines, quinolines, immunosuppressive agents (cyclosporin A), antibiotics (cefoperazone and rifampicin), steroid and hormonal analogs, reserpine, and surfactants (Ramakrishnan, 2003).

Results of the present work showed that verapamil (100 μ M) would give the best inhibitory effect on P-gp transporter of HMVECs of BBB and at the same time will be safer than 1000 μ M, so 100 μ M was chosen as an ideal inhibitory concentration in the subsequent experiments.

Our findings also showed evidence that the commonly used APs, in their IC₅₀, have various degrees of inhibitory effects on P-gp and BCRP transporters functions. The widely used anti-psychotic RIS was one of the most potent inhibitors of ABC transporters among the tested APs. These findings are in agreement with Wang et al. (2006 & 2008) who reported that several antipsychotics, including CPZ, RIS, quetiapine, paliperidone and CLZ, are dual inhibitors of both P-glycoprotein and BCRP.

In our study, it was difficult to evaluate the effect of APs on each transporter separately as the cells have both types and may be other types of ABC transporters.

Cytotoxic effects of verapamil and Ko134 in their effective transporters inhibitory effect (100 μ l for verapamil and 10nM

for Ko134) were excluded by AB assay. However, AB assays showed that the transporters inhibitors increased the cytotoxic effect of APs in all concentrations especially with RIS (Figures 5 and 6).

With the APs highest concentration, there was no significant increase in their cytotoxicity with addition of the ABC transporters inhibitors, which means that there is a higher limit for APs-induced cytotoxicity with no effect of further accumulation of APs intracellularly. Furthermore, this high concentration may block the transporters completely, so addition of the other inhibitors will have no more significant effect.

With the APs lowest concentration, addition of verapamil has no significant effect. This may be due to that the increased accumulation of more amounts of the APs intra-cellularly (caused by P-gp transporters blockade) is still tolerable with this low APs concentrations. On the other hand, addition of Ko134 significantly increased these low concentrations cytotoxic effects with CPZ and CLZ. This may be attributed to higher inhibitory effect of Ko134 on BCRP transporters than verapamil inhibitory effect on P-gp transporters. This finding may be also due to higher expression of BCRP transporters than P-gp transporter in HMVECs of BBB. This explanation is going with the findings of Eisenblatter et al. (2003). A third explanation of this re-

sult may be that the affinity of BCRP transporter to pump APs is higher than the affinity of P-gp to pump this group of drugs.

In general, the plasma levels of the APs in patients are relatively low compared to the current estimated IC_{50} values. However, the tested APs have extensive tissue accumulation, with the tissue to plasma ratios ranging from 1.8 to 97.7 folds (Wang et al., 2006). Based on this fact, the present findings suggest the existence of potential pharmacokinetic drug-drug interactions between ABC transporters substrates and several antipsychotics, especially RIS and CPZ.

The present results may have potential application for safety treatment of the antipsychotics. ABC transporters have long been recognized to confer multidrug resistance in cancer and inflammation chemotherapies. Modulation of BCRP may influence drugs with a variety of structural features and therapeutic purposes in terms of absorption, disposition, and ultimately, therapeutic outcome. Inhibition of BCRP's barrier function in the intestine by BCRP modulators, such as pantoprazole, omeprazole and gefitinib, has been associated with significantly increased oral bioavailability and elevated plasma levels of known substrates methotrexate, topotecan and imatinib (Reid et al., 1993 and Breedveld et al., 2006).

There are also supporting data for an important role of ABC transporters in drug excretion into bile, in transporting drugs across placenta (Jonker et al., 2000), in transporting drugs into human milk (Jonker et al., 2005) and in limiting drug penetration across the blood brain barrier (Cisternino et al., 2004). Accordingly, in combination with the present findings, it is suggested that toxic doses of APs may alter pharmacokinetics and pharmacodynamics of a lot of drugs which may be given to these patients for therapeutic purposes and they are substrates for ABC transporters. On the other hand, medications which act as inhibitors for these transporters will worsen the outcome of therapy as it will expose the neuronal cells to higher concentrations by blocking the outward pumping action of these transporters in the BBB endothelial cells.

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تأثير ناقلات الجدرالخلوية المرتبطة فى عملها بالأدينوزين ثلاثى الفوسفات على الأثر السمى لعقاقير مضادات الذهان على الخلايا البطانية للأوعية الدقيقة المغذية للمخ

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أثبتت العديد من الدراسات السابقة وجود ناقلات الجدرالخلوية المرتبطة فى عملها بالأدينوزين ثلاثى الفوسفات - وفى مقدمتهم ناقلتي البروتين السكرى (بى) و بروتين سرطان الثدي المقاوم - فى عدة مواقع فسيولوجية. ومن المتوقع أن تلعب هذه الناقلات دورا هاما فى التخلص من العديد من الأدوية والسموم البيئية. والهدف من هذه الدراسة التحقق من تأثير عمل هذه الناقلات على الآثار السمية لمضادات الذهان كالكلوربرومازين و الريسبيريدون و الكلوزابين والهالوبيريدول ، على خلايا الأوعية الدموية الدقيقة البطانية فى حاجز الدم فى الدماغ عند الإنسان.

فى البداية، تم دراسة التأثير السمى للأدوية قيد البحث على الخلايا البطانية باستخدام مادة الأمار الأزرق. ثم تم التأكد من وجود الناقلات فى الخلايا البطانية للأوعية الدموية الدقيقة كما تم التأكد من قيامها بوظيفتها المعتادة باستخدام العوامل المضادة لها كالفيراباميل (المثبط لناقل البروتين السكرى -بى) ومادة كى-أو- ١٣٤ (المثبط لناقل بروتين سرطان الثدي المقاوم) كما تم استخدام الرودامين-١٢٣ باعتبار ان كلا الناقلين يقومان بطرده الى خارج الخلايا فى الظروف العادية.

كما تم دراسة أثر مضادات الذهان على عمل الناقلات محل البحث وأخيرا تم تقييم تأثير الناقلات على التأثير السمى لمضادات الذهان عن طريق دراسة التأثير السمى لهذه العقاقير بعد إيقاف عمل هذه الناقلات باستخدام مثبطات عملها (فيراباميل بتركيز ١٠٠ ميكرومول ومادة ال كى-أو- ١٣٤ بتركيز ١٠ نانومول - كلا على حدة).

وقد أظهرت النتائج التأثير السمى للأدوية قيد البحث على الخلايا البطانية وقد كان عقار الكلوربرومازين أكثرها سمية. كما أكدت التجارب كيميائيا ووظيفيا على وجود ناقلتي البروتين السكرى (بى) و بروتين سرطان الثدي المقاوم. كما ثبت أيضا أن هذه العقاقير لها تأثير مثبط على هذه الناقلات فى جرعاتها السمية بدرجات متفاوتة. ومن ناحية أخرى أسفرت النتائج عن أن تشبيط عمل هذه الناقلات يزيد من الأثر السمى لهذه العقاقير وبناء على ما تقدم تشير النتائج الحالية إلى إمكانية حدوث تفاعلات دوائية بين مضادات الذهان فى تركيزاتها السامة و العقاقير الأخرى التى تعمل كمثبطات لهذه الناقلات أو مجموعة العقاقير الأخرى التى يتم ضخها إلى خارج الخلايا عبر هذه الناقلات.