CNS DRUG-DRUG INTERACTIONS MEDIATED BY P-GLYCOPROTEIN

by

XIN LI

(Under the Direction of Arthur Roberts)

ABSTRACT

Drug-resistance is one of major obstacles of CNS disorders in clinical settings. The efflux pump, P-glycoprotein, can be induced through chronic treatment leading to non-responsive patients. It is critical to figure out the underlying drug-drug interactions (DDIs) in order to reasonably modulate P-gp expression and function. In this study, I summarized in vitro and in vivo study of CNS drug-drug interactions as well as potential associations between P-gp and pharmaco-resistant CNS disorders. Aiming to establish a methodology in studying CNS DDIs, different model correlations, interplay of metabolism and transport, collaboration of efflux transporters, P-gp expression and function etc. should be considered. In addition, P-gp serves as an important target in drug development. P-gp inhibitors, inducers and even non P-gp substrates are important lead compounds in treating refractory CNS disorders mediated by P-gp.

INDEX WORDS: CNS drugs, BBB, P-gp, efflux pump, drug-drug interactions, drug resistance, P-gp inhibitors

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DEDICATION

I dedicate my Master's thesis to my parents, Hao and Yuhong. They support me all the time with their inclusive love. Because of you, I am able to have the courage to overcome all kinds of difficulties and insisted my belief to the end.

To the continent of North America, it brings to me too many precious memories. Thank you for letting me know the importance of being a person with independent spirit!

To my advisor, Arthur Roberts, it is my pleasure to gain your recognition. Your open mind motivates me to pursue the infinite world of science. I appreciate your support and encouragement!

Life is a long journey. "As heaven maintains vigor through movements, a man should constantly strive for self-perfection; as earth's condition is receptive devotion, a man should hold the outer world with broad mind."-The Book of Changes

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CHAPTER 1

INTRODUCTION

1.1 Central nervous system

Nervous system plays a leading role in modulating the physiological functions in the body. To achieve diverse activities, organs and cells are in close contact under the control of nervous system. Nervous system has regulatory effects on the whole organism through perceiving the changes from internal and external environment, and then generating or passing the signals through a series of positive and negative feedback loops. Nervous system is comprised by central nervous system (CNS) and peripheral nervous system (PNS).

Central nervous system, including brain and spinal cord, integrates information that it receives, coordinates the activities in all part of the bodies. Any damages involved in the CNS can lead to dysfunctions in sensory, emotion, study, memory, motion etc. Due to the conscious part (the cerebrum) in the brain [1], CNS disorders have more diverse forms of symptom than other diseases. There are approximately 600 types of CNS disordered affecting 1.5 billion people worldwide at any given time. And the trend is exponentially increasing. Thus in-depth research is urgently required in overcoming the complexity of CNS therapy.

1.2 Role of blood-brain barrier (BBB)

The brain is a special organ in the human body, which is isolated from the rest of the body by at least 4 barriers: meningeal barrier, blood-brain barrier, blood-CSF barrier and ventricular barrier [4]. Blood-brain barrier is the major challenge for drug delivery into brain. It is formed and organized by specialized cells that tightly control the movement of various substances in and out of brain [4]. Brain endothelial cells (BECs) pericytes and astrocytes; maintain the barrier

integrity [2]. BECs lack of fenestration, have few pinocytotic vesicles, also express a variety of metabolic enzymes and membrane efflux transporters [74].

Through numerous investigations on the BBB, it is widely accepted that lipophilicity and molecular size are not the main factors that determine whether the drug molecules are able to cross the endothelial cells or not. Increasing number of mechanisms in the drug penetration to the CNS has been found. These mechanisms act independently or collaborate in parallel and further impede the degree of penetration of a range of drugs, resulting in actual brain uptake lower than predicted physiochemical data [3].

1.3 The structure, function and classification of P-glycoprotein

Permeable glycoprotein (P-gp) is first identified to confer resistance to cancer cells against chemotherapeutic agent in vitro studies. P-gp is a 170kDa membrane-bound protein that is located on some important barriers such as blood brain barrier and intestinal epithelium. P-gp consists of 12 highly hydrophobic transmembrane domains, arranged as two bundles of six helices and linked by a highly charged extracellular linker.

The natural physiological functions of P-gp reflect in bioavailability, metabolism and neuro-protection. We do not deny the positive function of P-gp in the body. Instead, under the normal conditions, P-gp plays a critical role to preserve the homeostasis of plasma or cerebrospinal fluid. P-gp-mediated drug resistance can be understood as over protection for certain human tissues. However, the acquired P-gp on the tumor cells is another story. Tumor cells develop acquired P-gp when continually taken some chemotherapies and then exhibit multidrug resistance. P-gp-induced resistance mechanisms have been studied for 50 years. Medications or some symptoms of CNS disorders significantly affect the P-gp expression, function and binding affinity.

In my opinion, I would like to classify P-gp into three levels depending on their location. At intestinal or liver level, P-gp limits the bioavailability of drugs into blood. At brain level, substance transport is controlled by transporters on the BBB. Increased efflux for toxic substance

is demanding; whereas active compounds for certain CNS targets require increased influx or decreased efflux. At tumor level, P-gp expression is induced in malignant tumor cells and relevant pharmacological effects are supposed to be maintained by increasing the doses.

Table 1 Comparative features for three-level P-gp study

	Level 1	Level 2	Level 3
Locations	GI tract and metabolic organs	BBB, BTB, blood-placentas barriers	Tumor cells
Type of data	PK	PD	Cytotoxic data
Effects	Drug bioavailability	Metabolism and pharmacology	Enhanced anticancer effects
Organs	Intestines, liver, kidneys	Brain, testes, placentas	All kinds of tumor cells
Potential side effects	Peripheral side effects	Central side effects	Resistant to anticancer agents

1.4 Transporters at the blood-brain barrier

A number of transporters expressed at the BBB comprise a complex and dynamic barrier to control the exchange of substances which are structurally unrelated [43]. They protect the CNS from various exogenous and endogenous toxic molecules from blood [44].

P-gp was the first recognized efflux pump located at the BBB. P-gp belongs to ATP-binding cassette efflux transporter superfamily, encoded by ABCB1 gene. They have very wide distribution throughout the whole body, including bile canaliculi, intestine epithelium, kidney tubules, adrenal and gravid uterus as well as brain capillary epithelium. P-gp mediated transport for those substrates happened together with ATP hydrolysis. Efflux pumps enhanced elimination

and lower the desired pharmacological potency [45]. There are two cases of the expression of P-gp: physiological detoxification mechanism to pump xenobiotics from tissue to blood and "chemical stress protein" up-regulated by chemotherapeutics [52].

The localization as well as broad substrate profiles endues significances on the role of P-gp in drug disposition and absorption [52]. P-gp is highly polymorphic and expresses in the brain endothelial cells to limit substrate entry to the CNS, influencing the therapeutic responses of CNS drugs [108]. Not only antidepressants, but also some antipsychotics are recognized as substrates or modulators of P-gp. The potential drug-drug interactions have attracted public attention since the 90s of last century. Due to the altered P-gp activity and expression caused by one drug, the plasma or cerebral fluid levels of another drug can be easily increased or reduced. Subsequently, the cytotoxicity and/or unwanted effects bring great damage to health tissues. In this case, adjusted dosing or avoidance of co-administration is optional solutions. On the other hand, in order to increase one drug penetration into CNS, we are seeking strategies to increase the drug concentration into targeted sites through altering P-gp expression by pretreating with P-gp modulators. Mdr1a mainly express in microvessel endothelial cells and Mdr1b is found to express in the cerebral cells.

Besides P-gp, the effect of other transporter protein, such as MRP, oatp2/4, Bcrp cannot be ignored. The P-gp inhibition may be offset by other pumps. For instance, digoxin would not show apparent increased plasma level when co-administered bromocriptine which is a potent P-gp inhibitor [155]. Bromocriptine is a modulator for P-gp and oatp2. Thereby it is uncertain if changed or unchanged plasma level is caused by certain conditions. If not excluded the possible other transporters, the final cerebral drug concentration should be regarded as the synergistic consequences of influx and efflux process.

CHAPTER 2

MODELS THAT USED IN DDI STUDY

2.1 In vitro models

The regulation of human P-gp with drug-drug interaction in vitro studies has been widely reported. The most commonly used in vitro models are ATPase activity assay and monolayer transport assay.

As mentioned above, the transport of P-gp substrate accompanies with ATP hydrolysis catalyzed by ATPase. In other words, we have reasons to believe the tight association between ATP cleavage and the dimerization of two NBDs. The ATPase activity assay offers a direct and simple approach to determine the activity of efflux protein by measuring the amount of the inorganic phosphate (Pi) that is released when certain ligand and Mg-ATP are incubated in 30min at room temperature. Furthermore, ATPase activity assay is not limited to efflux protein. A good number of studies in relation to ATP binding apply with activity assay very successfully. For example, immunoglobulin heavy chain binding protein (BiP) purified from E.coli, which is a major endoplasmic reticulum protein, were characterized by ATP binding activity in this approach [82]. Km in the Michaelis-Menten equation [see below] can be used to identify the P-gp affinity with a single substance. In comparison with basal ATPase line, ATPase induction and inhibition are two conditions to help us identify the role of P-gp substrate, inhibitor or even inducer (although P-gp inducers are not commonly seen). Usually, vanadate, a phosphate analogue, is used as P-gp inactivator to keep the P-gp stay at one transition state so as to achieve a complete inhibitory control group [105].

$$v = \frac{d[P]}{dt} = \frac{Vmax[S]}{Km + [S]}$$

v: reaction rate;

[P]: concentration of product

[S]: concentration of substrate

Vmax: maximum rate at saturating substrate concentration

Km (Michaelis constant): substrate concentration when the reaction rate is half of Vmax

Table 2 Correlation between Km and binding affinity

Km	Affinity
<10uM	High
10-50uM	Moderate
>=50uM	Low

Monolayer transport assay is one of complicated in vitro systems consisting of two compartments separated by a porous support with a layer of tightly grow cells (Caco-2 and transfected MDCKII and LLC-PK1 cell lines are used often). The efflux ratio (apical-to-basolateral and basolateral-to-apical) is an important index to assess the flux of a ligand through the monolayer cell. The ratios above 2 and lower 0.5 are usually regarded as active transport process. Double transfected cell lines are introduced considering the transport happened through the intestinal epithelial cells [85]. It contains influx transporters and efflux transporters in apical and basolateral sides, respectively, allowing direct vectorial transport study. It has been applied in pharmaceutical research including predicting the ranking of absorption, identifying the mechanism of permeability and investigation on drug-drug interactions [85].

The discrepancy between ATPase activity assay and transport assay is attaching attentions. Shirasaka Y. et al [83] compared the results from ATPase activity assay and transport assay using MDCKII-MDR1 (Madin-Darby canine kidney strain II) cell lines. Interestingly, the parameter incompatibility was observed. Other literatures also reported contradicting data regarding to the P-gp affinity with one certain ligand [76]. It is doubted that the properties of different cells cause the different results. Also we boldly assume that the settings of experiments might change the

affinity between the ligand and P-gp. Namely, the membrane proteins have their unique reaction conditions which could play a crucial role in affecting the binding with substrates. In some papers, P-gp expression is prone to dependent on the type of tissues, confirming our speculation on the selection of cell lines exerting effects on the in vitro results. It is reckless to tell which method is more reliable. They complement to each other and a thorough profile is provided to assist us to make more objective conclusions.

2.2 In vivo models

In vivo models have more clinical significances than in vitro studies although differences between animal models and human cannot be ignored. It is a powerful methodology to demonstrate P-gp expression and function. On one hand, P-gp deficient mice and wide-type mice are applied to evaluate the role of P-gp as an efflux pump at important barriers (e.g. BBB and intestinal epithelium). In 1990s, gene knockout mice were bred with sensitive and unguarded brain endothelium. The brains of P-gp deficient mice are more sensitive to neurotoxin, ivermectin, than that of wide type mice. P-gp KO mice model is found to be a useful model to test the role of P-gp and its substrates. But the limitations of KO mice model are pronounced. We cannot determine if P-gp at the BBB or intestinal endothelium plays a role in altered drug pharmacodynamics responses. Moreover, the Bcrp mRNA level is almost 3-fold higher in P-gp deficient mice than wide type mice.

On the other hand, the well-known P-gp inhibitors, such as verapamil or CsA, are often co-administered with tested drugs. Worth to mention, P-gp inhibitors have totally distinct physicochemical properties. It is quite important to gain relevant information about their structures and binding events with P-gp. Various mechanisms of action may be involved even though their effects are the same in most cases.

2.3 Ex vivo models

Ex vivo refers to those experiments performed outside of the living organisms at the tissue or organ levels. One outstanding feature of ex vivo is to make it possible to carry on the experiments within the nature environment in an ethical manner. As expected, ex vivo study are efficient in some specific cases. In-situ rat perfusion technique on brain, intestines is a remarkable ex vivo study. In situ brain perfusion technique was carried to test the morphine transport through the BBB. Apparent distribution volume and transport coefficient are two important parameters [92]. Rahi M. used an open placentas perfusion system to study P-gp function as well as quetiapine transport through placentas [137]. This model shows good reproducibility with low variation in quetiapine and antipyrine transport. However, it is unable to carry on a continuous measurement for placentas. During pregnancy, the protective function of blood-placentas barriers varies, thus leading to varied P-gp function. Obtained placenta in this experiment is at its last stage. The P-gp function in this state cannot reflect the P-gp property in the entire process.

2.4 Positron emission tomography

Positron emission tomography (PET) is a non-invasive molecular imaging technique used to detect the metabolic process to diagnose specific diseases. In the late 1950s, the notion of emission and transmission tomography was established by David E. Kuhl. PET has been a promising methodology to investigate the function of P-gp at the BBB and underlying mechanism of drug distribution within the CNS since 1998 [33]. By measuring the concentration of biomarkers radiolabeled with positron emitting isotope in the tissues, P-gp function in living subjects can be assessed without the need of surgeries.

In the past two decades, P-gp PET tracers have been classified into two groups, tracers that are P-gp substrates and P-gp inhibitors [165]. [^{11}C]verapamil and [^{11}C]desmethylloperamide are the most commonly used tracers for P-gp binding studies. Besides verapamil, [^{11}C]flumazenil, [^{11}C]RWAY, [^{18}F]MPPF and [^{11}C]loperamide are evolved successful CNS tracers in PET studies.

When applied with P-gp substrate as PET tracers, a relative high partition ratio accounts for signals with high resolution. Low baseline signals can be resolved by giving a small dose of P-gp inhibitor such as tariquidar, leading to a slightly increased baseline. It should be noted that the dose of inhibitor is supposed to be controlled in order to avoid masking the altered function of P-gp caused by diseases [165].

Not like P-gp substrate, P-gp inhibitor serves as PET tracer is not frequently applied. The pre-or post-administration of P-gp inhibitors are inclined to present different identification with respect to the role of cerebral P-gp. Up-regulated P-gp activity involved in use of P-gp substrate as PET tracer may be overcome by the use of tracers that are P-gp inhibitors. Researches are attempting to find pure inhibitors (e.g. third-generation inhibitors). They do not have any P-gp substrate properties, which are ideal for exerting the totally opposite effects to the induced P-gp [165]. Nevertheless the boundary between P-gp substrate and inhibitor is obscure most of part. In addition to this, it is spatially unclear to differentiate the signals belonging to PET binding events at the BBB from the tracers residing in the blood or the fraction of tracers entering via passive diffusion.

New imaging probes are crucial to expand the study of efflux transporters [67]. The ideal PET probes should satisfy two requirements as Pike V [173] suggested: 1) comparatively hydrophilic metabolites would be produced; 2) these metabolites are unable to enter hydrophobic environment. Furthermore, it is observed that huge differences of brain concentration exist between rodents and human, suggesting the different characteristics of P-gp in different species.

CHAPTER 3

FACTORS THAT INVOLVED IN CEREBRAL P-GP STUDY

3.1 Complexity of BBB

BBB maintains the brain homeostasis and protects the brain from toxic substances. The specificity of BBB is the structure of endothelial cells and their connection by tight junction.

On one hand, cell culture is a critical step to make sure the integrity of BBB. In vitro study requiring incubated brain endothelial cells are integral without any destruction. Meanwhile, efflux/influx transporters should express and function well. Strict culture conditions are asked to satisfy all the requirements.

On the other hand, if the drug is easily metabolized by liver, transport study should focus more on their metabolites since these metabolites would interact with P-gp at the BBB instead of parent compounds.

3.2 The necessity of chronic study

In many investigations, acute and chronic study shows apparent inconsistent responses for CNS agents. Chronic study has attracted increasing attention in recent years. Drug-mediated resistance develops after drug administration for a while. Essentially, time is a must-condition for alter P-gp function. During this time, some signal pathways are induced or inhibited. Psychiatric drugs features chronic-term treatment and augmentation/combination regimen. The two characteristics raise the risk of drug-drug interactions and drug-resistance. Of great interest, chronic study is necessary to observe the P-gp changes in the whole administration process. At the same time, in light of the uncertainty of time, experiment design is limited by cost, especially for clinical trials.

3.3 Interplay between P-gp and CYP3A4

P-gp transport is not the only pathway affecting the interactions between CNS drugs. CYP3A4-mediated metabolism also plays a role. In a strict sense, the interplay between P-gp and CYP3A4 is based on altered protein dynamics and combined action of transport/metabolism, producing an influence on plasma/brain level of drugs. The overlapped substrate profile for P-gp and CYP3A4 indicates certain drugs that changed P-gp activity are able to alter the function of CYP3A4 at the same time. The finding of the internal correlation between P-gp and CYP3A4 presents not only challenges but also opportunities for the drug-drug interaction research. Prodrug strategy is a potent method to overcome limitation of BBB. Meanwhile, the optimized chemical structure alters the metabolism process. New drugs cannot recognize the previous binding sites on the CYP enzymes or reduced binding affinity is possible, resulting in increased plasma concentrations and decreased metabolism and elimination. It is unwise to judge if it is beneficial or harmful as a whole. Increased therapeutic responses often accompany with undesirable effects.

As for extensively metabolized P-gp substrates, CYP3A4 substrates or inhibitors have a profound effect on them. Eletriptan metabolism is partially blocked when co-administered with CYP3A4 substrates or inhibitors, because of the inhibition of CYP3A4-mediated reactions. Co-administration of eletriptan and CYP3A4 substrates is supposed to be avoided. Nevertheless the inhibitory effect on eletriptan metabolism to desmethyl-eletriptan is concentration-dependent. At low dose of furafylline (CYP1A2), S-mephenytoin (CYP2B6, CYP2C19 substrate), sulfaphenazole (CYP2C9, CYP2A6, CYP2B6 inhibitor) and quinidine (CYP3A4 substrate, inhibitor and inducer), the mechanism process was not significantly affected [153].

Sometimes, it is inevitable to combine two or more drugs to achieve better responses. Spina E. et al [135] assessed the pharmacokinetic interactions between risperidone and two mood-stabilizers, carbamazepine and valproic acid. Carbamazepine has anti-epilepsy activity and behaves as a P-gp substrate, inhibitor and inducer. Valproic acid serves as inhibitor for another two transport proteins, solute carrier and monocarboxylate transporter. No significant changes

were observed with valproic acid while decreased concentration levels of risperidone when co-administered with carbamazepine were detected. The role of the P-gp mediation can be determined. But they still speculated the induction of CYP3A4 metabolism.

3.4 Collaboration of P-gp and Bcrp

So far, the knowledge of Bcrp (Breast Cancer Resistant Protein) is insufficient. Some problems have to be addressed. First of all, neither Bcrp deficient model nor over-expression model is well-developed like P-gp deficient model. The experimental materials are not emphasized in almost every in vitro and in vivo study. I believe the propensity of cell models greatly affects the results leading to disagreements. Secondly, the collaboration of P-gp and Bcrp at the BBB is not easily explored in a direct manner. Usually a reasonable experiment design is hard to achieve since many factors are required to be considered. Last but not least, parameters reflecting inhibitory effect, binding affinity or efflux activity are not determinants for the synergistic effects between the two transporters.

One in vitro study provided evidences of the binding affinity of five analgesic drugs (norbuprenorphine, buprenorphine, methadone, ibogaine and THC), with P-gp and Bcrp. The results are at odds with each other. None of the tested drugs had been found to simultaneously inhibit both of them [151]. By using the P-gp and Bcrp overexpressing model, all drugs were not P-gp substrates, which is very suspicious.

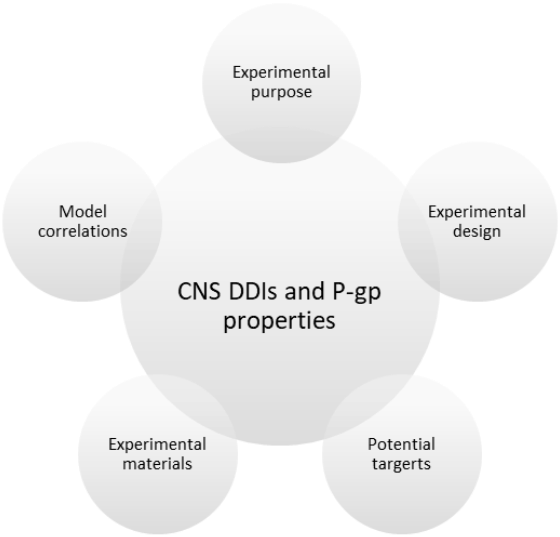

Figure 1 Factors in studying cerebral P-gp activity

3.5 Influence of ABCB1 polymorphisms

The ABCB1 gene variability is one of main contributors to varied clinical responses since single nuclear polymorphisms (SNPs) affect the expression of P-gp and P-gp affinity with ligands. Over 50 SNPs have been identified. Hung C. et al [90] established cell lines with different genotypes of ABCB1. In Rho123 uptake assays, the concentration of Rho123 decreased with increased methadone level in wide type mice, while Km value remains unchanged (affinity remains the same). For other polymorphisms (1236T-2677T-3435T and 1236T-2677A-3435T), P-gp affinity with methadone was lowered (decreased Vmax and Km). Methadone had significant inhibitory effect on Rho123 and Calcein-AM at the level of 1uM. Interestingly, although methadone inhibits variant type of P-gp as well, the potential is less than that of wide type, revealing that less P-gp would be limited and more methadone is required when patients carry with variant type of P-gp. They concluded that methadone concentration may affect the binding mode: it inhibits wide-type mice in non-competitive manner and variant type in uncompetitive manner.

The influence of genetic polymorphisms on quetiapine is not as sure as methadone. The association between ABCB1 polymorphism and quetiapine pharmacokinetic data was investigated in a bioequivalent study. Patients with 3435T/T genotype showed a higher AUC and Cmax than those subjects with C/T genotypes within 36hrs [139]. In a pilot study, however, different results showed that 3435T/T genotype led to much lower plasma concentration of quetiapine and nor-quetiapine than other genotypes [140]. Surprisingly, Kim K. et al [111] also performed clinical experiments on the influence of ABCB1 and CYP3A polymorphisms on the plasma pharmacokinetics of quetiapine in health individuals. They summarized that CYP3A5 polymorphisms significantly affected quetiapine plasma level and its pharmacokinetics while ABCB1 genotypes (including 1236C>T, 2677G>T/A and 3435C) did not. Hence, the influence of genetic polymorphism on PK parameters of quetiapine is uncertain since several factors (e.g. sex, small sample size and formulation) cannot be excluded.

Bozina N. et al [13] evaluated G2677T in exon 21 and C3435T in exon 26 genetic variants of MDR1 gene with any susceptible response for schizophrenia female patients. The haplotype G2677/C3435 represents lower responses. These two genotypes are closely associated with therapeutic consequences on the basis of positive PANSS (Positive and Negative Symptom Scale) percentage changes.

The inter-variability for patients taking cannabis is also remarkable. One clinical study described that C3435T genotype raised the risk of development of cannabis addiction. SNPs with CC genotypes are more relevant with higher P-gp expression [205]. Hence, cannabis dependence partly relies on SNP genotype C3435T.

Table 3 Impacts of ABCB1 genotypes on CNS disorders and treatments

Types	Drug	Description	References
Antipsychotics	Aripiprazole	For Japanese patients with schizophrenia, CYP2D6 genotypes have an influence on aripiprazole plasma levels. CYP3A5 and ABCB1 genotype (C3435T and G2677T/A) does not make any impact.	Suzuki T, 2014
	Risperidone	CYP2D6 and ABCB1 (G2677T and C3435T) are useful determinants for risperidone plasma concentrations, although the clinical significances are unclear.	Jovanović N, 2010
		For Chinese schizophrenia patients, only C1236T has the potential to predict the risperidone responses on the basis of the TT genotype showing great improvement than other types.	Xing Q, 2006
		For Japanese patients, The number of CYP2D6 variant allele and ABCB1 3435C>T genotypes affect risperidone and 9-OH-RIS plasma levels. ABCB1 2677T does not make any influence.	Suzuki Y, 2013
	Chlorpromazine	In South Indian population, patients with C3435T and G2677T/A resulted in better antipsychotic responses with increased doses.	Vijayan NN, 2012
	Quetiapine	A bioequivalent study showed that the 3435C>T genotype affected quetiapine plasma level. But 1236C>T haplotypes showed no significant changes.	González-Vacarezza N, 2013
		ABCB1 polymorphisms are a biomarker to predict quetiapine treatment in schizophrenia.	Nikisch G, 2011
		ABCB1 3435T allele in exon 26 may contribute to higher placental transfer than 2677G>T/A.	Rahi M, 2007
		In forty healthy male individuals, CYP3A5 polymorphisms but not ABCB1 genotypes (1236C>T, 2677G>T/A, and 3435C>>T) significantly influences the plasma level of quetiapine and its pharmacokinetics.	Kim KA, 2014

	Olanzapine	Lower representation of G2677/C3435 haplotype in schizophrenia female than controls. G2677 in exon 21 is associated with olanzapine responses. T and TT allele genotype leads to better responses.	Bozina N, 2008
Antidepressants	Citalopram/ escitalopram	C3435T, G2677T genotypes are related with increased P-gp expression.	Nikisch G, 2008
	Paroxetine	G2677T, C3435T are not related with paroxetine responses.	Mihaljevic PA, 2008
	Fluoxetine	G2677T ABCB1 genotype, T allele carriers showed apparent improved responses. CYP2D6 genetic variants impact the fluoxetine pharmacokinetics in children and adolescent patients.	Gassó P, 2014
	Morphine	Patients with C3435T genotype is associated with impaired P-gp activity. They hypothesized that acute renal toxicity was correlated with morphine accumulation due to inherited impaired P-gp activity.	Pogliani L, 2012
		They found that neither OCT1, ABCB1 (C3435T) nor UGT2B7 genotype affect morphine pharmacokinetics and pharmacodynamics in 37 healthy individuals.	Nielsen LM, 2017
	Methadone	Methadone shows less inhibitory potency on P-gp with 1236T-2677T-3435T, 1236T-2677A-3435T genotype through uncompetitive kinetics; while inhibits wide-type P-gp via uncompetitive manner.	Hung CC,2013
		Significant differences in genotype frequencies between higher and lower dose of methadone. 1236C>T is related with methadone doses.	Levran O, 2008
	THC	C3435T is related with higher P-gp expression.	Siddiqui A, 2003
Anti-epilepsy	Phenobarbital	ABCB1 C3435T genotypes greatly influence the CSF/S phenobarbital ratio and seizure frequency.	Basic S, 2008
	Carbamazepine	In Chinese patients, rs776746 and rs15524 in the CYP3A5 gene tend to affect carbamazepine metabolism, and rs2032582, rs10234411 in the ABCB1 gene may contribute to inter-individual variation in carbamazepine transport among patients with epilepsy when combined with phenobarbital.	Wang P, 2015

Anti-Parkinson Agents	Donepezil	CYP3A4 and CYP3A5 polymorphisms do not possibly influence donepezil clinical outcomes. ABCB1 genotypes (1236C/2677G/3435C 46% and 1236T/2677T/3435T 41%) may play a role in donepezil distribution and clinical consequences. Homozygous for the T/T/T haplotype has a little lower donepezil concentration-to-dose ratio than other types.	Magliulo L, 2011
Diseases	Alzheimer's disease (Amyloid-beta accumulation)	The amount of thymine has different effects on health subjects and AD patients. The more thymine presented in the C1236T, G2677T/A, C3435T, the higher binding affinity would be.	Van Assema DM, 2012
		For 234 AD patients and 225 controls, only C3435T displayed a significant association with increased risk of AD.	Feher A, 2014
	Schizophrenia	For Chinese schizophrenia patients, only C1236T has the potential to predict the risperidone responses on the basis of the TT genotype showing great improvement than other types.	Xing Q, 2006
	Epilepsy	C3435T with CC genotype relates with upregulated P-gp level.	Siddiqui A, 2003
		ABCB1 C3435T polymorphism is associated with the responses of anti-epileptics.	Taur SR, 2014
	Depression	A relationship between rs1128503 genotypes, sexual dysfunction and P-gp substrates (SSRI) use for women was established.	Thomas KH, 2013
	Dementia	ABCB1 genotype, allele and haplotype frequencies were neither different between patients with dementia nor age-matched controls. ABCB1 genotype frequencies are similar in younger populations.	Frankfort SV, 2006

CHAPTER 4

ASSOCIATION BETWEEN P-GP AND DRUG-RESISTANT CNS DISORDERS

4.1 Alzheimer's disease (AD)

Amyloid-beta clearance by P-gp avoids accumulation in the brain.

The incidence of AD rises with increasing trend of longevity [37]. Aging is the main stimulator in pathogenesis of AD. AD features extracellular amyloid plaques and neurofibrillary tangles from the perspective of histopathology. Swerdlow R. et al [37] established the all-round understanding of pathogenesis of AD. Since the role of AD is still quite controversial as either a condition of aging or an independent disease entity. They gave an interesting assumption: Centenarians are expected to suffer from AD, and non-AD elder people are the exceptions.

There are considerable debates about cause and symptom. Two hypotheses have been proposed: 1) Amyloid cascade hypothesis; 2) Mitochondrial cascade hypothesis. The debate mainly focuses on whether AD is a primary or secondary amyloidosis that induces the AD pathogenesis.

P-gp is able to efflux excessive Aβ accumulated in the brain tissues, facilitating the clearance across the BBB [38]. For example, studies found increased Aβ 1-40 level in the brain were observed in the absence of ABCB1 and ABCG2 genes [39]. Decreased clearance of amyloid-βvia efflux protein results in the ascending amyloid-β accumulation. The ABCB1 transport Aβ directly from brain tissue to systemic circulation while ABCA1, cholesterol transporter neutralizes aggregation depending on Apolipoprotein E (ApoE: a fat-binding protein mediated cholesterol metabolism in the brain). These two systems form an interactive model [33]. Transporters at the BBB are therapeutic targets for AD. Drug candidates enhancing P-gp activities are considered to ideally decrease Aβ build-up [33]. Herein the activation of P-gp

function is engaged in developing new Alzheimer's disease medications. Summarily, the promising drug development for AD should satisfy the requirements: good penetration across the BBB and are potent to increase P-gp activity. P-gp inducer could be an effective strategy for conventional AD therapy even though P-gp inducer is common like P-gp substrate or inhibitor.

Other evidences also confirm the involvement of mdr1 gene/P-gp. Not only ABCB1, increased level of ABCA1 gene also promotes Aβ efflux. The induction of vitamin D receptor (VDR) also called calcitriol receptor, causes P-gp upregulation [48]. Liver X receptors (LXRs) by its agonists mitigates the amyloid burden and restrains the development of neuro-degeneration in AD [49]. LXR agonists regulate ABCA1 gene expression which controls the balance between intracellular and extracellular cholesterol.

ABCB1 polymorphisms were assessed by Van Assema et al [47]. The amount of thymine (T) has different effects on expression of P-gp in health individual and AD patients. The more thymine is presented in the C1236T, G2677T/A, C3435T SNPs, the higher the binding affinity would be. Therefore, the genetic variants are conjectured to be linked to the disposition of Aβ in the brain.

4.2 Treatment-resistant schizophrenia (TRS)

The diagnosis of TRS is comprehensive process and requires opinions from family, community and social life. TRS patients experience persistent moderate to severe positive and negative symptoms, in spite of taken at least two antipsychotic regimens [183]. Prior to diagnosing TRS symptoms, it is necessary to distinguish inadequate duration of responses and insufficient dose of antipsychotic drugs. Generally speaking, TRS develops with the increased treatment time. If TRS happens, the cure would be life-long. TRS can be induced by the use of different antipsychotics or neurological medication. Broadly saying, the acquired resistant disorders usually do not respond to altered drug dosage, switching some other types of drugs, or adding auxiliary therapy.

The cornerstone of schizophrenia therapy is antipsychotic treatment. Antipsychotic treatments initially aim to modulate signs and symptoms at low possible dose. Given that complicated symptoms of schizophrenia and different patient groups, antipsychotics are often combined with antidepressants or anti-anxiety drugs so as to achieve ideal effects. An improvement would take a while to be noticed. And different mechanisms are involved in TRS symptoms. So far, clozapine is the only effective medication for TRS but without sufficient prescriptions in most countries [115]. People are worried about risks of clozapine use, including agranulocytosis, weight gain, cardiovascular morbidity, and dyslipidemia. Although the underlying mechanism of clozapine is unknown, factors affecting the clozapine responses include dopaminergic and serotonergic genetic variants, metabolizing enzyme (CYP1A2) and glutamate system. Glutamate exhibits higher level in the anterior cingulate cortex of TRS patients which may contribute to clozapine non-responses. Additionally, the variants GRIN2B, encoding 2B subunit of the glutamate N-methyl-aspartate receptor, will be further studied in the pathogenesis of TRS as well as clozapine treatment.

4.3 Refractory depression (RD)

Current antidepressant therapies face a high failure rate. The majority of depressed patients suffer from refractory depression, also called treatment-resistant depression. A characteristic of depression therapy is that it is required to take a long time to improve for symptoms. Additionally, suddenly discontinued medication induces relapse of the conditions. Thus it is notable that chronic treatment with antidepressant causes lower effectivity. The specific mode of mechanism is still unknown. However, some studies have been recognized that P-gp at the BBB may be the limiting factor in preventing antidepressants from reaching to their targets within the CNS. Compounds such as amitriptyline, imipramine, citalopram/escitalopram, fluoxetine, sertraline and nortriptyline display higher brain-to-plasm distribution ratios in P-gp knock-out mice relative to wild-type mice even though their structures are completely different [76]. Recent report believed

that numerous antidepressants are new type of P-gp reversing agents as a consequence of inhibiting P-gp [25].

4.4 Refractory epilepsy

Seizures and pharmacotherapy have a combined influence on epilepsy relapse.

Around 1-2% of population all around the world are affected by epilepsy based on reported in 1987 and 2002. This trend maintains stable from the last century to now. Simultaneously there are 20-30% of the patients that are non-responders to anti-epileptic drugs (AEDs) [51], even though these AEDs have different mechanism of action. To date, we are not so sure the causes involved in the incidence of epilepsy.

The development of refractory epilepsy induced by pharmacotherapies is a complicated process. Numerous hypotheses are concluded replying on animal studies and clinical results. NKCC1 (Na-K-Cl cotransporter: an active transporter and widely distributed through the whole body) expression in the hippocampus causing by dysfunction of GABA inhibitory pathway increased in patients with refractory epilepsy [196]. Abnormal function of adenosine kinase system may participate in the drug-resistant epilepsy [197].

As a well-accepted hypothesis, over-expression of P-gp in the epileptic focus tissues is associated with partial drug-refractory epilepsy. Isolated MDR gene expression from blood vessels located in temporal lobe of patients with refractory epilepsy was found. Over-expressed P-gp is the most accepted rational in refractory epilepsy. Clinical researchers are trying to explain the increased expression of P-gp at the molecular basis. An identified polymorphism of ABCB1, CC genotype in the mutation C3435T contributed to increased P-gp level [64].

Discussion about chronic AEDs study

In a week-long experiment, it is unable to find any P-gp over-expression, suggesting that a week administration of AEDs is not long enough to make significant changes [73]. Wen et al [72]

examined whether the chronic exposure of anti-epileptic drugs (phenobarbital, phenytoin and carbamazepine) would affect P-gp expression level in three weeks. After successive 21-day treatment with AEDs twice daily in rats, decreased tissue-to-plasma concentration ratios of Rho123 were detected in cortex and hippocampus which are believed as epileptogenic tissues. Simultaneously, no changes of drug level in plasma were found. Presumably, the cerebral drugs were remarkably decreased because of increased P-gp up-regulation. Immunohistochemistry and western blot study corroborated this hypothesis. They suggested that P-gp activity was correlated with P-gp levels in rats.

Phenobarbital administration has a time-, region- and species-dependent effect on P-gp function and expression [104]. The hippocampal kindled rat model is very powerful tool to test the drug potential for seizures. It has been accepted by National Institute of Neurological Disorders and Stroke. The advantages of this model are the utility of anti-epileptic drugs identification as well as the examination for complex brain network that can explain the seizure relapse. This model provides temporal frame for assessing drug safety and efficacy with short refractory period. Meanwhile, it can be utilized in fully blocking the kindled seizures through stimulus and obtain the elevate threshold to focal firing [199]. For instance, Mdr1a/1b concentrations in the cortex stimulated by kindling were higher than that in the hippocampus [104]. Longer time exposure causes great higher mRNA level.

4.5 Analgesic tolerance

Analgesic tolerance has been studied since last century. The long-time exposure of rat brain to opioids induces P-gp expression. After that, the disposition of opioids in brain has been significantly limited causing weakened pharmacologic activity of opioids. Increasing the amount of opioids is necessary to maintain same level of analgesic effects [102]. GABA inhibitory interneurons are found to be intimately involved in the morphine analgesic tolerance. These interneurons control the descending pain inhibitory signals. Further investigation should focus on

gender association, dose-dependency, and the cellular mechanisms to explain the morphine tolerance related to P-gp expression. Not only P-gp, opioids also affect the expression of breast cancer-resistance protein (Bcrp) [102].

The induction can be ascribed to several different levels of explanations: direct P-gp induction, increased gene transcription, increased protein translation or post-translation processing. Up-regulation of P-gp activities leads to reduced pharmacological effect of opioids which are P-gp or Bcrp substrates, mainly anticancer drugs and tyrosine kinase inhibitors.

Patients with opioids for pain relief or heroin maintenance therapy often undergo withdrawal syndrome. In order to explain opioid withdrawal, two elevated excitatory amino acids, glutamate and aspartate, are observed in morphine withdrawal. It is confirmed by Bauer B. et al [103] that P-gp modulates the glutamate signaling through NMDA receptors and COX-2 activity. COX contributes to the production of prostanoids and alleviates inflammation and pain. COX inhibition can reduce the prostaglandin synthesis and block the inflammation and analgesic effects. COX-2 inhibitors potentially improve the anticancer performance [146]. In short, morphine-induced P-gp expression is closely related to the two mechanisms [102].

Table 4: Association between P-gp and drug-resistant CNS disorders

Diseases	Biological etiology	Role of P-gp	Factors	Ref.
Alzheimer's disease	Extracellular amyloid plaques and neurofibrillary tangles	Efflux of Amyloid-beta by P-gp avoiding brain accumulation	hAβ42 level, vitamin D receptor, liver X receptor; ABCB, ABCA1, ABCG2	[33][39][48][49]
Refractory depression (treatment-resistant depression)	A complex set of physiological process is related to onset of depression: decreased levels of monoamines in the synaptic cleft	Glucocorticoids are transported by P-gp	Stress, HPA axis, genetics, immune system	[25]
Refractory epilepsy	Genetic influence; traumatic injuries; brain tumors or strokes; infectious diseases; prenatal injuries; autism and neurofibromatosis	P-gp expression greatly increased in the epileptic focus tissues	NKCC1, GABA inhibitory pathway, adenosine kinase system	[196] [197]
Analgesic tolerance	Pharmacotherapy-mediated upregulation of P-gp is remarkable; withdrawal syndrome	Long exposure of opioids induces P-gp expression; opioids distribution are limited with lowered pharmacological effects	GABA inhibitory interneurons, NMDA and COX-2 signaling pathways	[102] [103]
Treatment-resistant schizophrenia	Genetic predisposition; neurodevelopmental abnormalities and target features; further brain dysfunction and neurodegenerative influences	Antipsychotics induce the over expression of P-gp	Changes of presynaptic dopamine transmission; anterior cingulate glutamate pathway	[183]

CHAPTER 5

DRUG-P-GP INTERACTIONS AND DDIS MEDIATED BY P-GP

5.1 Antipsychotics

Antipsychotics are a class of drugs used to manage psychosis, mainly including schizophrenia and bipolar disorders. Typical antipsychotics are developed since 1950s. Atypical antipsychotics are discovered more recently. Both of them block the dopamine receptor activities.

The majority of antipsychotics are unable to cross the BBB because of their physicochemical properties. They have to overcome the expulsion of BBB. To this end, researchers are dedicated to inhibit the targets at the BBB and make better permeability of some drugs that are susceptible to these transporters. Clinically, in order to increase the safety and efficacy, multiple drugs are taken together, thus leading to undesired side effects (e.g. movement disorder, agranulocytosis and rapid major weight gain)[113].

5.1.1 Conventional antipsychotics

Domperidone (Motilium®)

Domperidone is a selective dopamine D_2 receptor antagonist. Unlike other prokinetic drugs (e.g. metoclopramide, sulpiride), domperidone hardly causes adverse effects within the CNS. Unlike metoclopramide, domperidone cannot cross the BBB. However, even the normal dose of domperidone can induce side effects in the brain tissues after other CNS disorders occur [122] or when P-gp inhibitors such as CsA [123] are concomitantly administered.

Domperidone is extensively metabolized by CYP enzymes in the liver and intestines via hydroxylation and N-dealkylation[124]. Although the extrapyramidal effects induced by domperidone can be neglected, the cytotoxicity related with predisposition to ventricular

arrhythmias caused by prolonged QT interval should be attached importance [126]. Domperidone induces catalepsy in a dose-dependent manner. The intensity of catalepsy induced by blockage of dopamine receptors has direct association with occupancies of $D_1$and D_2 receptors [98].

Food/herbs-domperidone interactions

In an in vitro study used non-everted rat intestinal sac model, suggested the apparent permeability from basolateral side to apical side rather than the opposite direction plays a critical role in DOM transport across the intestinal epithelial cells [125]. At the intestinal level, food-drug interactions or herb-drug interactions are being concerned in recent years. Due to the complex constitutes in the food and herbs, it is tough to determine which composition(s) play the main role in interacting with CYP enzymes or P-gp. Quinidine and extracted grapefruit juice have inhibitory effects on P-gp and the bioavailability of DOM has been significantly increased (ΔAUC=29%, 44%; ΔCmax=19%, 36% in 15min) in absorption phase. The inhibitory effect of grapefruit can be ascribed to some components that alter P-gp activity (absorption) or CYP enzyme activity (biotransformation) [52].

In male Wistar rats, the oral bioavailability of DOM across the intestinal epithelium was enhanced by pre-treatment with piperine after one week [125]. Piperine inhibiting CYP3A4 is involved in food-drug interactions [127]. Some in vitro evidences with Caco-2 (human colon carcinoma) cells confirmed the activity of CYP enzymes and P-gp were greatly inhibited by piperine [127]. In order to ensure that the P-gp affects the permeability of DOM in the case of integrity of intestinal barriers, a non-absorbed and a permeable marker, phenol red and propranolol are used. It turned out to be no impairments on the intestines [125]. It is implicated in clinical use: the concurrent use of piperine with DOM will raise the risk of cardiac side effects. Patients are recommended to avoid taking them together or reducing the doses of DOM correspondingly (under 30mg daily) [128].

5.1.2 Atypical antipsychotics

Compared with conventional antipsychotics, atypical antipsychotics have less neurological side effects [119]. The combination of typical and atypical antipsychotics is most commonly prescribed to treat schizophrenia [56]. Conventional antipsychotics are mainly dopamine D2 receptor antagonists while AAPs are partial agonists for D_2 and 5-HT_{1A} receptors [59].

Using ATPase assay as we mentioned above, it is predictable for the binding affinity between AAPs and P-gp. Both risperidone and quetiapine are P-gp substrates with low Km (high affinity) and high Vmax (high capacity). Olanzapine has moderate affinity [68]. Two conventional antipsychotics, chlorpromazine and clozapine cannot be identified as P-gp substrates. In vitro studies have limitations and cannot supersede in vivo experiments because of the complexity of BBB [77]. In vivo data offers more valuable clinical consequences and practical dynamic distribution information.

Antipsychotics (AAPs) show various degrees of inhibitory effects on P-gp

AAPs carry with some P-gp substrate or inhibitor characteristics. Risperidone, olanzapine and paliperidone are transported by P-gp at the BBB, based on increased brain-to-plasma ratios with Mdr1a knockout mice versus wide-type mice [66]. The combination with these AAPs is facing challenges in leading to undesired drug levels within the CNS. At a clinically relevant concentration, risperidone and olanzapine showed moderate inhibitory effects on P-gp activity. Other AAPs such as quetiapine, clozapine, showed mild or negligible effects. However, their exact cerebral concentrations are unknown [119]. Antipsychotics show different extent of binding affinity with P-gp [119]. It is suggested a competitive nature of the inhibitory effects of antipsychotics due to the same binding site inside the P-gp. Several studies have been confirmed the general inhibitory effects of antipsychotics, although different ranks are reports. Different drugs and cell lines are responsible for this phenomenon.

Aripiprazole

Aripiprazole is primarily used to treat schizophrenia and bipolar disorder. Aripiprazole shows low clearance and high distribution volume in mice and human [129]. Aripiprazole and its active metabolites, dehydro-aripiprazole are both P-gp substrates at BBB level [46/130]. In mice model, acute injection of aripiprazole is designed to determine the role of P-gp in transporting aripiprazole. Pharmacokinetics data indicated that 3.1- and 1.9-fold higher ratio of brain-to-plasma concentrations in P-gp deficient mice versus wide type mice [45]. They had the same results as that of Nagasaka Y. et al [86]. Besides, the binding affinity between aripiprazole and P-gp is moderate while ziprasidone has a relative lower affinity with P-gp.

Presently, this discussion is extended to general understanding of PD responses. Acute administration of aripiprazole and ziprasidone destroyed motor behaviors of mice in a dose-dependent fashion. The abcb1a/1b (-/-) mice had much more severe motor impairment than wide type group [45]. In other word, P-gp deficient mice bear more pressure from external stimulus. Unprotected extracellular fluid at steady-state is more easily disturbed. It helps us to better understand the protective functionality of P-gp.

RotaRod was used for locomotor performance of mice. It turns around with an adjustable speed and stops when mice fell off from the neutral position. Comparing the time of the pre-treated and post-treated with aripiprazole and ziprasidone, the PD changes can be estimated in an indirect way. P-gp substrate properties make some influence for aripiprazole on their PD responses.

The P-gp deficient mice model is a powerful and sensitive tool to investigate the P-gp distribution and the differences between drug levels in the brain and blood. Although P-gp is widely recognized as the main efflux pump at the BBB for aripiprazole, the Mdr1a1b double knockout mice as blank control is critical to confirm the consequences from the in vivo perspective. Evident higher drug levels in the brain tissues were detected in abcb1ab(-/-) mice(4.6-, 4.1- and 3.0-fold higher than wide type mice after 1h, 2h, 3h)[43]. The testes

concentration of aripiprazole also displayed palpable increase in the abcb1ab-/- mice than control mice, indicating P-gp is located at the testes as well. In contrast, the aripiprazole plasma level in P-gp deficient mice did not show any significant changes. In the absence of P-gp at the BBB, if there is any pharmacokinetics data provided, the retention of aripiprazole should be longer. However, the elimination half-life (t1/2) of aripiprazole in the brain was shortened in P-gp deficient mice by using WinNonMix, a population PK model. From my point of view, the shortened half-life time may rise from induction of other transporters at the BBB, altered intra-cerebral environment or binding affinity with serotonin/dopamine receptors.

P-gp is not the only transport protein for aripiprazole at the BBB. Numerous evidences have been expounded the putative concept of "Multiple efflux pumps collaboration". Although the content of Bcrp is much lower than P-gp at the brain microvessels, the role of Bcrp cannot be ignored. At some points, the expression of Bcrp is stimulated in the absence of P-gp. It is not surprising that P-gp and Bcrp could work cooperatively to limit the entry of drugs to the CNS. If the $K_{p,brain}$ value for dysfunctional P-gp and Bcrp is consistent than the combined values for each dysfunctional transporters[131], it can be presumed that there are some associations between them. Undeniably, the association varies. Are they complementary, synergistic, competitive or tandem? Further investigations are expected to focus on this area.

Although the unambiguous correlation between P-gp and Bcrp has not been established yet, some studies reported the situations without any link between them. One study focusing on how the plasma pharmacokinetics of aripiprazole would be affected in the absence of P-gp and Bcrp was conducted [86]. They found that the function and expression of P-gp is largely tissue-specific. At the intestinal tract, P-gp does not play a role in absorbing aripiprazole whereas Bcrp may contribute to a slightly increased fraction of absorbed drug in mice model. In human intestines, few attentions have been attached to BCRP. Conversely, Bcrp has little synergistic effect with P-gp in brain penetration of aripiprazole. From the above findings, we can firmly believe that the

aripiprazole and its metabolite, dehydro-aripiprazole, are both P-gp dependent substrates at the BBB level.

Risperidone/quetiapine/olanzapine

I will discuss three AAPs in this chapter. They are commonly prescribed antipsychotic medications often used to treat not only schizophrenia and bipolar disorder, but also dementia and behavior problems in different age groups. Compared with conventional antipsychotics, they are preferred when treating schizophrenia because of the lack of extrapyramidal side effects (drug-induced acute or tardive dyskinesia) [136] and well tolerated with good efficacy for positive and negative symptoms of schizophrenia [137]. In addition, they have other indications such as prolonging the QT interval (electrical depolarization and depolarization of the ventricles), leading to fatal arrhythmia.

They are all easily absorbed and metabolized by CYP enzymes. Generally saying, not only the parent compounds but also their main metabolites are able to cross the BBB and then reach the targets within the brain. The studies of them can be separated into two categories, preclinical studies with their metabolites and clinical trials with respect to the influence of ABCB1 genotypes. P-gp activity at the BBB greatly affects the brain concentration levels of risperidone (up to 13-fold higher) and 9-OH-risperidone (up to 29-fold higher) [66]. The plasma concentration difference between parent compounds and their metabolite contributes to this huge differences influence of P-gp.

The side effects are worrying and very different among them [132]. For example, some evidences reports overdosing of quetiapine may have fetal cytotoxicity, thus P-gp at the placentas being extensively investigated. Rahi M. et al [137] studied the effect of quetiapine on P-gp expression on the placentas. They confirmed whether there was any risk of quetiapine for pregnant women. Nevertheless, no any cytotoxic information for fetus has been reported by the drug manufacturer. In a dually perfused human placenta model, they drew a conclusion that the

quetiapine transport was not affected by P-gp inhibitors, PSC833 or GG918, indicating that P-gp may not play an important role in the transport process. Quetiapine is not affected by P-gp activity on the placentas (the prerequisite is quetiapine is limited by blood-placentas barrier). Although some opinions suggest quetiapine is a P-gp substrate, apparently this conclusion does not work for P-gp at the placentas. High protein binding affinity of quetiapine is one possible reason for this conflicting result.

Based on this experiment, initially conclusion can be drawn. Quetiapine is safe for pregnant women who suffer from psychotic disorders or when they take another P-gp inhibitor together. As an important organ in the uterus connecting with the developing fetus, placentas transports all kinds of nutrients, oxygen from mother and removes wastes of fetus, serving as exchanger and metabolizer linked with maternal and fetal circulation. The components of proteins on the placentas adapt to the function of placentas and the stages of pregnancy. P-gp is highly expressed in the syncytiotrophoblast of the placenta during the pregnancy. The expression of P-gp at the trophoblast cell layer of the placenta varies. Within the trimester of pregnancy, P-gp activity would reach the peak value. As far as I'm concerned, based on the tissue-specific principle of P-gp, the role P-gp inhibitors may be totally different. CsA, one P-gp substrates/inhibitor/inducer, displays increased transfer across the placenta while digoxin did not [138].

The inter-individual variability of dosing for this class of drugs is the primary problem in clinical studies. As for a standard dose of olanzapine for schizophrenia patients, 20-30% patients showed inadequate efficacy but 5-10% displayed overdosing [109].

5.2 Antidepressants

Antidepressant medication (ADM) is developed since 1950s. Before 1950s, depression has not been given considerable attention. Opioids and amphetamines were prescribed the most commonly. Nevertheless, their addiction and side effects limited their usage for depression. In 1952, scientists accidentally found isoniazid and iproniazid had anti-depressant activity in

patients with tuberculosis. The mechanism of action of isoniazid is poorly understood. But it is very likely to correlate with inhibition of diamine oxidase.

Hypothalamic-pituitary-adrenal axis (HPA axis)

Stress has been recognized as one stimulator for depression. The dysfunction of HPA axis is closely related with onset of depression. As a major neuroendocrine system, HPA axis controls a variety of reactions to stress and regulates the balance between different hormones, including three organs: hypothalamus, pituitary and adrenal glands. When HPA is activated, hypothalamus secretes two types of hormones, corticotropin-releasing hormone (CRH) and vasopressin (AVP). They act on receptors within the pituitary inducing pituitary to produce adrenocorticotropic hormone (ACTH). ACTH is then transported to adrenal glands and stimulates the release of cortisol. The function of HPA axis is completed through some loops: 1) negative feedback of cortisol: inhibiting the activity of hypothalamus and pituitary; 2) positive feedback of epinephrine/norepinephrine: increasing the ACTH and beta-endorphins levels. In addition to central nervous system, HPA axis also affects the function of other systems (e.g. immune system, cardiovascular system, metabolic system and reproductive system). Physical and psychosocial factors are integrated by HPA axis to maintain the normal operation of different systems.

The crosstalk between HPA axis, immune system and CNS is intricate. On one hand, CNS regulates immune system via HPA axis or some other endocrine pathways. On the other hand, immune reaction caused the abnormal of a number of pro-inflammatory cytokines can be transported to CNS or inactivate HPA axis. Mental state is direct inducing factor to HPA axis. In the case of stress, elevated glucose contributes to increased cortisol. Immune system is inhibited via blocking certain metabolic processes. Conversely, more glucose is further increased in the blood.

P-gp is intimately correlated with glucocorticoid levels involved in the HPA axis. Tariquidar has the potential to relief water-avoidance stress (WAS) which is a frequent psychological

symptom of mice. After administering the tariquidar, the mild stress showed significant improvement. Thoeringer C. et al [182] clarified for the first time that the inhibitory effect of tariquidar on P-gp at the BBB was facilitating the entry of corticosterone across the BBB. Increased corticosterone enhanced the negative feedback to hypothalamus and pituitary, eventually altering the HPA axis and mental conditions. Despite the promising application of P-gp inhibitor in treating stress-associated disorders, it is noteworthy that tariquidar is not very efficient for severe anxiety such as forced swim test (FST). P-gp inhibitor shows limited effect on reversing P-gp activity.

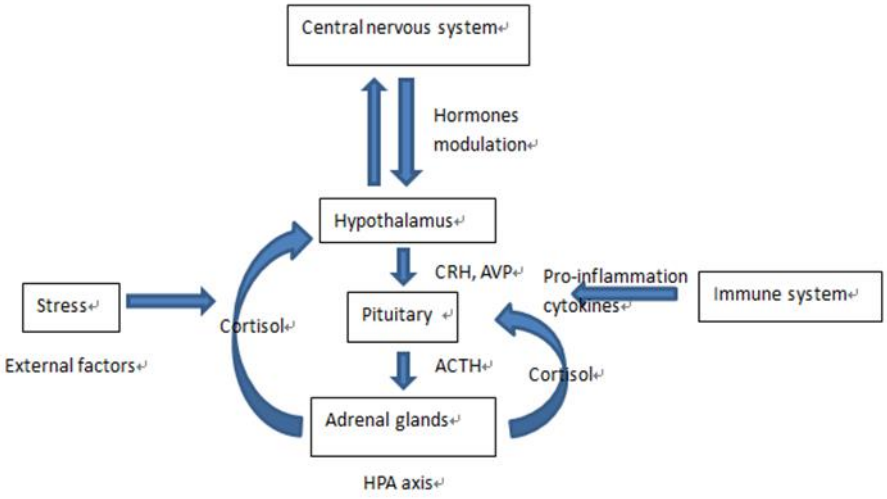

Figure 2 Crosstalk between HPA axis, immune system and CNS disorders

Development of ADMs

The development of first generation ADMs is followed by the establishment of monoamine hypothesis. Decreased monoamine including serotonin, dopamine and noradrenaline, leads to insufficient activation on the receptors on the postsynaptic neurons. Second generation ADMs are more diversified and selective to each receptor. Approximately 60% prescribed ADMs are selective serotonin reuptake inhibitors (SSRIs) which has full spectrum of depressive disorders.

Patients are well-tolerated even in overdoes. Similarly, serotonin-norepinephrine reuptake inhibitor (SNRIs) or norepinephrine reuptake inhibitors (NRIs) are under investigations for their efficacy. The potential cardiovascular side effect is the main barrier.

In this part, I am going to discuss the recent studies of tested antidepressants in vitro and in vivo to characterize their performances under different conditions. My goal is to explain the structural relativity in each class of ADMs as well as the interaction with P-gp function and expression.

5.2.1 Tricyclic antidepressants (TCAs)

Imipramine(Tofranil®)/desipramine(Norpramin®)

Imipramine is the first (in 1951) discovered tricyclic antidepressants (TCAs). TCA is a class of drug consisting of a tricyclic ring and an alkyl amine substitute. They inhibit norepinephrine and serotonin reuptake by blocking the reuptake transporters. Some of them also target histamine, muscarinic and cholinergic receptors suggesting that they give rise to some adverse effects. They have the ability to improve depressed signs, but also relieve chronic nerve pain and Attention Deficit Hyperactivity Disorder (ADHD).

Imipramine is rapidly absorbed through small intestines, and almost all metabolized by CYP proteins (CYP1A2, CYP3A4, and CYP2C19) in liver. The major metabolites of imipramine, desipramine, (can also be converted from imipramine within the body) is an active components for depression. Desipramine is deemed as the most potent and selective affinity with norepinephrine transporter. Meanwhile, desipramine displays minor antihistamine and anticholinergic actions compared with other TCAs.

Imipramine is identified as human P-gp substrate. Ex vivo study showed that pretreatment with verapamil made the imipramine concentration increased using intracerebral microdialysis technique without any changes in plasma; whereas desipramine is elevated in the presence of verapamil not CsA [160]. Using bidirectional transport experiment in MDCK-MDR1 cell line to

determine the role of imipramine (1 and 5 uM) as P-gp substrate (Transport ratio>=1.5)[23]. P-gp restricted the transport of imipramine across the BBB. This low concentration of imipramine reflects clear P-gp efflux effect from the therapeutic plasma levels. Furthermore, extended observation time gives more complete pharmacokinetics profile than data of previous studies [24]. The authors pointed out that the findings on imipramine were not able to extend to other antidepressants even in the same class. Amitriptyline is not P-gp substrate even though it has similar structures with imipramine. The possible explanation is the additional hydrogen-bond acceptors in the middle ring of imipramine play a crucial role when binding with P-gp, whereas there is a carbon atom in the analogous position of amitriptyline [23].

As far as I'm concerned, I am doubted that in vitro and in vivo studies are not clinically relevant. Imipramine has a half-life of 12h while desipramine has a half-life of 22.5h. As discussed above imipramine has extensive metabolism process and is inclined to bind with serum protein. It is important to measure how much imipramine can reach the BBB or bind with corresponding receptors within the CNS prior to all kinds of transport study. Unfortunately I did not find any pharmacokinetics data about imipramine concentration nearby the blood-brain barrier or the fraction being involved in the brain microvessels. It is believed that the fraction of imipramine that successfully reaches the BBB would not be as much as imagined. Further study should pay more attention on the disposition of desipramine or imipramine and protein bound complex in the brain.

5.2.2 Selective serotonin reuptake inhibitors (SSRIs)

SSRIs block the serotonin reuptake channels, increasing the serotonin levels in the synaptic gap and enhancing the function of postsynaptic neuron cells. Basically, SSRIs have different chemical structures but very similar pharmacological actions to serotonin reuptake channels. The flexibility of SSRI treatments makes it being widely used for depressive patients who present poor tolerance to other type of antidepressants.

Citalopram/escitalopram

Citalopram belongs to selective serotonin inhibitor (SSRIs) [126]. Citalopram and escitalopram are mutual chirality. However, escitalopram is safer and effective than citalopram and other SSRIs [163]. The pharmacological effects are largely due to the S-enantiomer, S-citalopram and S-demethylcitalopram [79]. Citalopram is approved by FDA to treat depression as well as mood disorders, smoking cessation, ethanol abuse, and diabetic neuropathy.

Citalopram is well absorbed in GI tract within 4 hours to reach peak plasma concentration. The bioavailability is pretty high (80%) and is not affected by food. Notably, it readily crosses the BBB due to its high lipophilic and large distribution. However, the main metabolite, desmethlycitalopram is not able to cross the BBB. The metabolism of citalopram includes N-demethylation, didesmethylation, N-oxide, and deamination. CYP3A4 and 2C19 are involved in the demethylation.

Escitalopram is recognized as P-gp substrate in the transport assay model [23/31]. As a control substrate, Bundgaard et al [41] assessed a series of new antidepressants (levomilnacipran, vilazodone and vortioxetine) penetration across the BBB. In P-gp knockout and wide-type mice, they measured the brain-to-plasma exposure ratios for both models after acute subcutaneous injection. Levomilnacipran (5.8) and vilazodone (5.4) showed higher values than the control substrate, escitalopram (3.1). Vortioxetine did not display any significant changes. In light of Kp,uu (unbound brain concentration/ unbound plasma concentration) at the steady-state, the involvement of P-gp in the brain distribution of levomilnacipran, vilazodone and escitalopram in vivo could be determined. In line with the results above, vortioxetine is not affected by P-gp.

Karlsson L. et al [142] investigated whether the enantiomers of citalopram and its metabolites are substrates of P-gp. They used P-gp genetic knockout (abcb1a1b-/-) mice and wild-type (abcb1a1b+/+) mice treated with citalopram in two regimens, single dose and two daily doses for 10 days. 3-fold higher of cerebral citalopram level in abcb1a1b-/- mice were detected

than that of wide type mice. Further clinical and toxicological studies are required to be elucidated.

O'Brien et al [31] put forward a broad concept of the role of escitalopram both in vitro and in vivo approach. They solved three problems before bringing forward the possibility of a new therapy. Escitalopram is transported by P-gp; escitalopram is restricted by P-gp at BBB in mice model; putative enhancement of escitalopram brain level would result in elevated pharmacodynamics activity. They found a moderately strong correlation between brain escitalopram concentration and immobility in the Tail Suspension Test (TST) which is one of the most widely used models for examining antidepressant-like activity in mice.

Chronic studies are also performed to measure the effect of escitalopram on P-gp function and expression. Co-administered verapamil with escitalopram in continuous 22 days, over 2-fold increase of escitalopram brain level was observed without altering plasma level, indicating P-gp inhibition might cause elevated brain level of antidepressants after chronic treatment [141].

Combined with P-gp inhibitors has advantageous of avoiding the risk of peripheral therapeutic responses due to high dose of certain antidepressants. It is worth noting that CNS side effects often inevitably occurred. Appropriate selection of P-gp inhibitors is able to overcome unwanted effects. Extrapolating in vivo data from mice to human is challenging.

The distribution of citalopram/escitalopram was elucidated in depressive patient who have different ABCB1 genotypes (including C3435T, G2677T) [89]. It is revealed that only the G2677T ABCB1 genotype gave rise to a significant effect on citalopram and its enantiomers disposition even though no changes of plasma and CSF ratios can be detected. This phenomenon suggested the lack of stereoselectivity of P-gp at the BBB. Furthermore, patients with 2677 GG/GT presented better therapeutic responses than others. To this end, it offers hope to the development of personalized dosing regimen in response to the genotypes of patients. Presently, the feasibility is challenging and the cost for clinical setting is unrealistic, but we are optimistic about the future replicated research on it.

Sertraline(Zoloft®)/paroxetine(Seroxat®)

Clinically, sertraline, paroxetine and fluoxetine are often prescribed to depressive patients either combined together or single use. Numerous clinical trials showed that they have similar efficiency for depressive symptoms. Thus I decided to discuss them as a part.

On the behalf of SSRIs, sertraline and fluoxetine were evaluated whether they affect the function of P-gp [11]. Sertraline has inhibitory effect on P-gp at the BBB [25] but is quite dependent on the dosing method. Acute administration of sertraline modulates P-gp activity at the BBB and BTB (blood-testes barrier). The modulation is biphasic: in a short period of time, the accumulation of P-gp substrate, digoxin, in the brain or testes would increase drastically followed by a falling trend in a slightly longer time. However, no significant effect of fluoxetine was observed on P-gp. In addition, sertraline inhibits P-gp expression in the heart (ventricular myocardium in human tissue) leading to digoxin accumulation and improved other cardiac drugs. Additionally, the primary metabolite of sertraline, desmethylsertraline, has the potential to inhibit P-gp in vivo [11].

Both paroxetine and duloxetine are transported by P-gp. Concomitant with verapamil, the efflux ratio of paroxetine and duloxetine decreased [133]. Paroxetine has more potential of inhibiting 5-HT reuptake than sertraline and fluoxetine but less than citalopram. Paroxetine also targets norepinephrine receptor at a weak level. The risk of paroxetine withdrawal such as suicidal thinking is worrying. Paroxetine is well absorbed and well metabolized by CYP2D6. It displays inter-individual variants in pharmacokinetic parameters (half-life, volume of distribution and clearance). Mihaljevic A. et al [174] investigated the effect of MDR1 polymorphisms on therapeutic response of paroxetine. According to HAMD-17 scale (Hamilton Depression Rating Scale, 17-item), they found G2677T and C3435T were not related with paroxetine response.

Fluoxetine(Prozac®/Sarafem®)

Fluoxetine is the first SSRIs. It has been used to treat depression, obsessive-compulsive disorder and panic disorder for a decade. The standard regimen of fluoxetine in treating with depression is 20-80mg/person per day [185].

Fluoxetine and anticancer drugs

Fluoxetine shows conflicting research potential when co-administration with other anticancer drugs. There is a debate about the pharmacological effect of fluoxetine on cancer cells. In some mice models, Fluoxetine has been proved to accelerate the tumor growth. On the contrary, some researchers believe fluoxetine can exhibit inhibitory effect on cancer cell proliferation. In my opinion, the binding event of fluoxetine on specific target may induce the signals affecting the tumor cell proliferation.

To date, chemotherapy for cancer is facing serious multidrug resistant problem. Long exposure to chemotherapeutic agents renders unavoidable tolerance to treatment. A chemosensitizer improves the drug-resistances to anticancer agents. Ideally, there should be no any effects on the cell viability [21]. Patients are recommended to take chemosensitizers with chemotherapeutic agents at the beginning of chemotherapy. At that time, the drug resistance has not appeared to be strong enough [81]. Functional and mechanistic aspects should be evaluated whether there are any correlations between the level of resistance and patterns and resistance modulation [21].

Fluoxetine is recognized as a mild and promising chemosensitizer [143]. Zhang Y. et al [143] explored the reversing effect of fluoxetine on P-gp expression and its influence on resistant/sensitive breast cancer cells. They elaborately described the decreased MDR1 mRNA and down-regulated P-gp expression after co-administering with fluoxetine. They concluded that fluoxetine altered the P-gp level, eventually enhancing the chemosensitivity of paclitaxel in MCF-7 cell lines. In monolayer transport assay using LLC-PK cell lines transfected by MDR1 gene, no inhibitory effects of fluoxetine were found during 1 hour [12]. However, when given fluoxetine and doxorubicin together to mice, tumor volumes were diminishing[18] and the

cytotoxicity of doxorubicin was increasing[21] compared with given doxorubicin alone. Argov M. et al [21] suggested that doxorubicin accumulation in HCT-15 cells was increased (32%) in the presence of fluoxetine and its efflux process was inhibited (70%) in vitro. In vivo, tumor progression can be slowed down by combination of fluoxetine and doxorubicin combination in a mild manner compared with other violent therapy, bevacizumab (avastin). For other P-gp inhibitors, PSC833 and cyclosporine A, are supposed to be at high dose of 50mg/kg body to reach the same modulatory effect of fluoxetine [184]. The different effects of chemosensitizers may be also dependent on the exposure time and frequency of treatment. Overall, fluoxetine is regarded as a highly potential chemosensitizer.

Fluoxetine and antimalarial agents

The development of P-gp reversing agents has been a hot spot with a rational outlook. Current mission is to develop newer, inert, pure and efficient P-gp inhibitors [201]. First-, second- and third-generation P-gp inhibitors are categorized due to their screening procedures (they can be obtained through screening marketed compounds, optimized compounds, or synthesized by combinational chemistry). Verapamil, trifluoperazine, cyclosporine A, quinidine, yohimbine are classified as first-generation P-gp inhibitors. They are accidently found to show some P-gp substrate characteristics when used as other indications. Hence most of them are not selective and causes a large variety of side effects. Second-generation P-gp inhibitors are optimized form of first-generation inhibitors. Dexverapamil(R-isomer of verapamil), PSC833 (also known as valspodar: cyclosporine A without immunosuppressing effect) are grouped in this class. Chemists are aimed at achieving better reversing consequences and greatly reducing the dose to the safe level in plasma. The altered chemical structure leads to changed metabolism efficiency of CYP enzymes and unpredicted metabolic pathways. Although second-generation inhibitors are facing many problems, they are widely used in preclinical tests. Combining the combinational chemistry and high throughput screening technique (HTS), third-generation inhibitors are far more effective

than the previous two types. Structure-based drug development (SBDD) makes the molecules more target-specific and highly sensitive. The remarkable characteristic for this type of inhibitor is the lack of interaction with CYP enzymes [201]. Nevertheless tariquidar has unwanted cytotoxicity; it is still one of most promising representative, blocking the ATPase via bound with P-gp at the modulatory sites.

Due to the paucity of adverse effects, fluoxetine is recognized as fourth-generation P-gp inhibitor [143]. In clinical use, fluoxetine is safer and milder than other type of inhibitors according to low IC_{50} value. Only when given 75 times of the therapeutic doses, series side effects can arise. Not only for anticancer agents, has fluoxetine also enhanced the effect of antimalarial agents. Fluoxetine has the potential to enhance the chloroquine and mefloquine (CQ/MQ) resistance. Chloroquine and mefloquine are used for patients with malaria. Occasionally, other medications are required so as to acquire better of pharmacological effects without weakened resistance. Malarial parasites reproduce asexually within the red blood cells by degrading hemoglobin to satisfy their need of essential protein and energy. Parasites transform heme which is one component of hemoglobin, into hemozoin to destroy the formation of porphyrin ring. Chloroquine gets into the red blood cells and binds to heme, blocking it from building up iron ion porphyrin complex, FP (Fe(II)-protoporphyrin IX). It prevents the parasites proliferation and digestive vacuole through passive diffusion. FP-chloroquine dimer is very toxic, leading to cell death and eventually parasites autodigestion[202]. Mefloquine is an effective alternative for chloroquine-resistant P. vivax malaria yet not for P. falciparum. It is often co-administered with primaquine to remove liver infections. However, mefloquine may induce seizures relapse and some psychiatric disorders.

Khairul M. et al [5] corroborated that 50% inhibitory effects of CQ and MQ were significantly lowered when co-administered with fluoxetine at different fixed concentrations. The synergistic effect between fluoxetine and CQ/MQ were fairly apparent in comparison with the positive control of verapamil, a classic P-gp inhibitor via isobologram construction. Moreover,

the combined effect of fluoxetine on CQ was more potent than that of MQ. Since fluoxetine inhibits P-gp activity, P-gp accounts for the cooperative resistant of fluoxetine to parasite, Plasmodium falciparum, in all likelihood. But the precise mechanism has not been clarified yet [5].

The effect of fluoxetine on P-gp has been discussed for years. Another study demonstrated [6] that P-gp inhibitors decreased the cell sensitivity to CQ and increased that to MQ, which is not in agreement with Khairul M. The disagreement may result from fickle in vitro experimental conditions.

Duloxetine(Cymbalta®)

Duloxetine belongs to thiophenen derivatives. Duloxetine and venlafaxine are representatives of serotonin and norepinephrine reuptake inhibitor (SNRIs). Since its safety and good tolerance, duloxetine have been introduced to treat chronic musculoskeletal pain to the market on Nov. 4, 2010.

Based on the chemical similarity with fluoxetine and paroxetine, Zhao R, Cao J and Peng W[25] concluded that duloxetine greatly inhibited the P-gp at the concentration of 5-10uM for the first time. The inhibitory effect is in a concentration-dependent fashion from Rho123 accumulation assay. In particular, the regimen of duloxetine is required to be adjusted when co-administered with P-gp inhibitors. In the clinical safe concentration of 0.2uM, duloxetine manifested weak inhibitory effects on P-gp (approximately 21% increase of talinolol bioavailability through the intestines). The in vivo data was in agreement with in vitro results. A positively charged nitrogen atom containing in the duloxetine as well as its lipophilicity are speculated to be related with the role of P-gp inhibitor. Although some people suggested that SSRIs and SNRIs used as P-gp reversing agents is lack of clinical relevance, Zhao R. et al [25] offered hope for the clinical effects of duloxetine on bioequivalence of talinolol.

5.2.3 Serotonin antagonist and reuptake inhibitors (SARIs)

Nefazodone(Serzone®)/trazodone

Both nefazodone and trazodone have ability of modulating the P-gp activity, however, they do not participate in the transport of nefazodone and trazodone across Caco-2 monolayers [26]. NFZ is taken as a P-gp inhibitor since Rho123 levels had decreased transport as the fraction of control at different concentrations (1uM to 100uM). After 72hrs incubation in LS180V cells with 10uM (3uM no effect) nefazodone and trazodone, P-gp expression displayed a 2.2-fold increase over control. Additionally, the Rho123 levels inside cells significantly reduced by 74% of vehicle control suggesting P-gp function had been inhibited. Ritonavir functioned as the positive control, caused 52% Rho123 reuptake increase.

5.3 Anti-epileptics (AEDs)

Current reports are insufficient to provide a broad P-gp substrate profile for all AEDs. In order to make generalized conclusions, many factors (such as the high cost of detection technology and inherent recessive pathogenesis of epilepsy) should be taken into account.

Evidences showed the synergism between efflux transporters at the BBB. It is a remarkable conclusion but at the same time, P-gp study is facing many difficulties by distinguishing from the effects from other efflux pumps. Nakanishi H. et al [22] evaluated how 12 anti-epileptic drugs (phenobarbital, phenytoin, ethosuximide, carbamazepine, valproic acid, clobazam, zonisamide, lamotrigine, gabapentin, topiramate, tiagabine, levetiracetam) were distributed in the brain, affected by P-gp and Bcrp at cerebral level. Results showed that P-gp participated in the brain distribution of phenytoin, topiramate, and tiagabine; whereas Bcrp contributed to limited penetration of phenobarbital, clobazam, zonisamide, gabapentin, tiagabine and levetiracetam. Notably, they injected several AEDs simultaneously, which means the potential drug-drug interactions may occur and subsequently affect the activity of P-gp and Bcrp.

Phenobarbital/lamotrigine/felbamate

Phenobarbital is the choice for generalized epilepsy and status epilepticus [80]. Considering old generation AEDs are prone to alter the plasma level of other class of drugs by changing the enzyme activity in the blood [110]. Lamotrigine and felbamate are two newer anticonvulsant drugs for epilepsy and bipolar disorders. Although they are structurally irrelevant, all of them show anti-epileptic effects. The efflux of phenobarbital, lamotrigine, felbamate, carbamazepine and phenytoin is regulated by P-gp in wide type and P-gp deficient mice. These AEDs have different chemical structures and mechanisms of action. Some are capable of regulating the voltage-dependent ion channels; others enhance synaptic inhibition and excitation [65/110]. Using microdialysis, Potschka et al [7] conducted experiments in P-gp expression in brain regions (detected by immunohistology experiment) of rat model to determine the effect of P-gp inhibitor, verapamil on brain penetration of a series of AEDs, including phenobarbital, lamotrigine, and felbamate. Their concentrations in the extracellular fluid were significantly increased after local perfusion of verapamil. Thus P-gp plays a pivotal role in the drug-refractory epilepsy. Conversely, phenobarbital up-regulates the expression of P-gp based on in vitro data [8/10]. The novel treatments of a combination of AEDs and P-gp inhibitors are investigated to improve the multi-resistant phenomenon.

Yang ZH and Liu XD [80] concluded that phenobarbital uptake into rat brain microvascular endothelial cells (rBMECs) was dependent on time, concentration and temperature. The uptake reached its peak plateau at 60 min or so. Lowered temperature leads to decreased uptake of phenobarbital. It may be resulted from inactivated transporter. At 10min, phenobarbital concentrations ranging from 10 to 80ug/ml, a non-linear pharmacokinetic curve indicated that saturated transport process participated in it. Cyclosporine A, ketoconazole, and dinitrophenol were applied. They found phenobarbital accumulation inside the cells was increased over 50% ($p<0.01$) in the presence of these P-gp modulators or metabolic inhibitor. However, the involvement of other transporters such as BCRP and MRP1 cannot be excluded. With the

treatment of rifampicin and dexamethasone, more than 20% decrease of phenobarbital uptake was seen. It is expectable results since these two drugs are able to enhance the P-gp activity. In addition, phenobarbital transport in the B-A direction is greater than A-B direction (1.9-fold) which confirmed the above findings. It is assumed that integrated results come from the combined action of uptake and efflux processes.

Carbamazepine

Carbamazepine is highly metabolized by CYP3A4 and CYP2C8 to CBZ-10,11-epoxide(CBZ-E) followed by epoxide hydrolase[42]. Carbamazepine is an inducer for CYP3A4 and P-gp [60]. A few studies refuted that carbamazepine was a human P-gp substrate [100].

Verapamil was concurrently administered with carbamazepine to overcome the refractory epilepsy [53]. As a calcium channel blocker, verapamil also lowers seizure burdens and improve the quality of life for patients to some extent. The results have been proved by clinical trials. An obvious increase of carbamazepine in plasma and decrease of 10,11-carbamazepine epoxide concentration and metabolite to parent ratios were presented in 6 drug-resistant epileptic patients. Lower dose of carbamazepine is recommended when taken verapamil together. Researchers explored the possibility of molecular basis of verapamil inhibition to P-gp activity. Some other mechanisms are also very likely to be responsible (e.g. metabolic alteration of carbamazepine or intrinsic properties of verapamil) [51]. It should be pointed out that some patients cannot endure higher concentration of carbamazepine before addition of verapamil.

From the PK perspective, Magnusson M. et al [42] explored carbamazepine-mediated induction of CYP (CYP3A4, CYP1A2) and P-gp in seven healthy individuals. These volunteers are dosed in over two weeks with carbamazepine. As the authors stated, midazolam, caffeine and digoxin were considered as probe substrates to test if there are any changes of CYP enzymes and P-gp. Midazolam is a biomarker for CYP3A4 activity. Caffeine is converted to paraxanthine by CYP1A2; while digoxin indirectly reflects the P-gp activities. They observed the plasma levels

before and after administration of carbamazepine. The main problem is: midazolam and caffeine are transported by P-gp as well. Their plasma levels affected not only by CYP enzymes but also P-gp at the intestines. Unfortunately, they did not describe it elaborately.

In this experiment, carbamazepine and its metabolite, carbamazepine-10, 11-epoxide functioned as the driving force of the induction, even though carbamazepine has narrow dose range [42]. As is well-known, an enzyme inducer increases the production rate but decreases the elimination rate, leading to increased retention in the body. It is promising to enhance the bioavailability in the presence of enzyme inhibitors. The results are based on inducer concentration-to-response relationship. A finely detailed description of the binding affinity, mRNA production levels, translation efficiency of mRNA cannot be obtained in this way. Surprisingly, Magnusson M. et al [42] did not found P-gp induction regulated by carbamazepine referring to unchanged digoxin levels. Though Giessmann T. et al indicated that carbamazepine induced P-gp activity [60]. Overall, an enzyme inducer elongates the half-life and improves the bioavailability of CYPs substrates. Nevertheless, the cessation may cause immediately reduced half-life which can be regarded as one risk of drug tolerance.

5.4 Analgesics

Opioid addictions have long-lasting, relapsing effects on physical and mental health [166]. Previous studies have shown that opioid analgesics and opioid analgesic peptides (e.g. DPDPE) are directly pumped by P-gp [54].

5.4.1 Natural analgesics

Morphine (MSIR®)

As the most potent opiate, morphine is used to treat acute and chronic pain [175]. Morphine is metabolized to morphine-6-glucuronide (M6G) and morphine-3-glucuronide (M3G).

In the CNS, morphine exerts the analgesic effect by inducing the opioid receptors. Morphine transports the BBB in transcellular pathway instead of paracellular considering its physicochemical properties [57]. There are at least three factors that closely modulate morphine penetration into brain: Passive diffusion, P-gp efflux protein, and low capacity activity influx protein [78]. Though active uptake is prominent among them, morphine is very sensitive to P-gp inhibitors at low concentration.

Dated back to the end of last century, researchers applied various methods to identify the role of morphine in vitro and in vivo. Xie et al [96] studied morphine transport across the BBB in mdr1a(-/-) and mdr1a(+/+) mice via retrodialysis and the dynamic-no-net-flux method to correct the microdialysis concentration for in vivo recovery. The results from the two approaches showed similar higher morphine levels in the extracellular fluid of mdr1a(-/-) mice than that of mdr1a(+/+) mice. After an i.v. bolus injection, the total brain distribution of morphine was 1.7-fold higher in P-gp deficient mice [97]. Thus, we could make initial determination that morphine is a good P-gp substrate. Furthermore, the binding affinity of morphine in the brain is not affected by the P-gp activity [96].

M3G, structurally related with M6G but non-active, is also suspected as a P-gp substrate at the canalicular membrane and renal tubule which are the sites for the conjugation formation [175]. Letrent S. et al [175] characterized the brain disposition of morphine and M3G. P-gp inhibitor, GF-120918, was employed and subsequently effectively increased the amount of morphine reaching to the brain. As a consequence, the antinociception effect increased. Unbound M3G in the extracellular fluid was 2-fold higher in the presence of GF-120918 (AUC value); while the ratio of the AUC of unbound M3G from brain to blood was not markedly changed. In some other studies, similar results are not found. They doubted the role of M3G as P-gp substrate [96].

Not like M3G, M6G is an active metabolite of morphine with potent analgesic action. However, the BBB permeability for M6G is 7 to 8-fold lower than morphine [93]. There are some debates for the role of M6G in binding with P-gp. The use of different inhibitors causes

completely different effects on the transport of M6G in the CNS. Bourasset F. et al [92] and Lotsch J. et al [95] drew conclusions that the plasma pharmacokinetics of M6G influenced by PSC833 instead of probenecid (MRP1/2 inhibitors), suggesting MRP1/2 can be ruled off in M6G transport through BBB. Bourasset F. et al [92] evaluated the M6G transport mechanism through the BBB. Considering the comparatively low level of M6G in the plasm serum, it is not easy to determine the role of M6G. They set forth that neither P-gp nor Mrp1 participated in the transport process. Instead, GLUT1 and Oatp2 may be involved.

Morphine interaction with quinidine, rifampicin

The oral bioavailability of morphine is increased when co-administered with quinidine, suggesting that P-gp or CYP3A enzymes at the intestines or liver may play a role in the absorption phase of morphine. The precise mechanism is vague. On the other hand, quinidine has no influence on i.v. morphine miosis. Morphine has to reach its targets presented in the CNS. Hence the assessment of the quinidine concentration-effect correlation was implemented, but eventually no changes of morphine pharmacological effects were found. It is assumed that quinidine inhibited P-gp activity at the intestinal level but not at the cerebral level. This finding was in agreement with the interactions between quinidine and methadone [170]. Quinidine is not a potent P-gp inhibitor. Using quinidine as a probe, there still exist a number of facts that need to be further validated.

Besides quinidine, rifampicin, a rifamycin antibiotic, also makes the oral morphine bioavailability decreased since rifampin induces the expression of P-gp at intestines, liver and brain in mice [91/169]. In light of the original function of rifampicin for bacterial infections and Legionnaire’s disease, it inhibits RNA production of bacteria by binding with RNA polymerase and preventing RNA elongation. In addition, rifampicin is a well-known CYP450 enzyme system inducer. With co-administration of rifampicin, the dosing frequency of warfarin for patients who suffer from thromboembolism or stroke are significantly is reduced [200]. The inhibitory effect of

rifampicin on P-gp may be explained from the molecular mechanism of transcription or translation.

As a P-gp inhibitor, rifampicin resistance rises from mutations that change the binding affinity between rifampicin and the binding pocket. The safety and efficacy of rifampicin are worrying. Successive drug-resistance is not expected when treating another drug-resistance disorder.

5.4.2 Synthetic analgesics

Methadone (Dolophine®)

Methadone is a widely used detoxification treatment for decreasing the development of tolerance to opioids and bypassing the withdrawal effect. The oral bioavailability is pretty high around 70-80%. The peak concentration can be observed at approximately 2 hrs [179]. Methadone is highly metabolized by CYPs (3A4, 2B6, 2C8 and 2C9) to 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP). (R)-Methadone targets μ and δ receptors with binding affinity of 10- and 50-fold higher than (S)-methadone [166]. (R)-methadone is responsible for the majority of indications, e.g. analgesia, respiratory depression, sedation and hypotension [172].

NMDA antagonists are identified to be effective for opioid addiction and tolerance. Glutamate is one of main excitatory neurotransmitters within the CNS. Glutamate modulates the excitatory transmission and memory formation. (S)-methadone binds with NMDA (N-methyl-D-aspartate) instead of opioid receptors, inhibiting the uptake of serotonin, norepinephrine and glutamate [179].

Methadone has wide distribution throughout the body because of its lipophilicity (LogP=3.93). The large volume of distribution results in a longer half-life than morphine (LogP =0.89). Meanwhile, the inter-individual variability is remarkable caused by distinguishable pharmacokinetics and pharmacodynamics among patients [164].

Methadone is a P-gp substrate. Wang et al [166] compared the brain distribution of methadone in abcb1a(-/-) and abcb1a(+/+) mice. Methadone concentration in abcb1a(-/-) was 15- and 23-fold higher than abcb1a(+/+) mice for (R)- and (S)-methadone. These data suggested that both enantiomers of methadone were good P-gp substrates in cerebral level.

Methadone interactions with other different types of drugs (Quetiapine, paroxetine, quinidine, telaprevir and HARRT)

The drug-drug interactions with methadone are complicated to explain. Uehlinger C. et al [9] found that quetiapine significantly increases (R)-methadone brain-to-plasma concentration ratios rather than (S)-methadone. Much higher (R)-methadone concentration than (S)-methadone might contribute to this phenomenon. On the other hand, quetiapine partly inhibits with CYP enzymes or P-gp, leading to increased plasma concentration of (R)-methadone. The genotypes of ABCB1 and CYP isoforms also have influence on methadone plasma level.

In another study studying the association between (R)-methadone and paroxetine [10], paroxetine inhibits the methadone metabolism and induces an increased plasma level. Similar with quetiapine, paroxetine is not only a P-gp inhibitor but also a CYPs inhibitor. It is not clarified yet if either P-gp transport system or CYP metabolisms play the main role. It is expected to resolve this problem by knocking down the expression of P-gp or CYP enzymes.

P-gp is expressed at the intestines and brain microvessels simultaneously, regulating the absorption process and drug penetration into the brain. Many facts manifested that giving a definite conclusion is not correct with respect to P-gp in the different tissues. Kharasch E. et al [170] found that quinidine, a known P-gp inhibitor, was unable to change the pharmacologic effect of methadone when taken intravenously (not affected by P-gp located on the intestines). Plasma methadone concentration was significantly increased (decreased t_{max}, unchanged C_{max} and AUC) when intravenously taken with quinidine. It indicates that the P-gp indeed assisted methadone to penetrate into the brain. From the pharmacological perspective, the pupil diameter

was decreased when only taken methadone, which is an indicative that is caused by methadone. Furthermore, quinidine does not show any effects on methadone transfer to other compartments from bloodstream.

P-gp regulates the entry of methadone into brain. However, why does not quinidine have any obvious effect on absorption phase of methadone? As discussed above, P-gp is a tissue-specific protein. It is very likely that the interactions between P-gp and quinidine have fairly different mechanisms.

Telaprevir is a protease inhibitor that is used to treat chronic hepatitis C combined with peg-interferon alpha and ribavirin. Fudin J. et al [164] demonstrated that telaprevir significantly inhibited CYP3A4 and P-gp activity and then affected the metabolism of methadone. Co-administered with telaprevir, 29% and 36% decrements of (R)-and (S)-methadone concentrations in the serum were observed, respectively [172]. Fudin revealed that methadone dose adjustment is necessary after concomitant with telaprevir in order to maintain the therapeutic responses. This conclusion was contradictory with previous study. Methadone can be accumulated or excreted for 2-3 weeks. A short-term study cannot provide enough evidences of the effects of telaprevir on methadone.

It has to be mindful in clinical practice when co-administered with HAART (Highly Active AntiRetroviral Treatment) therapy. HARRT is a dosing strategy combining multiple antiretroviral drugs in order to control HIV infections. The complexity of this regimen raises the risk of potential of side effects, such as viral resistance. First HARRT was introduced by Hammer et al and Gulick et al in 1996. The combination of two nucleoside reverse transcriptase inhibitors(NRTI) and one protease inhibitor, indinavir, displayed remarkable benefits(60%-80% death decline) for AIDs patients in clinical.

At this point, the DDIs between HIV treatments (e.g. indinavir, lopinavir, ritonavir and atazanvir) and methadone are investigated since various signaling pathways are involved. They can independently contribute to DDIs or collaborate with each other, which make the

identification tough to determine. In the meanwhile, some new mechanisms of action are being evaluated. Roux P. [116] found the opioid substitution treatment is potent to sustain the adherence of HARRT in HIV-infected patients. Although they did not provide any explanation, it is very likely to relate with P-gp inhibitory effects.

Naloxone(Narcan®)/naltrexone(Vivitrol®)

Both naloxone and naltrexone are synthetic opioid antagonists. They are both used to reduce the risk of opioid overdoes or acute alcohol poisoning. They do not emphasize selectivity to the types of opioid receptors. Naloxone undergoes glucuro-conjugation to naloxone-3-glucuronide, while naltrexone is rapidly and extensively metabolized by dihydrodiol dehydrogenase to 6-beta-naltrexol and excreted via renal pathway [188]. Moreover, 6-beta-naltrexol instead of naltrexone plays a critical role in maintaining long duration of action in plasma. They have similar lipophilicity while naltrexone has a higher PKa than naloxone. Passive diffusion is the primary mechanism of transport across the epithelium cells. Naloxone has comparatively poor bioavailability (5 to 40%) compared with naltrexone (2%) [186]. Naltrexone is about 2-fold potent and long-lasting than naloxone [187]. Suboxone is a combination of naloxone and buprenorphine that is used to cure the opiate addition. Naloxone reverses the adverse effects of buprenorphine.

Although they are rapidly absorbed in the GI tract and relative easily cross the BBB, it is speculated that P-gp at the intestines or liver may limit the entry of naloxone to systemic circulation, since animal study indicated that naloxone is capable of interacting with P-gp at high concentrations(>100 uM)[189]. At low concentration ranging from 1 to 50 uM, using bidirectional transport study, Kanaan M. et al [186] reported that naloxone and naltrexone are P-gp independent. Neither influx nor efflux process of the two opioid antagonists reply on P-gp activity. A P-gp inhibitor, GF120918, was also used to double confirm the involvement of P-gp. This time, still no significant changes of efflux transport and absorptive influx were observed.

Suzuki T. [198] investigated whether P-gp at the BBB takes part in the naloxone transport. The elimination of isotope labeled naloxone is not affected by addition of P-gp inhibitors (quinidine, verapamil, vinblastine and vincristine) in rat model. Thus naloxone and naltrexone do not directly contact with P-gp. I say in this way because naloxone exhibits inhibitory effect on P-gp phosphorylation in another study [190]. Collectively, naloxone may have interaction with P-gp through very weak binding affinity.

Dronabinol (Marinol®)

Dronabinol contains its synthetic substance of delta-9-tetrahydrocannabinol (Δ9-THC), one of psychoactive compositions in cannabis. Dronabinol is approved by FDA as an adjunctive therapy for weight loss in AIDS patients or antiemetic for patients who take chemotherapy. The target, cannabinoid receptor CB1 and CB2 are widely distributed throughout the body. The activation of CB1 and CB2 is linked with cardiovascular function, inflammatory pain, memory storage, immune function and emotion modulation.

Because of the rapid absorption and high metabolism, only approximately 20% parent compounds arrives systemic circulation. However, cannabinoids modulate ABC transporters by weakly stimulating ATPase hydrolysis and inhibiting Bcrp [204]. Additionally, both mice deficient P-gp and Bcrp expression displayed accumulated THC in blood and brain than wide-type mice. Absence of P-gp and Bcrp shortened the time of the THC peak concentration in the brain (1hr earlier) but delayed elimination time. And the incidence of THC-induced hypothermia is rising without the protection from these transporters. Thus THC is suspected to be a dual P-gp and Bcrp substrate [203]. Worth to mention, the affinity with P-gp is much lower than Bcrp. Yet Tournier N. et al disagreed and their in vitro bidirectional transport assay proposed THC was not transported by Bcrp. Instead, THC as well as methadone, buprenorphine, ibogaine inhibited Bcrp concentration-dependently [151].

THC-related drug-drug interactions are problematic for schizophrenia patients or other antipsychotic disorders. THC is considered as a competitive P-gp substrate that might block the efflux of risperidone or olanzapine in the brain causing excessive accumulation [108]. The cerebral THC level is probably influenced by other CNS agents.

5.4.3 Buprenorphine(Subutex®)/Norbuprenorphine

Buprenorphine is derived from thebaine with longer half-life than morphine. It is highly metabolized to norbuprenorphine and glucuronide-conjugates. Norbuprenorphine and glucuronide conjugates are active metabolites of buprenorphine with few antinociception and higher respiratory suppression. Buprenorphine-resistance does not easily develop because of the lack of affinity to delta opioid receptor.

Norbuprenorphine is a P-gp substrate in vitro and in vivo. Distinct models are applied, but the result is unity. Suzuki T. et al found that buprenorphine concentration in brain tissues were increased in the presence of cyclosporine A and quinidine by means of brain uptake/efflux techniques. The inhibition of cerebral P-gp is measured (32%-64%) [147]. They suggested P-gp must participate in the efflux transport of buprenorphine in rats. In mdr1a(-/-) and mdr1a(+/+) mice model, antinociception was assessed using hot-water tail-flick assay and respiratory depression was examined using whole-body plethysmography. Only the efflux ratio of norbuprenorphine in P-gp deficient mice is much higher than wide-type mice. The antinociception effects were increased correspondingly [144]. However, P-gp at the placentas is not involved in buprenorphine transport from maternal-to-fetal direction [149]. It is reasonable to speculate the activity of other transporters at the placentas that contributes to the efflux of buprenorphine. This conclusion conform the fact that P-gp is tissue-specific.

As a matter of fact, the role of P-gp interacting with buprenorphine is controversial. In 2009, a comparative study focusing on the binding affinity of methadone and buprenorphine was conducted. Since buprenorphine is used as opioid addition just like methadone. Results indicated

buprenorphine was not likely to be a P-gp substrate while methadone was able to stimulate ATPase hydrolysis as a P-gp inducer in vitro and methadone concentration increased in P-gp gene knockout mice in vivo. They convinced buprenorphine did not have any drug-drug interactions mediated by P-gp [150].

5.5 Anti-migraines

5.5.1 Triptans

Triptans are tryptamine-based compounds with the ability of alleviating migraine and headaches. However, triptans are abortive medication without any preventative efficacy. Specially, they are very effective in treating tension-type migraine. It is reported that triptan class of compounds have poor brain penetration characteristics compared with typical CNS marketed drugs [157]. Sumatriptan is the first approved triptan in 1991. Since then, ergotamines have been substituted by triptans. Most of migraines can be obtained without any prescription since its few side effects. The drug-drug interactions involved in triptan is compelling. For instance, monoamine oxidase inhibitors should not be taken with some triptans in the case of serotonin syndrome induction. Serotonin syndrome includes a series of symptoms (high body temperature, agitation, confusion, diarrhea and muscle rigidity) induced by serotonergic agents.

The pathology basis of migraine is unknown. “Neuropeptides excessive release” hypothesis is proposed. Trigeminovascular afferent nerves are activated leading increased neuropeptide release to stimulate the inflammatory reaction. The classic neuropeptide includes CGRP (Calcitonin gene-related peptide which is a peptide vasodilator with effects on nociception transmission), substance P and neurokinin A. As selective agonists with high affinity to 5-$HT_{1B/1D}$ receptor, they are prescribed for moderate to severe migraine attacks for patients who are non-responders for NSAIDs. 5-HT agonists binding with the receptor on the blood vessels results in vessel constriction and prevents the neuropeptide from releasing.

Eletriptan (Relpax®)

Eletriptan belongs to second-generation triptan drug. In rat, dog, and human models [153], eletriptan is demethylated by hepatic CYP3A4 to N-desmethyl-eletriptan, and then oxidized on the pyrrolidine ring. Eletriptan has superior effect than naratriptan, sumatriptan, and is equivalent to almotriptan, rizatriptan and zolmitriptan referring to the majority of efficacy parameters. Eletriptan is more economic than sumatriptan and well-tolerated as well [156].

Of great interest is the effect of P-gp on regulating the eletriptan uptake into brain due to the central mechanism of action [2]. Eletriptan is considered as the best P-gp substrate at the BBB among the triptan class drug. The transport of eletriptan across the LLC-PK1 cell (Epithelial-like pig kidney cells) monolayers was higher (over 10%) than other triptan drugs. Referring to basolateral-to-apical/apical-to-basolateral ratios of typical P-gp substrates, dexamethasone, ritonavir and verapamil in LLC-MDR and LLC-Mdr1a cells, eletriptan was shown to be ideal substrate (11 and 9.1 for MDR, Mdr1a expressing cells) of human and mouse P-gp in vitro [153]. On the other aspect, P-gp reduces the brain uptake of eletriptan by approximately 40-fold. Brain/plasma ratios were 13 and 0.3 in Mdr1ab(-/-) and Mdr1ab(+/+) mice, respectively.

In ATPase activity assay [154], eletriptan activates P-gp-mediated ATP hydrolysis approximately 2-fold, while sumatriptan slightly inhibits the release of inorganic phosphate by around 10%, indicating that they have different transport mechanisms even though they have similar chemical structures [154].

Intrinsic fluorescence approach is used to illustrate tertiary conformational change of multi-resistant protein [106]. Applying acrylamide quenching of P-gp tryptophan fluorescence with the drugs and a non-hydrolysable ATP analog, it is possible to recognize the sumatriptan-P-gp dimer that undergoes large conformational change and faces more steric hindrance in ATP hydrolysis and transport across the BBB in comparison with eletriptan [154].

5.5.2 Nonsteroidal anti-inflammatory drugs (NSAIDs)

NSAIDs are able to enhance the effectivity of anticancer drugs by inhibiting the P-gp function or expression [62/148]. These NSAIDs include diclofenac, curcumin, ibuprofen, NS-398, indomethacin and celecoxib etc. Many distinct pathways may be involved in the trigger mechanism, though P-gp inhibition is the most widely accepted explanation.

In Sanchezcovarrubias L.'s study, diclofenac was able to alter the permeability of BBB, ending up with altered morphine transport into the brain. Diclofenac treatment causes increased P-gp expression via western blot analysis. Rats suffering from PIP showed attenuated symptoms after taken diclofenac compared with saline control group without any NSAIDs treatments. Diclofenac has influence on pain and inflammation [88]. In order to avoid or extenuate drug-drug interactions, they suggested that it was critical to understand how the therapeutics agents are modulated to exert their ability to control the P-gp activity [88]. P-gp expression was threatened from pathological factor and pharmacotherapy, diclofenac. Peripheral inflammatory pain (PIP) resulting from λ-carrageenan stimulates P-gp expression and function at the BBB. Diclofenac pretreatment renders increased P-gp expression in the absence of PIP symptoms. So the direct association between improved PIP symptoms and P-gp expression at the BBB is being questioned. One possible explanation is that PIP may disrupt the integrity of BBB due to damaged TJ protein.

Angelini A. et al [145] found that three NSAIDs (curcumin, ibuprofen, NS-398) at their therapeutic concentration enhanced the doxorubicin resistance in uterine sarcoma cells (MES-SA/Dx-5). Increased cytotoxicity and apoptosis were witnessed compared with treated with doxorubicin alone. They suggested that NSAIDs and their derivatives were newer generation of chemosensitizers to improve the responses of anticancer drugs. But P-gp inhibition is not the only predicted mechanism. In another paper, activation of heat shock factor 1 (HSF1) with increased heat shock factor 1 was suspected to account for this result. Finally the autophagic and apoptotic mechanism system is induced leading to up-regulated cytotoxicity [148]. Ye CG et al [146] shed light on that indomethacin (one NSAID) and SC236 (one COX-2 inhibitor) are two potent chemosensitizers in hepatocellular carcinoma cell lines and the drug-resistant sub lines. Elevated

doxorubicin retention and decreased IC50 value in vitro agreed with the in vivo data. They boldly assumed the partially reversed P-gp expression was responsible for it since the P-gp and MRP1 is induced by doxorubicin treatment in HepG2 cells.

5.6 Other types of CNS drugs

5.6.1 Anti-Parkinson agents

Bromocriptine(Parlodel®)

Bromocriptine is an antiparkinsonian ergot, serving as dopamine receptor D_2 agonist in the CNS [155]. Bromocriptine is used to treat hyperprolactinemia, acromegalia and Parkinson's disease [50]. Bromocriptine stimulate D_2 dopamine receptor in the striatum to improve the symptoms of Parkinson's disease. On the other hand, hyperprolactinemia, a pituitary disorder, can be treated with bromocriptine by blocking the release of prolactin from the pituitary gland. It further lowers the growth hormone levels.

BCT has very poor brain permeability due to certain active transport mechanisms at the BBB, such as P-gp[161]. BCT is 10 times lower in the striatum than in the pituitary. The two regions are under and not under the protection of BBB, respectively [159]. Not only the drug distribution in the brain matters, but the metabolisms in the liver and/or kidneys also determine how much BCT and its metabolites can get into the circulatory system before it enters the CNS. In a clinic setting, the plasma level of BCT is pretty low since it undergoes extensive first pass effects when taken orally. BCT is metabolized by hepatic CYP3A and converts into two main metabolites via hydroxylation, reaching the peak plasma level at 1 to 2 hrs after dosing [158].

BCT is often administered with other medications. Co-administered drugs that modulate P-gp activity could first modify BCT cerebral concentration, and then modify the neurotoxicity [155]. BCT per se is also able to modulate P-gp expression, leading to increased or decreased cytotoxicity/resistance of drugs (e.g. vinblastine [50]) which are transported by P-gp. Furuya K.

et al [181] demonstrated that BCT was prone to induce the expression of P-gp as a result of increased MDR1 mRNA during transcription in H35 Reuber hepatoma cells.

In vitro and in vivo studies are not consistent and even contradictory. In vitro studies are performed on the basis of cellular level. The cells are mainly from liver, kidneys, and lung, reflecting the characteristics of P-gp located in these regions. I will discuss from two aspects.

BCT as P-gp reversing agent on tumor cells

BCT is identified as a good P-gp reversing agent. Orlowski S. et al [50] firstly tested BCT as a MDR-reversing agent vincristine in a MDR cell line, DC-3F/ADX. BCT had shown to inhibit verapamil-induced ATPase hydrolysis competitively (mutually exclusive interactions) and vinblastine-dependent P-gp activity non-competitively, respectively. Particularly, they found BCT and verapamil were in contact with P-gp by reversible equilibrium bindings, indicating that their binding sites on P-gp could be very close or even overlapped. At the same experiment conditions, BCT and progesterone binding with P-gp are mutually non-competitive. This phenomenon allows me to assume it is possible to speculate how BCT binds with P-gp by observing inhibition constant (Ki) and dependence curve of P-gp ATPase. Apparent affinity of BCT binding with P-gp is very likely dose-dependent. Higher concentration of BCT (60nM) did not show the ability to affect the vinblastine levels.

Shiraki N. et al [180] gave a comprehensive explanation on the reversing effect of BCT. The K562-DXR cell lines are MDR tumor cell line with resistance to anticancer drug, doxorubicin. Rho123 accumulation was increased in K562 cells at BCT concentration of 10 uM, while doxorubicin was totally stored in both types of cells under the treatment of 1uM BCT. They also compared the inhibitory effects of BCT with other common P-gp inhibitors, cyclosporine A and verapamil in L-MDR cells. BCT showed greater inhibitory effect than verapamil but less than cyclosporine A.

Additionally, the IC_{50} ratio of anticancer drugs (doxorubicin, vincristine, vinblastine, vinorelbine, etoposide) in L-MDR1 cells without or with BCT elucidated that BCT reversed the anticancer effect of these drugs in P-gp highly-expressing cell lines. Notably, the reversing effect of BCT on vinca alkaloids reached to 400 to 2000-folds suggesting affinities of vinca alkaloids with P-gp may be easily reduced when pre-treating with BCT. The ability of inhibition can reflect the binding affinity of drugs with P-gp to some extent. The reversing effect of BCT is specific for P-gp. In order to prove this, Shiraki et al performed another experiment in A549 cells (MRP expressing cells); BCT had few inhibitory effects on cytotoxicity of anticancer drugs.

Is BCT a P-gp substrate, inducer or inhibitor?

In CF1 mice model (wide type and knockout type), BCT was studied as P-gp substrate, inducer and inhibitor in 3 separated and independent experiments. BCT is transported by P-gp without any inducing or inhibitory effect on cerebral transfer of digoxin which is a well-known P-gp substrate and poorly metabolized and weakly bound with plasma proteins [155]. P-gp modulators such as digoxin, probenecid and PSC833 may interact with more than one transporter (e.g. oatp2) [94]. The drawback in this experiment is that they took rifampicin as standard P-gp inducer. Although the role of rifampicin is controversial, the inhibitory effect of rifampicin has reported in more literatures. They suggested that it was understandable that BCT acts as P-gp inhibitor in vitro model but identified as P-gp substrate in vivo. The differences between chemically and genetically inhibited mice were not fully described in those studies. In my opinion, the ability of chemical inhibition varies among inhibitors while genetic inhibition is complete and thorough. Though in vitro models they did not show any differences in both types, we still can predict that it could have discrepancy when using diverse P-gp inhibitors.

In summary, experimental materials, experiment conditions and the procedures of drug additions are very crucial and even can affect the role of BCT binding with P-gp. At this point, in vivo data seems more valuable with more clinical significances.

5.6.2 Depressants (sedative-hypnotic drugs)

In this class of CNS agents, the well-studied drugs includes barbiturates, benzodiazepine. Sedative-hypnotic drugs help the patients calm down, reflecting in suppressed respiration, inhibited brain activity, regulated sleep conditions or even coma and death if overdosing. Barbiturates are preferred to treat anxiety and sleep disorders; while benzodiazepines are often used to cure seizures and work as anesthetic during surgery. Relatively speaking, benzodiazepines are safer than barbiturates when higher effect is pursued. Withdrawal effects appeal to deep research. Combination with alcohol caused over-sedation, memory impairment and damaged motor coordination.

Barbiturates

Barbiturates such as phenobarbital are more common used for epilepsy or as anticonvulsants. Due to too many unexpected effects and risk of overdosing, barbiturates have been replaced by benzodiazepines. Since the AEDs part has illustrated this class of drug, I would not make elaborated description here.

Benzodiazepines

Benzodiazepines are a class of compounds with a benzene ring and a diazepine ring in the center. They are outstanding tranquilizers. They take place of barbiturates as hypnotics in clinic because of their less cytotoxicity even in overdose.

The interactions between bromazepam, chlordiazepoxide, diazepam and flurazepam and P-gp were assessed by Lima S. et al. They established an induced-fit mechanism to describe the benzodiazepine interactions with P-gp. Using acrylamide and iodide ion as quenchers to characterize the protein conformation status when binding event occur with benzodiazepine, they advocated that all of the four benzodiazepines lead to a conformational change of P-gp-ATP dimers. However their binding sites varied. Bromazepam and chlordiazepoxide have binding sites

situated at the protein membrane-surface interface and surface region, respectively, while diazepam binds with P-gp on the membrane region. The binding sites of flurazepam cannot be detected [106]. It has been borne out that either benzodiazepine or ATP causes conformational change of P-gp, depending on their binding order and binding site. It is confirmed the rationality of flippase model. Flippase model was put forward to illustrate substance extrusion process by ABC transporters. P-gp substrates bind with the hydrophobic pocket within the phospholipid bilayer. At the same time, 2 molecules of ATP bind with NBD region inducing a conformational change of the transporter. Eventually, an outward-facing shape was formed to eject substances to the extracellular environment, simultaneously preventing them from flowing back to the cell as well. The whole process is driven by energy supplements. ATP releases one inorganic phosphate and is converted to ADP, followed by separation of the NBD dimers [32].

Regarding to the role of flurazepam, Lima S. et al [105] found that it had inhibitory potential to P-gp by observing 80% increased accumulation of daunorubicin in human cancer cell line, KB-V1, with high content of expressed P-gp. The data from ATPase activity with a colorimetric assay is consistence with that of the anticancer uptake assay. Interestingly, the other three benzodiazepines (bromazepam 1.3-fold; chlordiazepoxide 1.9-fold; diazepam 1.2-fold increase of ATPase activity were obtained) were considered as P-gp activators. They did not reveal any interference to the daunorubicin transport across the cell membrane. Accordingly this is the first time to prove that anxiolytic drug is inclined to efficiently raise the concentration of anticancer drugs in tumor cells. It is questioned the viability of clinical application of flurazepam. Miwa B. and Garland W. [101] found that the required flurazepam levels to make success in enhancing daunorubicin accumulation in KB-V1 cells are far higher than that in the plasma. The primary obstacle for some potent chemosensitizers is that they have to stay in high plasma concentration in order to play a role. That is very unfavorable property for translating the in vitro findings into clinical practice. Undesired damage to other tissues occurs often.

Table 5 CNS drug-drug interactions mediated by P-gp

Type of drugs	Drug name	CNS targets	Log P	Drug-drug interactions	Method	P-gp	Citation
Antipsychotics	Domperidone	D2 antagonist	3.9	CsA	Clinical study	Substrate	[123]
				Piperine	Non-everted rat intestinal study	Substrate	[125]
				Quinidine grapefruit juice	Clinical study	Substrate	[52]
	Aripiprazole	5-HT/D2 antagonist	4.5		P-gp deficient mice model	Moderate substrate	[45][86]
					Double KO mice	Substrate	[43]
	Ziprasidone	5-HT/D2 antagonist	3.8		P-gp KO mice	Poor substrate	[45]
	Risperidone	5-HT/D2 antagonist	3.2		Mdr1a KO and wide-type mice	Substrate	[66]
	Quetiapine	5-HT/D2 antagonist	2.9	PSC833, CG918	Placentas perfusion study	Non-substrate	[137]
Antidepressants	Imipramine	Noradrenaline transporter inhibitor	4.5	Verapamil	Intracerebral microdialysis	Non-substrate	[160]
					Bidirectional transport assay	Substrate	[23][24]
	Desipramine	Noradrenaline/serotonin transporter inhibitor, 5-HT antagonist	4		Intracerebral microdialysis	Substrate	[160]
	Citalopram	Serotonin transporter inhibitor	3.5		Transport assay	Substrate	[23][31]
					P-gp KO mice model	Substrate	[142]

	Escitalopram	Serotonin transporter inhibitor	3.58		Clinical study	Substrate	[31]
					Clinical study; BBB	inhibitor	[141]
	Levomilnacipran	Noradrenaline/serotonin transporter inhibitor	1.42		In vivo, BBB	Substrate	[41]
	Vilazodone	Unknown	4.21		In vivo BBB	Substrate	[41]
	Vortioxetine	Serotonin transporter inhibitor, 5-HT antagonist	4.51		In vivo BBB	Non-substrate	[41]
	Sertraline	Serotonin transporter inhibitor	5	Digoxin	In vivo BBB BTB	Inhibitor	[11][25]
	paroxetine	Serotonin transporter inhibitor, 5-HT antagonist	3.1	Verapamil	In vivo	Inhibitor	[133]
	Duloxetine	Serotonin transporter inhibitor	4.7	Verapamil	In vivo	Inhibitor	[25][133]
	Fluoxetine	Serotonin transporter inhibitor	4.1	Paclitaxel	Cytotoxicity assay	Inhibitor	[143]
				Doxorubicin	Monolayer transport assay; In vivo	Inhibitor	[18][21]
	Nefazodone Trazodone	Serotonin transporter inhibitor, 5-HT antagonist	3.7	Rho123	Caco-2 monolayers	Inhibitor	[26]
Anti-epileptics	Phenobarbital	GABA potentiator	1.4	Verapamil	Micro-dialysis rat model	Substrate	[7]
				Cyclosporine A, ketonazole, dinitrophenol	In vitro, rBMECs cell lines	Substrate	[80]
	Carbamazepine	Sodium channel	2.45	Verapamil	Clinical study	Substrate	[100]

		inhibitor					
				Digoxin, midazolam, caffeine	Clinical study	Non-inducer	[42]
Analgesics	Morphine	Mu/Kappa/ Delta-type opioid agonist	0.9		P-gp deficient mice model; retrodialysis, dynamic-no-net-flux method	Substrate	[96][97][175]
				Rifampicin	Intestinal level	Substrate	[91][169]
	Methadone	Mu-type opioid agonist	4.1	Quinidine	Clinical study; BBB	Substrate	[170]
					P-gp KO mice; BBB	Substrate	[166]
				Telaprevir Quetiapine Paroxetine	Clinical study	Substrate	[164][9][10]
	Naloxone Naltrexone	Mu/Kappa/ Delta-type opioid agonist	1.47	GF120918	Bidirectional transport assay	None	[186]
				Quinidine, verapamil, vinblastine, vincristine	Rat model; BBB	None	[198]
	Tetrahydrocannabinol	Cannabinoid receptor 1 agonist	5.6		P-gp KO mice; intestinal level	Substrate	[203]
	Buprenorphine Norbuprenorphine	Kapp-type opioid antagonist/ Mu-type opioid partial agonist	4.5	Quinidine, CsA	Brain uptake/efflux techniques	Substrate	[147]
					P-gp KO mice	Substrate	[144]
				Methadone	ATPase assay; P-gp KO mice model	Non-substrate	[150]

Anti-migraines	Eletriptan	5-HT agonist	3.8		Monolayer transport assay; P-gp double KO mice	Substrate	[153]
					ATPase assay, intrinsic fluorescence method, acrylamide quenching	Substrate	[154]
	Diclofenac	Prostaglandin G/H synthase inhibitor	4.98	Morphine	Rat model; BBB	Inducer, inhibitor	[88]
	Curcumin ibuprofen NS-398	Prostaglandin G/H synthase inhibitor	N/A	Doxorubicin	MES-SA/Dx-5 cell lines	inhibitor	[145]
	Indomethacin	Prostaglandin G/H synthase 2 inhibitor	4.25	Doxorubicin	hepG2 cell lines	Inhibitor	[146]
Depressants	Flurazepam	GABA potentiator	3.81	Daunorubicin	Acrylamide and iodide ion quench assay; KB-V1 cells	Inhibitor	[105]
	Bromazepam Chlordiazepoxide Diazepam	N/A	N/A	Daunorubicin	Acrylamide and iodide ion quench assay; KB-V1 cells	Inducer/substrate	[105]
Other types	Bromocriptine	D2 agonist	3.5	Rifampicin, digoxin,	In vivo, CF1 mice model	Substrate	[159][155]
					In vitro, H35 cell lines	Inducer	[181]
				Vinblastine, verapamil	In vitro, DC-3F/ADX cell lines; ATPase assay	Inhibitor	[50]
				Rho123, doxorubicin; verapamil, CsA	In vitro, K562-DXR cell lines; L-MDR cells	inhibitor	[180]

CHAPTER 6

P-GP EXPRESSION AND FUNCTION

6.1 Diet

When it comes to the effects of food, undoubtedly, physicochemical factors of GI tracts make an influence on drug-drug interaction. Factors including pH, temperature, enzymes activities, and gastric motility contribute to the disparity between fasting and fed conditions. Furthermore, active transporters such as P-gp at the intestines are affected by altered GI environment. From the perspective of food, the challenge of studying food-drug interaction is to determine single active substance involved in food. In addition, food-drug interactions at the intestinal level are classified into reduced, delayed, increased and accelerated absorption types [192].

Deferme S. and Augustijns P. [192] summarized that some dietary constitutes including grapefruit, orange juice, garlic, strawberry, milk, piperine, mint, and apricot with inhibitory effects on P-gp. In vitro experiment with P-gp inhibitors (CsA, Rho123, and digoxin) is the most commonly designed strategy. They discussed flavonoids as another type of components due to its rich content in the meals and the wide array of constitutes. Not only increased bioavailability is observed when taken flavonoids, but the drug penetration into brain is sometimes influenced. For example, bioflavonoids affect vincristine entry into brain in a biphasic manner. Higher concentration leads to inhibited P-gp function, while low concentration up-regulates the P-gp activity [191].

Usually, Food-mediated P-gp expression and function is more complicated. The contradictory results are more commonly obtained. To the best knowledge, in vitro studies on food-drug interactions are preferred compared with in vivo or clinical study. Partly because the

concentration of active substance contained in food is not very high. Observed phenomenon is minimal.

6.2 Age and gender

Aging is a direct stressor to many neurodegenerative disorders. Simultaneously, aging is relevant to cell dysfunction, including a decline in P-gp function. Thus CNS disorders and P-gp dysfunction are believed to correlate with each other. Reduced P-gp function causes the toxic substance accumulation which may accelerate the aging process and deteriorate the intracerebral environment. Once the trigger conditions of certain disease are met, the incidence of disease greatly increased.

Gender disparity has been ascribed to dynamic interplay between drug transporter and metabolic enzymes. Bebawy M. and Chetty M. discussed that P-gp as an active mediator at the molecular level contributes to the gender difference in drug distribution and therapeutic outcome [152]. We believe that gender does play a role in MDR1 gene transcription or translation and P-gp expression or function, but there is not enough clinical data provided. One PET study aimed to explore potential different P-gp function in young, middle-aged and old groups with men and women, respectively [162]. The brain-to-plasma concentration ratios of PET tracer, (R)-[^{11}C]verapamil, were decreased with increased age but only for men group. As for women group, P-gp functions were very similar among three groups. This kind of difference is the most significant in young groups between male and female, yet gradually disappears in the middle-age and old groups. Gender difference is often neglected by clinical researchers. Hence the importance of it is not noticed and gender-related confusion is barely seen in the reports. In primate model [171], P-gp function is the most efficient for young monkeys. Overall, P-gp function presents a bell-shaped curve versus age. The difference curve between genders shows a downward trend with the increased age.

6.3 CNS disorders

Not only neurodegenerative disorders, but also other traumatic central nervous system diseases affect P-gp expression. With the development of modern diagnostic technology, we are able to detect the minor changes within the brain induced by abnormal signs and symptoms without any surgery. A large amount of PET studies shows P-gp function at the BBB decreases in the presence of Alzheimer's disease and Parkinson's disease while increases under the condition of epilepsy and schizophrenia [165].

Brain ischaemia

Basically, brain ischaemia is caused by inadequate blood supply that is required by metabolic needs in the brain. There is a large amount of inducing factors involved. The limited nutrients and oxygen is fatal damage to the brain tissue. MRI is the most frequent diagnosing technique for it. It is found that brain ischaemia gives rise to energy shortage for P-gp. Nevertheless direct evidences have not been found to show the correlation between brain ischaemia and P-gp. Other active transporters are also susceptible to the energy shortage. In the meanwhile, considering interference factors, the ATP contained in the microvessels may partly offset the insufficient energy that is required for P-gp basal activity [122].

An ex vivo study employed in-situ rat brain perfusion technique, the distribution of domperidone in the brain was evaluated in two cases: 1) pre-treatment with verapamil; 2) brain ischaemia. They provided new explanations for P-gp dysfunction, ATP depletion that caused by some brain diseases. The brain distribution of domperidone was increased 3-fold in the case of brain ischaemia. Particularly, they excluded the possibility of altered brain vascular volumes and ensured that the impaired pumps were responsible for increased permeability coefficient values.

Alzheimer's disease

The ability of efflux transporters for Aβ declines with aging even for health people [40]. P-gp expression is very likely age-related. Alzheimer's disease patients tend to have lower P-gp expression on the endothelial cells of hippocampal vessels than health individuals [42].

Conversely, Aβ accumulation suppresses the expression of P-gp and worsens the neurodegenerative process. That can be called a form of vicious circle between Aβ accumulation and P-gp dysfunction. P-gp impairment at the BBB is an important phenomenon in neurodegenerative disorders, including Alzheimer's disease or Parkinson's disease [114].

Huntington's disease (HD)

Huntington's disease is an inherited disease that causes brain cell death. The production of Huntingtin protein is harmful for all kinds of mechanisms in the brain, though less information are obtained for its effects on cell signaling, intracellular pathways, even the apoptosis mechanism. The only diagnosis for Huntington's disease is genetic testing to test the triplet repeats of CAG (cytosine- adenine-guanine). As far as I'm concerned, the potential of P-gp expel the Aβ is very likely to be extend to the Huntington's disease. Can Huntingtin protein or other neurotoxin involved in Huntington's disease be transported by P-gp? It is waiting to be solved. Kao Y. et al [134] evaluated the altered function of P-gp in brain capillaries in Huntington's disease. R6/2 HD transgenic mice containing human mutant Huntington protein gene have showed higher levels of P-gp. Under this condition, the concentrations of antipsychotics in brain extracellular fluid, such as risperidone and paliperidone, were extremely lower. When co-administered with IKK inhibitor and BMS-345541 which are two selective, ATP-competitive inhibitors of IκB kinase (An enzyme complex affecting cellular response to inflammation), P-gp mRNA level along with ATPase activity decreased, enhancing the availability of antipsychotics that are P-gp substrates.

Epileptic seizure

Seizure induced by epilepsy is caused by abnormally excessive or synchronous neuronal activity [206]. The most prevailing epilepsy, the temporal lobe epilepsy, is induced by the abnormally active hippocampus [104]. Elevated NKCC1 expression in the hippocampus caused

by dysfunction of GABA inhibitory pathway potentiates refractory epilepsy [196]. Abnormal function of adenosine kinase system may participate in the drug-resistant epilepsy [197]. Experimentally-induced seizures are caused by over-expressed P-gp at the BBB. Jing X. et al [194] demonstrated that combined action of epileptic seizures and drug effects caused overexpression of P-gp on the brain microvessels for patients with refractory epilepsy. Short-term phenobarbital treatment represented good anti-epilepsy action. During a long-term dosing regimen, phenobarbital did not exhibit any anti-epileptic effects with decreased concentration and overexpressed P-gp in hippocampus. Also, one paper published in 2008[195] mentioned the use of P-gp inhibitors may not always enhance the anti-epileptic effects of one certain AED. The effect of P-gp inhibitors following the increased P-gp expression is time-dependent.

6.4 Underlying molecular mechanisms

In this part, I will briefly introduce some mediators affecting P-gp expression at molecular level. They have same increasing trend with overexpressed P-gp in tumor cells. However, it is not clear yet what is cause and what is effect.

Mediator 1: hPXR

As a transcriptionally modulator of P-gp, human pregnane X receptor (hPXR) can up-regulate P-gp expression in previous study [167]. Rifampin and hyperforin are used, which are two hPXR ligands, as inducers to increase the expression of P-gp in mouse model. The underlying mechanism is a cause and effect between these hPXR ligands and hepatic CYP3A11 expression [168]. In this case, the brain uptake for methadone decreased by 70%, yet the plasma methadone concentration almost did not change. In addition, an assay testing the mouse tolerance to electrical stimuli with or without methadone was carried out. After dosing methadone for 35 min, the tolerance level to a stimulus was approximately 3 times greater than without dosing methadone. On the contrary, rifampicin did not lead to increased tolerance of electric stimuli. The control experiment aimed to prove the involvement of hPXR pathway. Rifampicin potentiated the

P-gp expression while the seizures occur. Thus methadone is inefficient to ease the degree of seizure. However, it is uncertain about the extent to P-gp expression as well as the pharmacodynamics effects on P-gp substrates. The efficiency of hPXR ligands to alter the permeability of BBB is dependent on the drug exposure level, ligand affinity with receptors and pharmacokinetics and pharmacodynamics of the drug compounds.

Mediator 2: CAR

As a member of nuclear receptor superfamily, constitutive androstane receptor (CAR) plays a role in drug metabolism and distribution, and cancer development [169]. Current progress on CAR stated a protein complex in the cytoplasm is formed to influence several intracellular signaling pathways when CAR is activated. CAR has been identified as inducer for CYP3A4 and CYP2B6, inhibitor for CYP1A1 and CYP1A2 [207]. CAR study has been extended to uridine diphosphate glucuronosyltransferase (UGT) isoforms, multidrug resistance proteins (P-gp), multidrug resistance-associated proteins (MRPs) and organic anion-transporting polypeptide 1(OATP1). A thorough understanding to functions of CAR is demanding in predicting drug-drug interactions and xenobiotic-metabolism.

CAR is able to sense the xenobiotics, coordinate several physiological and pathophysiological conditions [169]. Many AEDs lead to P-gp induction and CYPs activation via CAR pathway. CAR can be activated directly or indirectly by different cellular signals, such as p38 mitogen-activated protein kinase (MAPK), extracellular signal-regulated kinase (ERK), AMP activated protein kinase (AMPK) and epidermal growth factor (EGF) etc. As a sister receptor with PXR, they have overlapped substrate profile and share common characteristics. Substantial evidences indicate CAR behaves as a sensor activated via classical ligand binding and ligand-independent mechanism and up-regulating other protein transcription and translation process (e.g. ATP-driven xenobiotic efflux pump).

CAR has the potential to stimulate the P-gp, Mrp2, Bcrp expression at the BBB. Phenobarbital is recognized as a CAR activator and has the capability to upregulate P-gp expression through a long time exposure to the brain endothelial cells. This induction can be avoided by treating with protein phosphate 2A inhibitor or TCPOBOP (a mouse-specific CAR ligand). As a consequence, the permeability of BBB can be modulated by CAR ligands [208].

Mediator 3: GST-π proteins

Glutathione S-transferase π (GST-π) belongs to GSTs enzyme family that serves as a glutathione reducer by conjugating with hydrophobic or electrophilic substances. It is drug-resistant biomarker and a prognostic marker for cancers [209].

GST-π may regulate P-gp expression in multidrug resistant cell lines. This hypothesis is established on the fact that P-gp and GST-π protein expression is both overexpressed in resistant cells. As discussed previously, fluoxetine is a mild reversing agent for P-gp. Fluoxetine reverse the doxorubicin/paclitaxel resistance of breast cancer cells via inhibiting GST-π protein levels [143]. Similarly, in gallbladder cancer (GBC) tissue samples, P-gp and GST-π protein is higher than normal indicators (health individuals) and positively correlated with each other. Ma Q. et al [209] suggested detection of P-gp and GST-π protein levels in the small biliary ductules might be considered as a reliable prognostic marker for diagnosis of drug-resistant gallbladder cancer. Not only gallbladder cancer, but the same results also apply to the majority of leukemia and lung cancer [210].

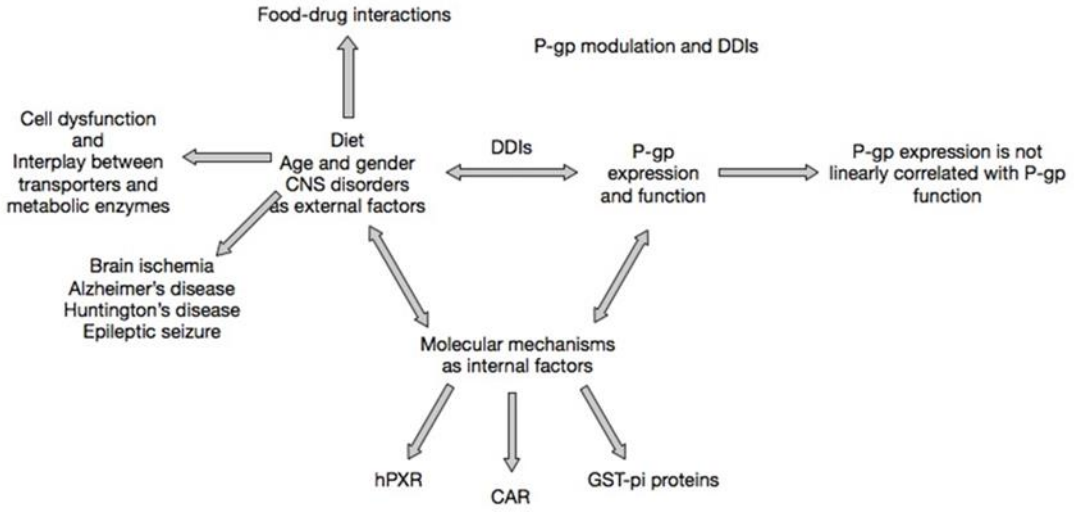

Figure 3 Factors that affect P-gp expression and function

6.5 Insights into P-gp expression and function

Increased P-gp expression does not correspond to improved P-gp function

From the perspective of PET, an interesting finding is that expressed P-gp does not always function well. In other words, P-gp expression is not always consistent with P-gp function in a linear manner. Syvanen S. And Ericsson J. [165] indicated that it is supposed to carry on more study about the P-gp function instead of P-gp expression when predicting drug penetration into brain. Strictly speaking, one important analysis of PET study using P-gp inhibitor as tracers has been applied on P-gp expression but not function.

A similar conclusion is obtained by Peer D. et al [28] about the effects of fluoxetine on increased MDR1 mRNA and elevated P-gp expression. Fluoxetine can monitor P-gp and Glutathione S-Transferase-pi (GST-π) levels, improving the capability of chemotherapy. The combination of fluoxetine and doxorubicin slows down tumor progression in vivo [28]. Based on the observed incomparable levels between MDR1 mRNA and P-gp, they proposed that high expression of P-gp is not necessarily caused by increased expression of MDR1 mRNA [143].

Significance of P-gp induction

Research on P-gp induction is not common. It can be attributed to two reasons. Firstly, P-gp inducer is rare compared with the considerable amount of P-gp substrates and inhibitors. Then in general, induction is unable to change the pharmacokinetics of drugs but give rise to observable changes of pharmacodynamics within the CNS. In most circumstances, the goal for P-gp induction is often target some toxic substances and expulse them out of body. P-gp induction at the BBB cannot be achieved in "genetically unchanged" mice by Kalvass et al [58]. In vivo experiment about P-gp induction is tough and ineffective to estimate the possible drug-drug interactions most of the time. But there is always an exception. Efflux of amyloid-beta accumulation in brain is the goal for treating Alzheimer's disease. Thus P-gp inducer for AD is a demanding research area.

5.8 P-gp reversing agents as new therapy

P-gp reversing agents are also known as P-gp inhibitors/modulators. The finding of P-gp inhibitor has a profound meaning in the research and development of new CNS drugs. Most inhibitor researches stop at the preclinical study. The drugs that can go to market must be clarify their safe therapeutic level in the plasma and brain in clinical settings. Unfortunately, this part of drugs is rare. Four-generation P-gp inhibitors are suitable for different experiment conditions, which also mean the limited possibility to be clinical-relevant.

Preclinical study including in vitro and in vivo experiment aims to investigate the P-gp expression and function, or drug-drug interactions by making use of P-gp inhibitors. When using the P-gp inhibitors such as verapamil, quinidine, cyclosporine A, and PSC833 etc., the restriction of intestines barrier or BBB can be overcome. The protective function of efflux pump may be destroyed, enhancing the elevated drug concentrations within the cells. If the goal for increasing the concentration for certain drugs can be achieved, the following study is driven to solve two

problems, “does increased drug concentration make clinically-relevant” and “how to bypass the side effects”.

Reversing effects is tissue-specific

P-gp is tissue-specific. P-gp reversing effect is also tissue-specific from substantial literatures investigating P-gp inhibitor and drug-drug interactions medicated by P-gp. The effects of P-gp inhibitors are not consistent in different models for methadone transport assay. Methadone can be transferred by P-gp located in the placental trophoblast tissue brush-border membranes. During pregnancy, the concentration of methadone in the fetal circulation is affected by the expression and activity of P-gp that partly determines the incidence and intensity of neonatal abstinence syndrome. The expression of P-gp in placentas varied between term placentas. P-gp inhibitor, GF120918, does not show pronounced reversing effects on methadone. Since methadone has better permeability even without taking any reversing agents. One report used colon adenocarcinoma cell line Caco-2 monolayers in a transwell model system and studied methadone transport across the intestinal epithelium. They found that verapamil did not affect methadone transport [16]. However, in the everted rat intestine model, verapamil increased the transfer of methadone by 60% [17]. It can be concluded that the used models are critical in methadone transport ability. The above experiments are experimentally-based results without any clinical data to confirm their conclusions.

Prodrug strategy for better brain penetration

Prodrug is one of strategies to bypass the extrusion of the efflux pump. In a very recent study [113], chemists made efforts to design a set of dimers that connects antipsychotics and P-gp substrate, quetiapine, with shortest tether length. These dimers were created as prodrugs of quetiapine. They are supposed to break down to monomer when crossing the BBB. In Rho123 transport assay, it is corroborated that these dimers are pharmacologically equivalent with the

monotherapy. Furthermore, they are more potent. After optimizing the structures of these dimers, they found that the addition of two blocking methyl groups manifested ideal stability and better inhibitory effects. Using immortalized human brain capillary endothelial cells (hCMEC/D3), dimer $QT_2C_2Me_2$ exhibited 100- and 20-fold more potent than the QT monomer in inhibiting the P-gp activity by measuring the concentration of fluorescent P-gp substrate Rho 123 and Calcein-AM with gradient concentrations of QT and its dimers.

With the aid of the new dimer, both antipsychotics and verapamil levels into the brain shows an obvious increase (20 to 30-fold increase than monomeric quetiapine). Moreover, they found the interactions between P-gp and the dimers were located in the H-and R-binding sites. However, the dimers are easily broken down by liver esterase which means they are probably not suitable to be taken orally.

The notion of precision medicine

Integrated the genetic, epigenetic, and behavioral information of patients, precise regimen is achievable to treat CNS disorders with drug-resistant characteristics. Pharmacogenetics has an increasing trend in studying resistant-type CNS disorders such as TRS. Up to now, it is facing challenges in many aspects. First of all, the genomic diagnosis is costly and susceptible to other confounding factors. Secondly, a life-long integrated health record is easily interrupted. Lastly, the clinical significance is limited. The deep mechanisms are studied widely in preclinical rather than clinical treatment. Thereby it is not surprising to see the influence of sample size on the conclusions [115].

CHAPTER 7

CONCLUSIONS

P-gp is the most commonly expressed at many physiological barriers, including the intestinal epithelial, hepatocytes, renal proximal tubular cells, the adrenal gland and endothelial capillaries of the brain comprising the blood-brain barrier, as well as being over-expressed in tumor cell lines[69/70]. P-gp is energy-dependent hydrophobic efflux protein. The expression of P-gp in many organs results in reduced drug penetration and enhanced extrusion [71]. According to the location of P-gp, it does not only affect the oral bioavailability, but also limits the drug penetration into brain. The study of P-gp should be conducted in a level-based manner. I focused on the DDI study for CNS drugs in three levels: a) P-gp on the intestinal epithelial/hepatocytes/ renal proximal tubular cells determining the oral bioavailability; b) P-gp on the BBB, BTB and placentas preventing the drug penetration from reaching the specific targets; c) P-gp expressing on the tumor cells increasing resistance of the tumor tissues in long-term treatment.

In this review, the practical significances manifest in: 1) characterize the involvement of P-gp in refractory CNS disorders; 2) identifying the role of CNS drugs interacting with P-gp in different models; 3) revealing unknown mechanism for enhanced drug resistance; 4) providing suggestions on CNS drug development when targeted P-gp.

A comprehensive understanding of DDIs prevents patients from experiencing drug dose dumping, withdrawal or intoxication [90]. Drug-resistant of CNS disorders includes two important factors, long term therapy and polypharmacy strategy. They can be regarded as external driving force of drug-drug interactions. In some cases, two or more drugs taken simultaneously are necessary due to symptom-directed therapies or special needs for specific population. Furthermore, special population needs more considerations. Elder people tend to have more

complications than young people, while it is inevitable to give special consideration when pregnant women are diagnosed with psychiatric or depression [98], since P-gp also situated on the placentas. However, in the first place, safety and efficacy should be taken into account when dose adjustment implements.

Both in vitro and in vivo models are necessary to fully understand the role of CNS compounds. Nevertheless they have preferences and blind zone. In vitro models identify the interactions between ligand and protein; while the ultimate determination of P-gp influence on drug ADE process (absorption, distribution and elimination) require the in vivo evaluation.

Meanwhile, the discovery of Bcrp changes the long standing role of P-gp as the sole efflux pump at the BBB. The cooperation between Bcrp and P-gp are well-studied. Bcrp displays overlapped substrate profile with P-gp. Thus dual substrates of them are largely affected by this kind of collaboration. Not only Bcrp, but also metabolic enzyme, CYP3A4, affected P-gp substrates. They are functional integrated and coordinately regulated in some instances, leading to a synergistic effect.

P-gp, serving as a promising target, should be attached enough attentions. P-gp inhibitors are co-administered to enhance antipsychotic, antidepressant, antiepileptic activity. P-gp inducers have potential to facilitate the clearance of amyloid beta from brain tissues. New opioids candidates without any P-gp affinity are investigated in order to overcome the analgesic tolerance. Prodrug strategy is aimed to maintain the original pharmacological but reduce the binding affinity with P-gp at the BBB. Although the proposed theory is facing many challenges, it is still a critical area for CNS drug development.

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