CHAPTER I
INTRODUCTION

1.1 Statement and significance of the problem

During the past five decades, the development and strategic use of anticancer drugs has become one of the most important ways of controlling malignant disease. However, the emergence of drug resistance has made many of the currently available chemotherapeutic agents ineffective. Many studies using tumor cell lines as model systems have demonstrated that exposure of cells to one drug often results in cross-resistance to many other structurally, chemically, and functionally distinct agents. This phenomenon is broadly known as the multidrug resistance (MDR) phenotype [1, 2].

One of the major mechanisms of MDR is the enhanced ability of tumor cells to actively efflux drugs, leading to a decrease in cellular drug accumulation below toxic levels. Active drug efflux is mediated by several members of the ATP-binding cassette (ABC) superfamily of membrane transporters which have now been subdivided into seven families designated A through G [3, 4]. Among these ABC families, the classical MDR is attributed to the elevated expression of ABCB1 (P-gp), ABCC1 (MRP1), and ABCG2 (BCRP or MXR) [5, 6].

ABCB1, or as it is more commonly referred to in the literature, P-gp, which is normally expressed in tumors derived from epithelial tissues including cancers of the kidney, liver, colon, and brain, has been associated with intrinsic drug resistance of these cancers [7]. Some other tumors (for example breast, ovarian and small cell lung cancers) exhibit generally low levels of P-gp expression at diagnosis. However, the P-gp expression can be induced during the course of treatment, causing the cancer to become resistant to anticancer drugs [7]. P-gp has been proven to responsible for the resistant to a variety of structurally and functionally unrelated antitumor drugs such as vinblastine, vincristine, doxorubicin, daunorubicin, etoposide, teniposide, paclitaxel and many others[1, 2, 4]. Attempts to predict P-gp-mediated transport of a substrate from its chemical structure have not been particularly successful. However, P-gp substrates in general appear to be lipophilic and amphiphatic [1, 8].

ABCC1, or MRP1, is broadly expressed in the epithelial cells of multiple tissues including the digestive, urogenital, and respiratory tracts, endocrine glands, and the hematopoietic system [9]. MRP1 expression has been demonstrated in multiple tumor tissues and has been implicated as a component of the MDR phenomenon in leukemia and cancers of the lung, colon, breast, bladder, and prostate. With regards to the role of MRP1 as a drug transporter, MRP1 has been shown to transport glutathione conjugates of several drugs, including alkylating agents as well as etoposide and doxorubicin. Additional agents in the MRP1 profile include the vinca alkaloids, methotrexate, and certain arsenic and antimonial centered oxyanions [10].

ABCG2, or MXR, is expressed the highest in the placenta, heart, ovary, and kidney, with lower levels of expression in the liver, colon, small intestine, prostate, and brain. In tumor cell lines, ABCG2 is expressed in breast, colon, stomach, myeloma, and fibrosarcoma cell lines and appears to mediate resistance to mitoxantrone, anthracyclines, topotecan and SN-38. However, sensitivity to cisplatin, paclitaxel, and vinca alkaloids appears to be retained [11, 12].

The clinical importance of P-gp, MRP1 and MXR for multidrug resistance and cancer treatment has led to the investigation of the inhibiting properties of several compounds on these transporters. The calcium channel blocking agent verapamil was the first drug described as an inhibitor of the P-gp efflux mechanism [13]. After this discovery, several other second generation compounds [14] have been studied for their inhibitory effects on P-gp (e.g. valspodar, GF120918, LY335979) [15, 16]. Although these agents are effective, one of the major problems with most of them is that the *in vivo* plasma concentrations required to inhibit P-gp are too high, and resulting in severe toxic side effects. For example, the effective dose of verapamil to inhibit P-gp function is 8-10 μM, but the highest steady state plasma level that can be achieved when using continuous infusion is less than 5 μM. Dose limiting toxicity includes cardiovascular problems, congestive heart failure, and hypertension [17, 18].

The development of inhibitory modulators for MRP1 is currently being explored. Many of them, including indomethacine, probenecid, and sulfinpyrazone, have been reported, but these inhibitors modulate the activity of many transporters,

including organic anion transporters that do not belong to the ABC superfamily. MK-571, the widely used LTD_4 receptor antagonist, ONO-1078, the peptide leukotriene receptor antagonist [19], LY475776 and LY402913 have also been reported to inhibit MRP1-mediated transport. Although numerous inhibitors [19] that inhibit MRP1 transport activity more directly have been described, the specificity of many of these compounds has not been defined. Moreover, there is little available information on these compounds that can readily be used for applications in animals and patients.

For the ABCG2, or MXR modulator, Rabindran et al. [20, 21] discovered earlier that FTC (Fumitremorgin C), a fumitremorgenic mycotoxin produced by the fungus *Aspergillus fumigatus*, effectively reversed drug resistance and increased cellular drug accumulation in MXR-expressing cells. FTC effectively inhibited MXR *in vitro* at concentrations (1-5μM), well below those toxic to cultured cells (IC_{50}> 80 μM), and had little effect on P-gp or MRP1 mediated drug resistance, making it very useful for cell pharmacological studies of MXR. However, its neurotoxic effects precluded any meaningful use in animals and patients. A series of FTC structural analogues (mostly pentacyclic diketopiperazines) was generated and analyzed [22], unfortunately they were frequently cytotoxic. Whether any of these analogues might be applied in animals or patients is unclear.

At present, due in part to the disappointing results associated with the many side effects of modulators that have been used in clinical trials, current research efforts are directed towards the identification of novel compounds with attention to dietary natural products or dietary herbs. The advantage is that these are dietary herbs exhibit little or virtually no side effects, and do not further increase the patient's medication burden. Moreover, many groups have studied the beneficial effects of natural products as holding anti- tumorigenic, anti-proliferative, anti-inflammatory as well as various immunological properties. The accumulative effect of these studies has convinced patients to compliment their conventional medication with dietary supplements.

Turmeric is composed of about 3-4% of curcuminoids, three major forms of curcuminoids; curcumin I, II and III have been reported [23]. All of them exhibit antioxidant, anti-inflammatory, and antiangiogenic properties [23-25].

In previous studies in the human cervical carcinoma cell line, KB-V-1 cells, curcumin in mixture form (which contains ~77% curcumin I, ~17% curcumin II, and ~3% curcumin III) significantly lowered MDR1 gene expression in a concentration-dependent manner [26] and increased rhodamine 123 accumulation in a concentration-dependent manner [27, 28], indicating curcumin in a mixture form can lead to a new reversal of P-gp. In this study researcher provided further details from a study on the purification of the three major curcuminoids and characterized their effect on P-gp, MRP1 and MXR function and expression.

To investigate the effect of curcuminoids on the ABC transporters function, researcher evaluated by the following criteria: (i) Ability to potentiate the cytotoxicity of anticancer drugs in P-gp, (ii) Ability to restore the intracellular accumulation of fluorescent substrates and (iii) Ability to modulate the MRP1 and MXR expression. ATPase assay and photoaffinity labeling was also carried out to gain additional biochemical information of the curcuminoids on the transporters.

Researcher hopes that the results presented in this dissertation will lead to an increase in the value of turmeric, which is a plentiful Thai medicinal herb, in cancer research. Also the information from this study will serve as strong additional supporting evidence to develop curcumin as an MDR modulator by using in combination with conventional chemotherapy to overcome MDR in cancer patients.

1.2 LITERATURE REVIEWS

1.2.1 How do tumor cells become resistance to anticancer drugs [29]

Possible resistance mechanisms to anticancer drugs have been review frequently, some of these mechanisms such as loss of a cell surface receptor or transporter for a drug, or alteration by mutation of the specific target of a drug. In such cases, use of multiple drugs with different mechanisms of entry into cells and different cellular targets allows for effective chemotherapy and high cure rates. However, cancer cells express mechanisms of resistance that confer simultaneous resistance to many different structurally and functionally unrelated drugs. This phenomenon, known as multidrug resistance, can result from changes that limit accumulation of drugs within cells by limiting uptake, enhancing efflux, or affecting membrane lipid composition such as ceramide. These changes block the programmed cell death (apoptosis) that is

activated by most anticancer drugs, activation of general response mechanism detoxify drugs and repair damage to DNA, and alterations in the cell cycle and checkpoints that render cells relatively resistance to the cytotoxic effects of drugs on cancer cells [2, 4, 8, 18, 30-32].

1.2.2 The ABC transporter family

The expression of ATP-dependent transporters, known as the ATP-binding cassette (ABC) family, is a major mechanism of multidrug resistance in cultured cancer cells [2, 8, 31].

The ABC transporter superfamily is among the largest and most broadly expressed protein superfamilies known. The vast majority of its members are responsible for the active transport of a wide variety of compounds across biological membranes, including phospholipids, ions, peptides, steroids, polysaccharides, amino acids, organic anions, bile acids, drugs, and other xenobiotics. In humans, 48 ABC genes that are organized into seven subfamilies (A–G) have been reported, as shown in Table 1. They play diverse biological roles in cells [2-4, 7, 8, 18, 29, 31, 33], and several of which have been involved in well-defined genetic disorders as indicated in Table 2.

Table 1. Human ABC transporters and their basic features [31].

Family	Member	Alias	Expression	Function
ABCA	ABCA1	ABC1	Ubiquitous	Removal of cholesterol and phospholipids onto HDL particles
	ABCA2	ABC2	Brain	
	ABCA3	ABC3, ABCC	Lung	
	ABCA4	ABCR	Rod photoreceptor	
	ABCA5		Muscle, heart, testes	
	ABCA6		Liver	
	ABCA7		Spleen, thymus	
	ABCA8		Ovary	
	ABCA9		Heart	
	ABCA10		Muscle, heart	

Table 1. Continue

Family	Member	Alias	Expression	Function
	ABCA11		Stomach	
	ABCA12		Low in all tissue	
ABCB	ABCB1	MDR, P-gp	Adrenal, kidney, brain	MDR
	ABCB2	TAP1	Ubiquitous, ER	Peptide transport
	ABCB3	TAP2	Ubiquitous, ER	Peptide transport
	ABCB4	P-gp3, MDR3	Liver	Phosphatidylcholine transport
	ABCB5		Ubiquitous	Iron transport
	ABCB6	MTABC3	Mitochondria	Heme transport
	ABCB7	ABC7	Mitochondria	Heme transport
	ABCB8	MABC1	Mitochondria	
	ABCB9		Heart, brain	
	ABCB10	MTABC2	Mitochondria	Bile salt transport
	ABCB11	SP-gp, BSEP	Liver	
ABCC	ABCC1	MRP1	Ubiquitous	Drug resistance
	ABCC2	MRP2,cMOAT	Liver	Organic anions transport
	ABCC3	MRP3,cMOAT2	Lung, intestine, liver	Drug resistance
	ABCC4	MRP4	Prostate	Nucleotide transport
	ABCC5	MRP5	Ubiquitous	Nucleotide transport
	ABCC6	MRP6	Kidney, liver	
	ABCC7	CFTR	Exocrine tissues	Chloride ion transport
	ABCC8	SUR	Pancreas	Sulfonylurea receptor
	ABCC9	SUR2	Heart, muscle	
	ABCC10	MRP7	Low in all tissue	
	ABCC11	MRP8	Low in all tissue	
	ABCC12	MRP9	Low in all tissue	
ABCD	ABCD1	ALD	Peroxisomes	
	ABCD2	ALD1,ALDR	Peroxisomes	

Table 1. Continue

Family	Member	Alias	Expression	Function
	ABCD3	PMP70, PXMP1	Peroxisomes	
	ABCD4	PMP69, P70R	Peroxisomes	
ABCE	ABCE1	OABP	Ovary, testes, spleen	Oligoadenylate-binding protein
ABCF	ABCF1	ABC50	Ubiquitous	
	ABCF2		Ubiquitous	
	ABCF3		Ubiquitous	
ABCG	ABCG1	ABC8	Ubiquitous	Cholesterol transport
	ABCG2	MXR,BCRP	Placenta, intestine	Drug resistance
	ABCG4	White2	Liver	
	ABCG5	Sterolin 1	Liver, intestine	Sterol transport
	ABCG8	Sterolin 2	Liver, intestine	Sterol transpot

Table 2. Human diseases associated with ABC transporter [34]

Disease condition	ABC transporters
Cancer	ABCB1(P-gp), ABCC1 (MRP1), ABCG2 (MXR)
Cystic fibrosis	ABCC7 (CFTR)
Stargardt disease and AMD	ABCA4 (ABCR)
Dubin-Johnson syndrome	ABCC2 (MRP2)
Pseudoxanthoma elasticum	ABCC6 (MRP6)
Persistent hypoglycemia of infancy	ABCC8 (SUR1), ABCC9 (SUR2)
Sideroblastic anemia and ataxia	ABCB7 (ABC7)
Adrenoleukodystrophy	ABCD1 (ALD)
Sitosterolemia	ABCG5, ABCG8
Immune deficiency	ABCB2 (Tap 1), ABCB3 (Tap2)

1.2.3 ABC transporter-mediated multidrug resistance in cancer cells

Cancer cells frequently have resistance or develop resistance to anticancer drugs during treatment. One form of drug resistance is observed against a variety of chemically unrelated agents and is known as multidrug resistance (MDR), a phenomenon that causes by overexpression of ABC proteins. Although 48 human ABC transporters has been reported, further studies have suggested that many ABC transporter have restricted substrate profile, and to date only 14 have been shown to transport chemotherapeutic drugs [35]. Among these, only three have been pursued as potential transporters responsible for the resistance in clinic [35]. These three are ABCB1 (P-gp/MDR-1), ABCC1 (MRP1) and ABCG2 (MXR).

1.2.3.1 ABCB1 or P-glycoprotein (P-gp)

Structure and function of P-gp [8]

ABCB1 or P-gp was the first ABC transporter described. It is the most extensively studied ABC transporter. The link between P-gp expression and cancer has evolved since the initial observation that Ehrlich ascites cells actively decreased their intracellular concentration of daunorubicin [36, 37], and subsequently P-gp was discovered in multidrug-resistant cells [38].

P-gp is encoded by *MDR1* gene which is located on chromosome 7 and encodes 1280 amino acid residues (170 kDa). The structure of P-gp has been proposed as a single polypeptide consisting of two homologous halves, each containing six transmembrane domains and an ATP binding site, separated by a flexible linker region (Figure 1).

The glycosylation sites of P-gp are most likely important for the proper trafficking of the P-gp to the cell surface; however, glycosylation is not required for the transport function [38].

The study of P-gp mutants has been reported to help elucidate the functional unit of the P-gp molecule. The mutation sites in mammalian P-gp that affect substrate specificity are predominantly in the transmembrane domains 4, 5, 6 and 10, 11, 12. However, they are also found throughout the rest of the molecule [8]. Replacement of nonfunctional regions to the last transmembrane region (TM12) markedly impaired resistance to actinomycin D, vincristine and doxorubicin but not to colchicine. In

contrast, replacement of the transmembrane loop between TM11 and TM12 appeared to create a more efficient drug pump for actinomycin D, colchicine and doxorubicin but not for vincristine [39]. The point mutations in either ATP binding domain abolish drug transport function suggesting that both nucleotide binding domains are essential for the proper function of P-gp [40-42]. Moreover, separate expression of cDNA encoding the carboxy-terminal half molecules of human MDR in Sf9 cells revealed that each half molecule had basal ATPase activity but drug stimulated ATPase activity was not present until the full-length human MDR1, was expressed [42].

Taken together, the two halves of human P-gp molecule are believed to interact to form a single transporter and the major drug binding domain residue in or near transmembrane domains 4-6 and 10-12. Both ATP binding sites are necessary for a functional molecule. In fact, interaction between the ATP binding sites and the drug binding domain is essential for drug transport. A wide variety of structurally unrelated compounds such as vinka alkaloids, anthracyclines and epipodophyllo-toxins are transported by P-gp, as shown in Table 3.

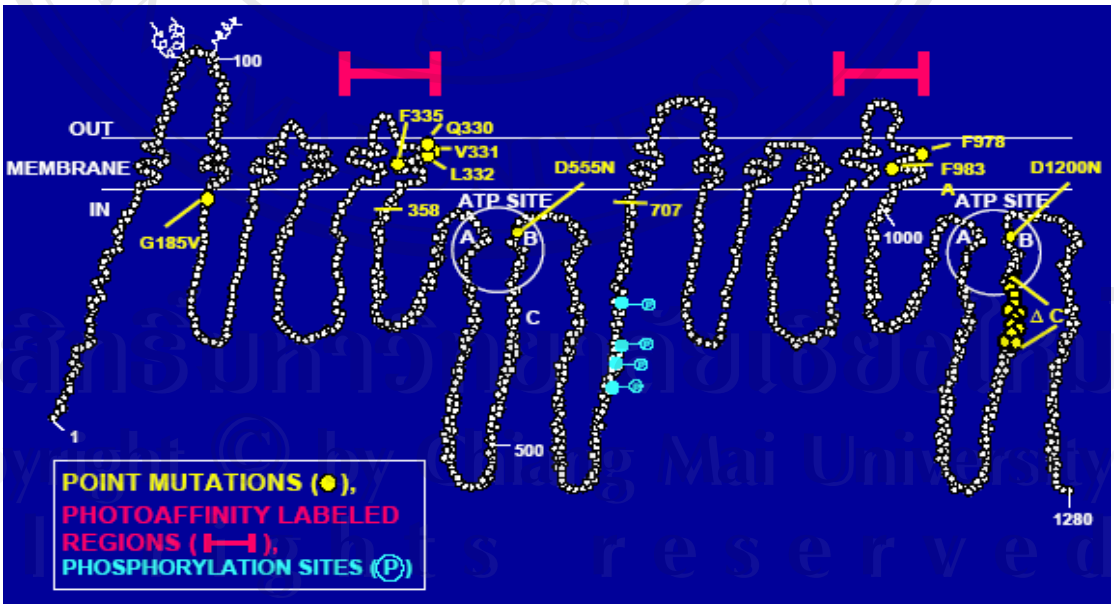


Figure 1. Two-dimensional hypothetical model of human P-gp structure [8]. The ATP binding domains are circled with the Walker A, B, and 'linker dodecpeptide' or 'signature sequence' (LSGGQ) motifs are designated by the letters 'A', 'B' and 'C'.

Putative glycosylation sites are represented by squiggly lines. The regions known to bind photoaffinity drug analogues are designated by bars, and serine residues that are known to be phosphorylated, are shown as circles with an attached an encircled “P”. Each circle represents an amino acid residue. The filled blue circles show many of the position of point mutations that change substrate specificity in human P-gp.

Table 3. Compounds which interact with P-gp [1]

Anticancer drugs	**Other cytotoxic agents**	**HIV protease inhibitors**	**Cyclic and linear peptides**
Doxorubicin	Colchicine	Ritonavir	GranicidinD
Teniposide	Puromycin	Indinavir	Valinomycin
Vinblastine	**Other compounds**	Saquinavir	
Vincristine	Rhodamine123		
Paclitaxel	Calcein AM		

Substrate binding sites for P-gp [8]

A direct approach to elucidate the regions of P-gp that interact with drugs is the use of photoaffinity analogues of drug substrates. This has been an active area of research for over a decade, allowing the drug substrate-binding regions of P-gp to be extensively mapped. Recently, using Edman sequencing of radiolabeled peptides [43], [^{125}I]iodoarylazidoprazosin (IAAP, prazosin analog) was found to bind to three sites on the hamster P-gp, amino acids 248–312 (TM4–TM5), 758–800 (beyond TM8), and 1160–1218, which are located within the second cytosolic nucleotide binding domain (NBD) [44]. A comprehensive summary of the localization of drug binding sites of P-gp from studies with photoaffinity analogues is given in Table 4. These data suggest that several of the transmembranes play a role in substrate binding, and that different substrates have different, but perhaps overlapping, binding sites on P-gp.

The mechanism of action of P-gp [8]

The most widely accepted model of P-gp action is as an active transporter using energy provided by ATP hydrolysis for the transmembrane translocation. This

model predicts the substrates (cytotoxic drugs) bind to the specific domain which subsequently undergoes an energy dependent conformational change allowing the substrate to be released [1]. Building of the vanadate (Vi) – induced trapping and chemical modifications at the ATP site, the catalytic cycle of ATP hydrolysis by P-gp have been proposed [5]. The essential features at the cycle are illustrated in Figure 2. The drug and ATP first bind to P-gp without energetic requirement. The prior binding of ATP is not essential for drug interaction with P-gp. Thus, ATP binding could precede, follow, or accompany the binding of drug. The hydrolysis of ATP is accompanied by a large conformational change that drastically reduces the affinity of both drug and nucleotide. Following hydrolysis, ADP is spontaneously released. The dissociation of ADP is accompanied by a conformational change that allows nucleotide binding but not substrate binding. A second ATP hydrolysis is initiated which is kinetically indistinguishable from the first. The subsequent release of ADP completes one catalytic cycle, bringing the P-gp molecule back to the original state where it can bind both substrate and nucleotide to initiate the next cycle.

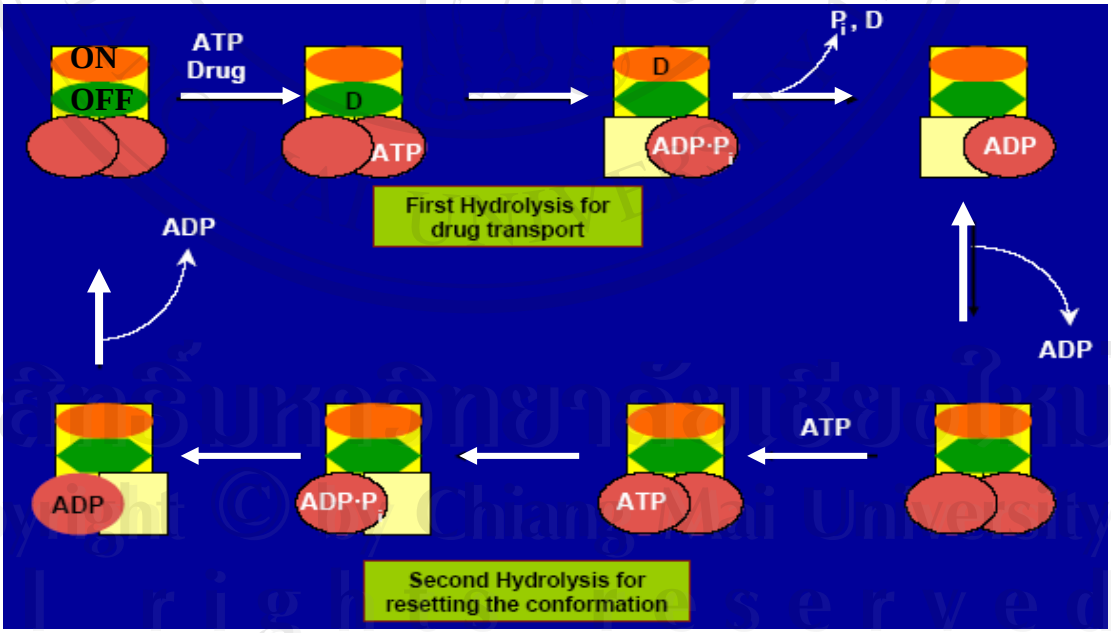


Figure 2. A proposed scheme for the catalytic cycle of ATP hydrolysis by P-gp [5]. The ellipses represent the substrate binding sites, the "ON" and the "OFF" site. The hexagon portrays the "ON" site with reduced affinity for the drug. Two circles

represent the ATP site and the circles are shown overlapping to indicate that both sites are required for ATP hydrolysis. The empty square represents the ATP site with reduced affinity for nucleotide

Table 4. Drug binding sites on P-gp identified using photoaffinity analogs [8]

P-gp source	**P-gp drug substrate**	**Photoaffinity analogue**	**Binding site (amino acid residue)**
Human	Azidopine	Azidopine	198-440 and 102-1028
Mouse	Paclitaxel	3'-p-Benzoyldihydrocinnamoyl	985-1088
		7'-p-Benzoyldihydrocinnamoy	683-760
Human	Dexniguldipine	Dixniguldipine-HCl	468-527
Human	Dexniguldipine	Dixniguldipine-HCl	468-527
Hamster	Cyclosporin A	Diazirine-cyclosporin	953-1007
Hamster	Daunorubicin	Iodomycin	230-312
Hamster	Azidopine	Iodoaryazidoprazosin	230-312
Hamster	Prazosin	Iodoaryazidoprazosin	248-312,758-800, and 1160-1218
Human	Prazosin	Iodoarylazidoprazosin	1135-1169

Physiological function of P-gp [8]

P-gp has a number of physiological functions; however, its main activity seems to be the protection of the body and the specific cell types from toxic agents. It is preferentially located on the apical membrane of epithelial cells of the intestine and renal proximal tubule, canalicular membrane of the hepatocytes and endothelial face of the blood brain barrier as illustrated in Figure 3.

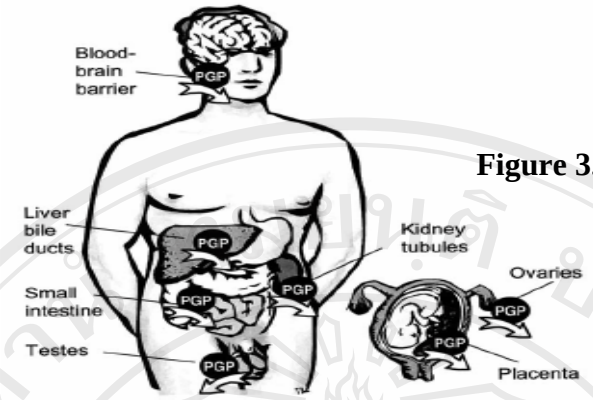


Figure 3. Tissue distribution of P-gp.

1.2.3.2 ABCC1 (Multidrug resistance protein (MRP1) [45, 46]

The MRP family entered the drug resistance scene in 1992 when Susan Cole and Roger Deeley cloned the multidrug resistance associated protein gene, now known as MRP1 and was classified to ABCC1 [47]. Since then 13 genes for ABCC family have been reported and designated ABCC1 to ABCC13. In 2002, Yabuuchi et al [48] reported that ABCC13 is predicted to encode a non-function protein. As a result, ABCC family contains only 12 functional proteins. The discovery of the MRP family has considerably broadened the study of MDR in tumor cells and has led to widespread interest in the possible function (s) of the members of this family in normal metabolism.

Structure and function of MRP1

MRP1 has an additional NH_2- terminal domain, MSD1, with five TMs and extracellular NH_2-terminus (Figure 4). It is predicted to contain three MSDs with 5+6+6 TM helices [10].

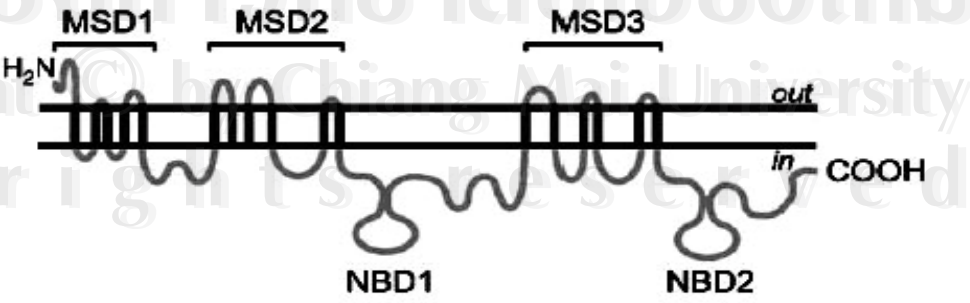


Figure 4. Predicted secondary structure of human MRP1. MSD, membrane spanning domain; NBD, nucleotide binding domain [49].

Similar to most of ABC transporters, MRP1 requires ATP-hydrolysis for its transport, the interaction of ATP with MRP1 was studied by photoaffinity labeling and vanadate-induced trapping experiments using ^{32}P-labeled 8-azido-ATP. Binding of azido-ATP was exclusively found at NBD1, while trapping of azido-ADP was predominantly observed at NBD2. The two NBDs show co-operatively in the binding and trapping of the nucleotide: trapping of ADP at NBD2 requires the presence of a functional NBD1 [50, 51], and binding of ATP at NBD1 was reduced when NBD2 was mutated. In addition, ADP (putatively bound to NBD2) stimulated specific binding of ATP at NBD1, suggesting an allosteric interaction between the two NBD's. Thus, although both NBDs seem to be functionally non-equivalent, they are interdependent. Mutation of the highly conserved lysine residues in the Walker A or B motifs from either NBD resulted in impaired binding and trapping of the nucleotide and reduced LTC4 transport [50, 51]. Remarkably, mutations in NBD2 were more effective in reduction of LTC4 transport than mutations in NBD1. This may indicate a more critical role for NBD2-mediated ATP hydrolysis for substrate transport [50, 51].

Mechanism of MRP1-mediated drug resistance [46, 52, 53]

Experiments with membrane vesicles from MRP1-overexpressing cells demonstrated that MRP1 is a transporter for the unmodified anticancer drugs vincristine and daunorubicin, but only in the presence of physiological amounts of glutathione (GSH) [54, 55]. These results extend the earlier observations that GSH is a critical factor in MRP1-mediated drug resistance. It has been proposed recently that MRP1 may interact with GSH by at least four different mechanisms as illustrated in Figure 6 [56]. First, GSH may be a direct low affinity substrate for MRP1 (Km~10 mM). Second, GSH is required for the co-transport of certain MRP1 substratres for examples in the case of daunorubicin, vincristine and aflatoxin (Km ~ 0.1 mM). Third, GSH stimulates the transport of certain compounds on MRP1, but itself is not transported, and, finally, the transport of GSH is accelerated by certain compounds that are not themselves substrate for MRP1.

Substrate spectrums of MRP1 are shown in Table 5. MRP1 transports a wide variety of substrates which include drugs conjugate with GSH (GS-X pump),

glucuronide and sulfate, and some anticancer drugs such as anthracyclines, vinca alkaloids and epipodophyllotoxin.

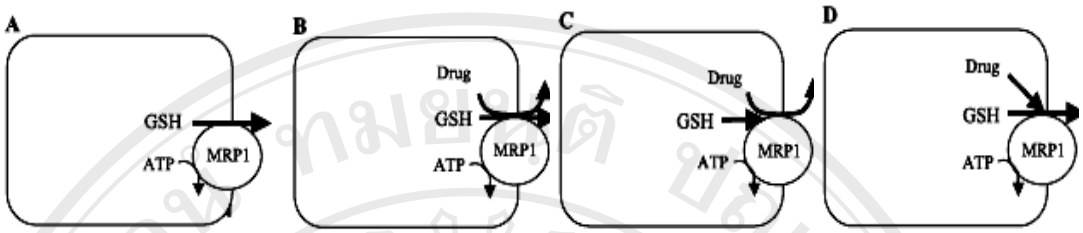


Figure 5. Proposed for a model that GSH is transported and co-transported by MRP1. A. GSH itself is substrate for MRP1, B. GSH acts as a cotransport compound with drug, C. GSH stimulates transport of certain compounds but itself is not co-transport, and D. GSH transport is accelerated by certain compounds that are themselves not transported by MRP1 [56]

Table 5. Substrate specificity of MRP1 [53].

Substrate	Efflux	Substrate	Resistance	Efflux
GSSG	+	***Anticancerdrug***		
GSH	+	Daunorubicin	+	
GSH+Vincristine	+	Daunorubicin + GSH		+
GSH+Verapamil		Epirubicin	+	
Glutathione S-conjugates		Etoposide	+	
LTC4	+	Vincristine	+	
LTD4	+	Vincristine + GSH		+
LTE4	+	Vinblastine	+	
N-acetyl-LTE4	+	Methotrexate	+	+
S-Glutathionyl PGA_1	+	***Heavy metal***		
S-Glutathionyl PGA_2	+	Sodium arsenite	+	
S-Glutathionyl ethaacrynic acid	+	Sodium arsenate	+	
S-Glutathionyl4- hydroxynonenal	+	Potassium antimony tartrate	+	
S-GlutathionylaflatoxinB1	+	Potassium antimonite	+	
Glucuronide conjugates		***Anions***		
Monoglucuronosyl bilirubin	+	Calcein		+
Bisglucuronosyl bilirubin	+	Fluo-3		+
17-beta glucuronosyl estradiol	+	Fluo-4		+

Physiological function of MRP1 [57]

It has been proposed that a physiological function of MRP1 is the extrusion of endogenously formed GSH-dependent detoxification products to prevent cellular damage. The generation of *Mrp1* -/- knockout mice has significantly contributed to the understanding of the physiological role of MRP1. Mice lacking Mrp1 show a poor response to inflammation induced by arachidonic acid, probably due to impaired export of LTC4 from LTC4-secreting cells. In addition, MRP1 plays a role in the protection against etoposide-induced damage in the testicular tubules, tongue, cheek, and the urinary collecting duct. Recently, the presence of MRP1 in mouse and rat choroids plexus (CP) was shown. It is located on the basolateral site of CP epithelial cells. Functionality of Mrp1 was strongly suggested by a rapid elimination of E2 17βG from the CP and by MK571-mediated inhibition of translocation of 99mTc-sestamibi through CP epithelial cells. Comparison of double (*Mdr1a/Mdr1b*) and triple (*Mdr1a/Mdr1b/Mrp1*) knockout mice clearly demonstrated that Mrp1 eliminates the anticancer drug etoposide from the CP. Thus Mrp1 appears to function also as part of the blood-cerebrospinal fluid barrier by preventing drug entry into the brain.

1.2.3.3 ABCG2 (MXR/BCRP/ACP)

Structure and function of ABCG2 [58]

ABCG2 was first cloned and sequenced from mitoxantrone-resistant S1-M1-80 human colon carcinoma cells and from MCF-7 AdrVp human breast cancer cells selected in doxorubicin (adriamycin) [59, 60]. This gene is designated ABCG2 by the new nomenclature system but is also referred to as BCRP (breast cancer resistance protein) [60], MXR (mitoxantrone resistance protein) [59], or ABCP (placenta-specific ABC transporter) [58].

The human *ABCG*2 gene is located on chromosome 4q22 and encodes a 655 amino acid polypeptide with a predicted molecular weight of 72 kDa. Therefore, ABCG2 is proposed to be a half-transporter, containing only one set of six transmembrane (TM) domains and one nucleotide binding site as shown in Figure 6.

Recently, it has been reported that amino acid 482 is an important determinant of substrate recognition by ABCG2 [12, 61-66]. For example, wild-type MXR with

an Arg at position 482 does not transport daunorubicin, rhodamine123, and lyso-tracker green; whereas, these compounds can be transported by mutants with a Thr (T) or Gly (G) at this position [67]. On the other hand, substances such as mitoxantrone, bodipy-prazosin, and hoechst 33342 are substrates of both wild-type MXR and the 2 mutants [65, 67]. Recently, Miwa *et al*. [68] generated a large number of mutants in the transmembrane segments and examined the effect of these amino acid substitutions on drug resistance conferred by ABCG2. They found that amino acid substitutions of Glu (E) at position 446, which is predicted to be located within or proximal to the TM2 of ABCG2, resulted in complete loss of drug resistance to SN-38 and mitoxantrone. Cells transfected with mutant ABCG2 cDNA with substitution of Asn (N) residue at position 557 to Asp (D) (N557D) exhibited comparable resistance to mitoxantrone but significantly reduced resistance to SN-38 relative to wild-type protein. Position 557 is predicted to be located within or proximal to the TM5 segment. These data again provided strong evidence that the drug binding sites are likely located in the MSD and; therefore, amino acids in or proximal to the TM segments are important for substrate recognition by ABCG2. Alternatively, amino acid substitutions in the TM segments may alter the substrate recognition and/or translocation pathway of the protein. The position 557 is a putative N-glycosylation site of ABCG2. Whether glycosylation is important for ABCG2 function is not known at the present time.

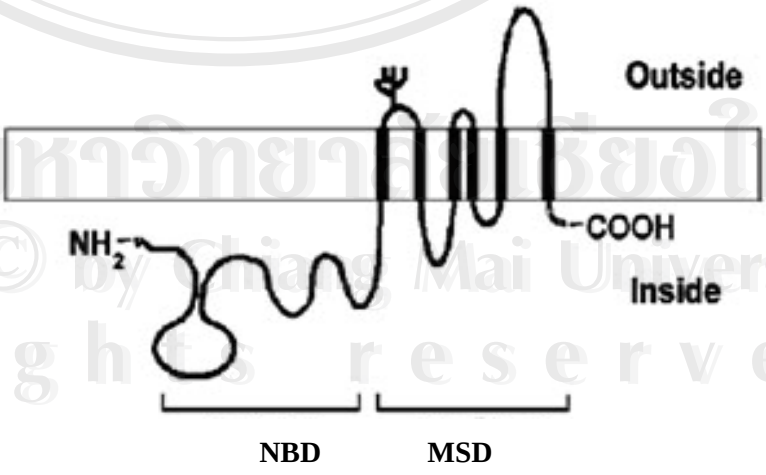


Figure 6. A predicted model for ABCG2. ABCG2 is predicted to contain one nucleotide binding domain (NBD) and one membrane-spanning domain (MSD) with

6 transmembrane (TM) helices. The putative N-glycosylation site (position 557) is predicted to be in the third extracellular loops as indicated in the Figure.

ABCG2-mediated drug resistance

ABCG2 confers resistance to several P-gp substrates such as mitoxantrone, the anthracyclines such as daunorubicin and doxorubicin, the camptothecins, bisantrene, topotecan, rhodamine123, prazosin and SN-38 [6, 59, 60, 69]. In contrast, ABCG2 does not efflux other known P-gp substrates such as taxol, colchicine, verapamil, vinblastine and calcein-AM, nor the MRP substrates calcein and glutathione-conjugated monochlorobimane [6]. Substrates of ABCG2 are summarized in Table 6.

Table 6. Substrates of ABCG2 [70]

Anthracyclines	**Camptothecin derivatives**	**Nucleoside analogs**	**Conjugates**
Daunorubicin Epirubicin Anthracene Mitoxantrone Bisantrane Aza-anthrapyarazole	SN-38 9-aminocamtothecin Irrenotecan Diflomotecan Topotecan	Zidovudine (ZVD) ZVD 5'-monophosphate Lamivudine (3TC)	Estrone-3-sulfate E3040G $E_2$17βG DNP-SG
Fluorophores	**Polyglutamates**	**Other drugs**	**Other molecules**
Bodipy-prazosin Hoechst 33342 Rhodamine123 Lysotracker Green	Methotrexate Methotrexate-Glu_2 Methotrexate-Glu_3	Prazosin Indolocarbazole Flavopiridol ErbB1 tyrosine kinase inhibitor (C11033) Imatinib mesylate (STI571)	Phosphatidylserine Pheophorbide α Protoporphyrin IX GV196771 PhIP

Physiological roles of ABCG2

ABCG2 is endogenously expressed at high levels in human placenta and to a lesser extent in liver, small intestine and colon, ovary, vein and capillary endothelia, kidney, adrenal and lung, with little to no expression in brain, heart, stomach, prostate, spleen and cervix [6, 58, 60, 62, 66, 71-73]. Based on its localization, it has been suggested that the physiological roles of ABCG2 may be to protect cells from potentially toxic substances and to prevent absorption of xenobiotics ingested in our diet by actively transporting compounds from cells.

In the placenta, ABCG2, along with P-gp and members of the MRP family, is thought to protect the fetus from xenobiotics by creating a maternal-fetal barrier. ABCG2 has been shown to be expressed at the cell surface in immature hematopoietic stem cells [72]. Although the function of ABCG2 in this cell type is not clear, several hypotheses have been offered: ABCG2 may simply be to protect sensitive stem cells from toxins [125] or may maintain immature cells or regulate stem cell differentiation, apoptosis or growth by actively removing regulatory lipophilic molecules from the cells.

1.2.4 Role of ABC transporters in clinical practice

P-gp is expressed in acute myelocytic leukemia (AML) cells in approximately 30% of patients at diagnosis and over 50% at relapse. A lower expression rate (17%) of P-gp is found in leukemic cells from patients less than 35 years of age, compared with rates of expression in the elderly (39%), however this may be explained partly by the better response to therapy seen in younger patients [74]. MRP1 and MXR are also found in AML[74], but MRP1 is more frequently found in chronic lymphocytic and prolymphocytic leukemia [75, 76]. Studies in solid tumors are more heterogeneous. A breast cancer meta-analysis concluded that P-gp expression could be detected in 41% of patients with breast cancer, with increased levels post therapy [77]. The expression of MRP1 in breast cancer is common, but may represent tumor contamination due to its presence in normal tissue. High levels of MRP1 were found in lung cancer, with incidences of approximately 80% and 100% in small cell lung cancer (SCLC) and non small cell lung cancer (NSCLC), respectively. P-gp expression was found in 25% of lung cancer samples. A low rate of expression of P-gp was found in ovarian cancer,

which could explain the apparent ineffectiveness of P-gp inhibitors in current studies in those tumors [78]. Several studies have evaluated P-gp expression in bladder cancer. In one study, P-gp was highly expressed in half of all normal urothelial samples. Surprisingly, low-grade tumors expressed P-gp infrequently, while high-grade tumors expressed P-gp in 27% of cases, with a mean MDR1 mRNA level twice that of low-grade tumors. This is in contrast to MRP expression, which was frequent (55%) in low-grade bladder tumors and infrequent (8%) in high-grade tumors [78]. In paired samples, both P-gp and MRP1 expression levels, measured using quantitative RT-PCR, were found to be greater post chemotherapy, with P-gp expression 5.7-fold greater and MRP1 expression 2.4-fold greater than in untreated patients [79].

ABCG2 expression has been detected in numerous types of human cancers, including hematological malignancies and solid tumors. ABCG2 expression was first reported in AML, with about 50% of AML patients exhibiting relatively high levels of ABCG2 [71]. Subsequently, several laboratories also reported ABCG2 expression in human leukemia patients, however; the association of ABCG2 expression with relapsed/refractory leukemia remains controversial [80-84]. Recently, 20 paired clinical samples from diagnosis and relapsed/refractory AML patients was examined [83]. The study reported that there was no consistent up regulation of ABCG2 protein expression or activity with relapsed/refractory AML.

ABCG2 expression in acute AML patients has been reported to correlate with cell viability in the presence of 250 nM flavopiridol and with apoptosis induced by flavopiridol; whereas, MDR1 mRNA level did not correlate with either flavopiridol toxicity or induction of apoptosis by flavopiridol [85]. This led to the conclusion that, unlike P-gp, ABCG2 may play a role in leukemia resistance to flavopiridol [85]. All of these studies suggest that ABCG2 may be involved in clinical drug resistance of some subgroups of leukemia patients. ABCG2 expression was also observed in other tumor types with apparently higher frequency in gastric carcinoma, hepatocellular carcinoma, endometrial carcinoma, colon cancer, small cell lung cancer, and melanoma [86].

1.2.5 Reversal of ABC drug transporter-mediated MDR by chemosensitization

There are essentially three basic ways to reverse MDR mediated by ABC transporters. First, inhibition of function at the protein level. Second, suppression at mRNA levels, and third, suppression of gene expression via transcriptional repression. However, by far most of the effort aimed at reversing MDR has focused on inhibiting ABC transporter function. These so called chemosensitizers or MDR modulators.

1.2.5.1 MDR modulators of ABCB1 (P-gp)

Some selected P-gp modulators are given in Table 7.

First generation MDR modulators

The history on the studies of MDR modulators began more than 2 decades ago with the discovery by Tsuruo and co-workers that calcium channel blocker, verapamil, can reverse MDR [87]. Later, it was reported that verapamil inhibits P-gp activity via direct competition with P-gp substrates [88]. Other first generation MDR modulators include the anti-malarial drug quinidine, the calmodulin antagonist trifluoperazine and the immunosuppressant cyclosporin A [89]. The cyclosporin A is proved to compete with P-gp substrates for binding to a common drug-binding site of P-gp [89]. There were promising results in phase I clinical trials with some of the first generation of MDR modulators, but most required high doses [90], and non-specific side effects were noted. As a result their clinical applications in cancer patients have been limited, and this has led to generation of so-called second and third generation MDR modulators.

Second generation MDR modulators

The second generation MDR modulators include dexverapamil, PSC 833, dexniguldipine, and VX-710. Among these, the most studies are with PSC833 and VX-710.

PSC833 (valspodar) is an analog of cyclosporin D, and the results to date suggest that PSC 833 acts as a non-competitive inhibitor by binding to site(s) other than the substrate-binding site to alter conformation of P-gp [91]. Numerous studies

have been reported for its clinical trials including phase III clinical trials. Although PSC 833 exhibited increased potency, and thus required lower doses to achieve effective *in vivo* plasma concentrations to modulate MDR, but it retained some properties that limit its clinical usefulness. For instance, PSC 833 was found to inhibit the cytochrome P450 3A4-mediated metabolism of paclitaxel and vinblastine, resulting in patients overexposing to increased serum concentrations of the cytotoxic drugs [92, 93].

VX-710 (biricodar) is an amido-ketopipecolinate derivative which has been shown to block both P-gp and MRP activity [94]. However, similar with PSC833, the use of VX-710 is limited by its unpredictable pharmacokinetic interactions with cytotoxic agents. For instance, study in patients with solid tumors showed that upon administration of VX-710, the clearance of paclitaxel is greatly decreased [95].

Third generation MDR modulators

Most of the third-generation MDR modulators have been developed based on structure-activity relationships and combinatorial chemistry, in the hope of overcoming limitations exhibited by the second-generation molecules [96]. The third generation MDR modulators, which are currently in clinical development, are LY335979, XR9576, laniquidar (R101933), GF120918 and ONT-093 [96]. Both LY335979 and XR9576 are amongst the most studied in this group of modulators.

LY335979 (zosuquidar) is a difluorocyclopropyl dibenzosuberane derivative [97] which is highly specific for P-gp and not a modulator for either MRP or ABCG2. The exact mode of action of LY335979 is unclear, although there is suggestion for non-competitive inhibitory mechanism, since the modulator is not a substrate for P-gp and cannot be transported by the ABC transporter [97]. In preliminary clinical studies, LY335979 has been shown to significantly enhance survival rate and reduce tumor mass of mice engrafted with MDR-bearing human tumors [97], besides; it had no effect on the pharmacokinetics of doxorubicin, etoposide, daunorubicin, vincristine, or paclitaxel [93].In clinical studies, it is still under investigation [93].

Another promising third generation molecule is XR9576 (tariquidar) [9]. It is believed that XR9576 acts in a non-competitive manner with P-gp substrates, such as vinblastine and paclitaxel [98]. Preliminary clinical studies, XR9576 showed no

effect on the pharmacokinetics of paclitaxel, vinorelbine, or doxorubicin when it was administered to patients with solid tumors [98], and appears to be well tolerable to patients at concentrations effective in inhibiting P-gp [99].

Table 7. Selected modulators of P-gp with ability to reverse MDR [100-102].

Calcium channel blockers R-verapamil (5-10 μM) Dexniguldpine (0.1-1 μM) Gallopamil (5 μM) Roll-2933 (2-6 μM) PAK-200 (5 μM)	**Cyclic peptides** Cyclosporin A (0.8-2 μM) SDZ PSC 833 (0.1-1 μM) SDZ 280-446 (0.1-1 μM) FK 506 (3 μM) Rapamycin (3 μM)
Calmodulin antagonists Trifluoperazine (3-5 μM) Fluphenazine (3 μM) Trans-flupenthixol (3 μM)	**Vinca alkaloids analogous** Vindoline (20-50 μM) Thaliblastine (2 μM)
Protein kinase C inhibitors Calphostin (250 nM) Staurosporine (200 nM) Safingol (20-50 μM)	**Miscellaneous compounds** GF120918 (0.02-0.1 μM) Tolyporphin (0.1-0.5 μM) Dipyridamole (5-10 μM)
Steroidal agents Progesterone (2 μM) Tamoxifen (2-10 μM) Toremifene (5-10 μM) Megestrol acetate (5 μM)	BIBW22 (1 μM) S9788 (1-3 μM) Terfenadine (3-6 μM) Reserpine (5 μM) Amiodarone (4 μM) Quinidine (10 μM) Methadone (75 μM)

Concentrations in parentheses are those shown to have effect in reversing MDR *in vitro.*

1.2.5.2 MDR modulators of ABCC1 (MRP1)

A variety of MRP1 inhibitors have been reported as shown in Table 8. For instance, LTD_4 analogue MK571, probenecid, cyclosporin A and PSC 833. These compounds are mostly not specific to MRP1 and they need to be used at relatively high concentrations to overcome the MDR mediated by MRP1 [103-108].

Table 8. Selected modulators of MRP1 [49].

General inhibitor of organic anion transport	Inhibitors of MRP –related transporters	Inhibitors of MRP1 and P-gp	GSH-dependent inhibitors of MRP1
Probenecid Sulfinpyrazone Indomethacine	MK-571 ONO-1078 Glibenclamide Some GSH conjugates	VX-710 Agosterol A PAK-104 Verapamil Cyclosporin A Genistein Quercetin	LY475776 LY402913

1.2.5.3 MDR modulators of ABCG2 (MXR)

A variety of MXR inhibitors have been identified (Table 9). It has been reported recently that [109], GF120918, a second-generation P-gp inhibitor is also a potent inhibitor of MXR. Various studies showed that GF120918 can be tolerated in human and animals at concentrations sufficient to inhibit MXR [110-112]. The natural product fumitremorgin C (FTC) secreted from the fungi *Aspergillus fumigatus* is another potent modulator of MXR that able to completely reverse mitoxantrone- and topotecan-resistance in MXR-overexpressing cells at 1 to 5 μM concentrations [113, 114]. Many studies [60, 67, 73] showed that this compound is highly specific to MXR and did not reverse P-gp- or MRP1-mediated drug resistance. Recently, several FTC analogs such as Ko132 and Ko134 have been developed [110]. These compounds could potentially be further developed as clinically useful MXR inhibitors because they were more potent than FTC, the IC_{50} are in the range of 85-270 nM. Several of the tyrosine kinase inhibitors for example CI1033 have been also shown to

be potent inhibitors of MXR inhibits MXR -mediated efflux of topotecan and SN-38 at low μM concentrations. Recently, HIV protease inhibitors ritonavir, saquinavir, and nelfinavir were also found to be effective inhibitors of MXR [115]. Collectively, although a large number of MXR inhibitors has been described, whether any of these compounds are clinically useful in reversing MXR -mediated multidrug resistance has yet to be determined.

Table 9. Selected modulators of MXR [70, 110]

Compound	Effective concentration	Comments
GF120918	IC_{50} ~ 50 nM	Potent P-gp inhibitor, well tolerated *in vivo*
FTC	1-5 μM	Low activity against P-gp and MRP1 Relatively specific inhibitor for MXR
		Neurotoxin
Ko132, Ko134	IC_{50} ~ 85-110 nM	Potent inhibitor for ABCG2
Cl1033	~ 3 μM	Tyrosine kinase inhibitor
Novobicin	50- 100 nM	Potent inhibitor for ABCG2
Reserpine	Used at 5 μM	Inhibit P-gp

Concentrations in parentheses are those shown to have effect in reversing MDR *in vitro.*

1.2.6 Turmeric

1.2.6.1 Botanical description and origin of turmeric

Turmeric is the yellow color powdered rhizome which was described as *Curcuma longa* by Linnaeus and the taxonomic position is as follows:

Class Liliopsida

Subclass Commelinids

Order Zingiberales

Family Zingiberaceae

Genus *Curcuma*

Species *Curcuma longa*

1.2.6.2 Chemical composition of turmeric

Turmeric contains protein (6.3%), fat (5.1%), minerals (3.5%), carbohydrates (69.4%), water (13.1%), essential oils (5.8%) and curcuminoids (3–4%). Three major forms of curcuminoids, curcumin I (diferuloylmethane), curcumin II (demethoxycurcumin) and curcumin III (bisdemethoxycurcumin) have been reported. Their chemical structures are shown in Figure 7, and some of their physicochemical properties are indicated in Table 10.

1.2.6.3 Actions and pharmacology of turmeric and curcuminoids

Consumption of turmeric and curcuminoids have been reported extensively with the association of beneficial effects on human health; prominent among them are anti-oxidant, anti-inflammatory and anticarcinogenic activities [25]. The antioxidant properties of curcuminoids are thought to be imparted by its β-diketone moiety, cleavage of C-C bond at the methylene carbon between two carbonyls in the β-diketone moiety, and the formation of relatively stable free radicals due to its extended conjugated double bond structure.

The possible anticarcinogenic activity of curcuminoids may be accounted by a few mechanisms. These include inhibition of angiogenesis, upregulation of apoptosis, interference with certain signal transduction pathways that are critical for cell growth and proliferation, inhibition of colonic mucosa cyclooxygenase (COX) and lipooxygenase (LOX) activities and inhibition of farnesyl protein transferase [116]. In addition to its possible activity in preventing malignant transformtation and inhibiting tumor growth, curcuminoids have been found to inhibit matrix metalloproteinase-9 in a human hepatocellular carcinoma cell line [117-120]. The possible anticarcinogenic activity of curcuminoids may be attributes, at least in part, to their ability to inhibit activation of the transcription factors NF-kappa B and AP-1. Curcuminoids have been also been found to target the fibroblast growth factor-2 (FGF-2) angiogenic signaling pathway and inhibit expression of gelatinase B in the angiogenic process. The proposed mechanisms of curcuminoids as illustrated in Figure 8 [121]**.** Extracellular growth factors, cytokines, or tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) binds to membrane receptors, such as epidermal growth factor receptor (EGFR), tumor necrosis factor receptor (TNFR), or protein kinase C (PKC), resulting

in the activation of a number of serine, threonine or tyrosine kinases, which include ras, NFκB inducing kinase (NIK), mitogen-activated protein kinase (MAPK), extracellular response kinase (ERK), MAPK/ERK kinase kinase (MEKK1), IκB kinase (IKK) and c-jun N-terminal kinase (JNK). JNK is activated by MAPK kinase (MKK4), causing activation of the c-jun protein which forms a heterodimer with the c-fos protein thus enhancing the activity of the transcription factor AP-1. Recent studies indicated that both IKK and PKC are important for activation of NFκB which leads to enhancement of the expression of c-myc, iNOS and other cellular proliferation genes. Reactive oxygen species (ROS) are considered to be endogenous mitogenic factors (or apoptotic factors under certain conditions) that can activate NFκB and other transcription factors in the nucleus. Ultimately, activation of the MAPK family members causes activation of specific transcription factors, such as NFκB, AP-1, serum response factor (SRF) and others which help determine the fate of cell such as proliferation, carcinogenesis, inflammation or apoptosis. Curcuminoid(s) (Cur) has been demonstrated to block several sites of these multiple signal transduction pathways as indicated by the blockade symbol in Figure 8.

1.2.6.4 Pharmacokinetic studies of curcuminoids

The pharmacokinetics of the curcuminoids remain incompletely understood. Of the curcuminoids, curcumin (curcumin I) has been most studied, mainly in animals [25, 121]. Curcumin is poorly absorbed following ingestion in mice and rats. In these animals, 38 to 75% of an ingested dose is excreted direcly in feces [23]. In mice, curcumin was first bio-transformed to dihydrocurcumin and tetrahydrocurcumin, and that these compounds subsequently were converted to monoglucuronide conjugates (Figure 9) [122]. It has been proposed that, the major metabolites of curcumin are curcumin glucuronide, dihydrocurcumin glucuronide, tetrahydrocurcumin glucuronide and tetrahydrocurcumin [122]. These metabolites are formed in liver. Animal studies and the pharmacokinetics of curcumin are continuing.

Human pharmacokinetic studies are mostly still under investigation. Recently [123], Upon taken orally of curcumin for 3 months with starting dose at 500 mg/day. If no toxicity was noted, the dose was escalated to 1,000, 2,000, 4,000, 8,000, or 12,000 mg/day in order. It was reported that [123], there was no treatment-related

toxicity for doses up to 8000 mg/day. Beyond 8000 mg/day, the bulky volume of the drug was unacceptable. The serum concentration of curcumin usually peaked at 1 to 2 h after oral intake of curcumin and gradually declined within 12 h. The average peak serum concentrations after taking 4000 mg, 6000 mg, and 8000 mg of curcumin were 0.51 ± 0.11 μ*M*, 0.63 ± 0.06 μ*M*, and 1.77 ± 1.87 μ*M*, respectively. Urinary excretion of curcumin was undetectable.

1.2.6.5 Safety evaluation with turmeric and curcumin

The major findings on the safety evaluation of turmeric and curcumin are presented as following;

Turmeric

The average intake of turmeric from 0.5 to 1.5 g/day/person produces no toxic symptoms [124]. Male and female Wistar rats, guinea pigs and monkeys were fed with turmeric at much higher doses (2.5 g/kg body wt) than normally consumed by humans. No changes were observed in the appearance and weight of kidney, liver and heart. Also, no pathological or behavioural abnormalities were noticed and no mortality was observed [125].

Curcumin

Curcumin was given to Wistar rats, guinea pigs and monkeys at a dose of 300 mg/kg body wt. No pathological, behavioural abnormalities or lethality was observed [126]. No adverse effects were observed on both growth and the level of erythrocytes, leucocytes, blood constituents such as haemoglobin, total serum protein, alkaline phosphatase, etc.[126]. Human clinical trials also indicate that curcumin I has no toxicity when administered at doses of 1–8 g/day and 10 g/day [25].

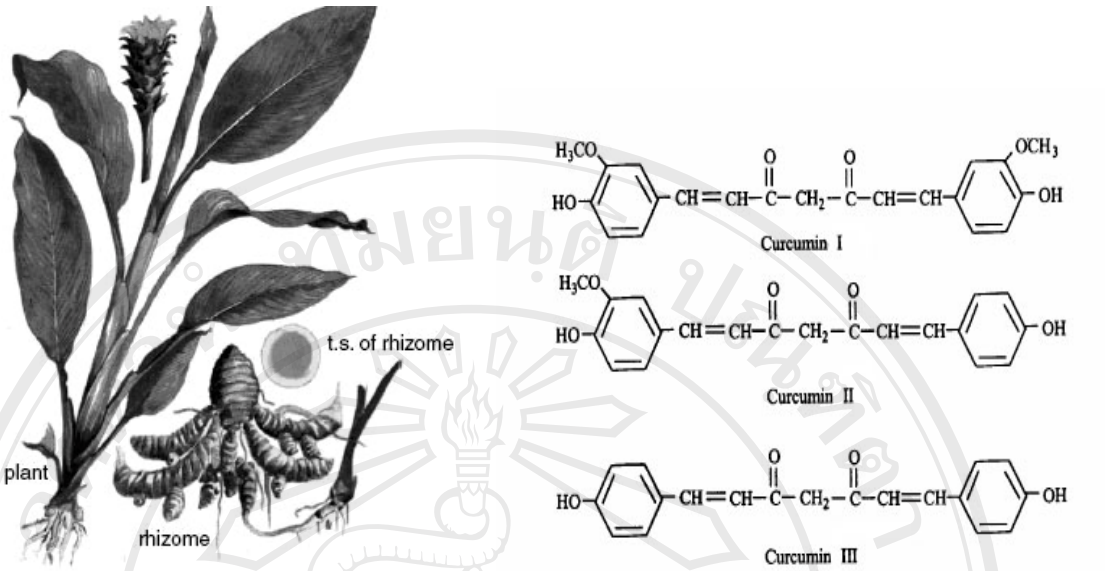
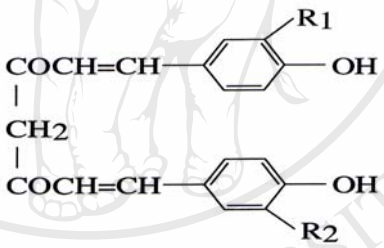


Figure 7. Turmeric and chemical structures of curcuminoids.

Table 10. Physicochemical properties of curcuminoids [127]



R1, R1	**OCH_2, OCH_3**	**H, OCH_3**	**H, H**
Chemical name	Di-cinnamoyl (Feruloyl) methane	4-hydroxycinnamoyl (feruloyl) methane	Bis-4-hydroxycinnamoyl (feruloyl) methane
Common name	Curcumin, curcumin I	Demethoxycurcumin, Curcumin II	Bisdemethoxycur-cumin,CurcuminIII
Molecular weight	368.4	338.0	308.1
Melting point (^oC)	184-186	172.5-174.5	224
Absorption maxima λmax; (ε)	430 nm ; (54950) 268 nm ; (12300)	423 nm ; (51290) 251 nm ; (13490)	418 nm ; 37150 248 nm ; 11750

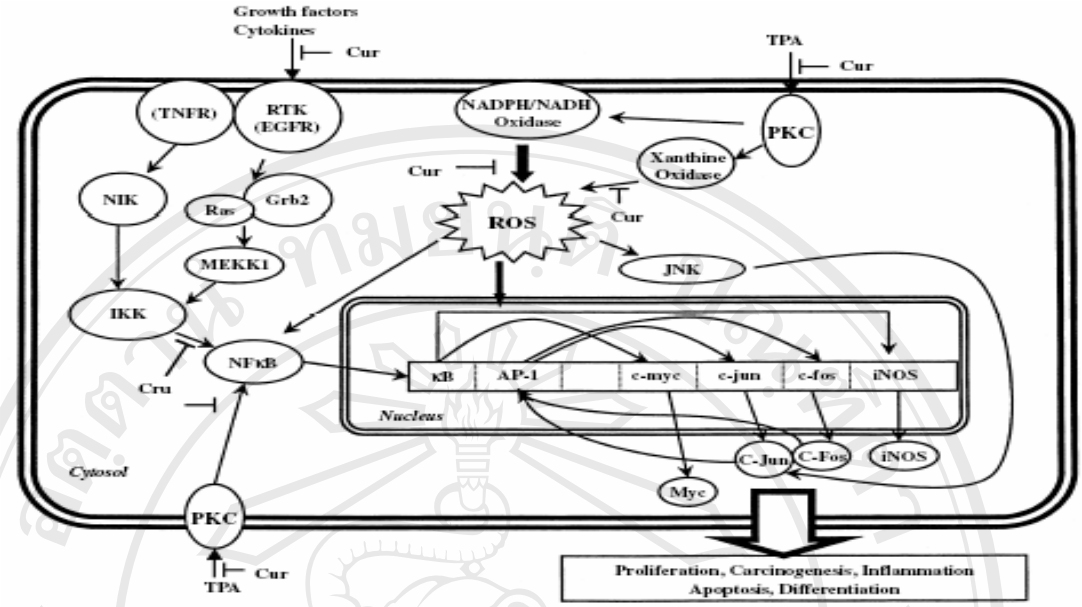


Figure 8. Proposed the mechanism of curcuminoids for their anti-oxidant, anti-inflammatory and cancer chemopreventive activity [116]. Curcuminoid(s) (Cur) has been demonstrated to inhibit several sites of signal transduction pathways as indicated by the blockade symbol.

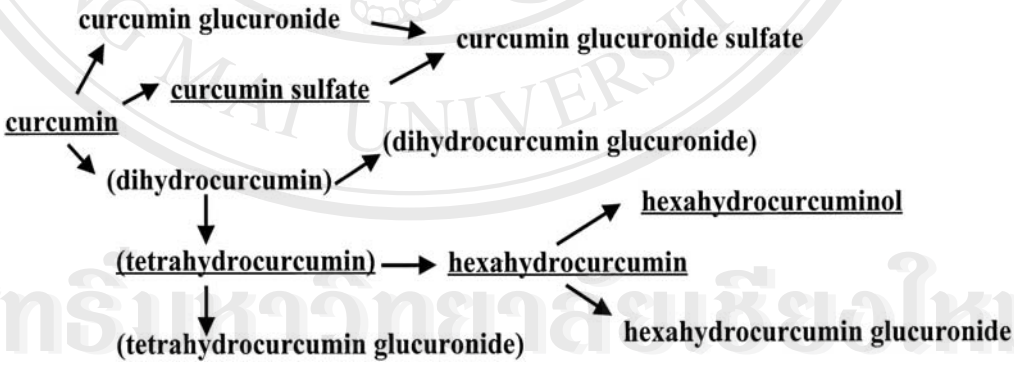


Figure 9. Biotransformation of curcumin I (curcumin) in mice proposed by Pan et al [122]. Major metabolites form of curcumin are curcumin-glucuronide, dihydro-curcumin-glucuronide, tetrahydrocurcumin-glucuronide, and tetrahydrocurcumin.

1.3 OBJECTIVES

To study the mechanism of action of curcuminoids purified from turmeric on ABC transporters P-gp, MRP1 and MXR- linked to the development of MDR in cancer cells.

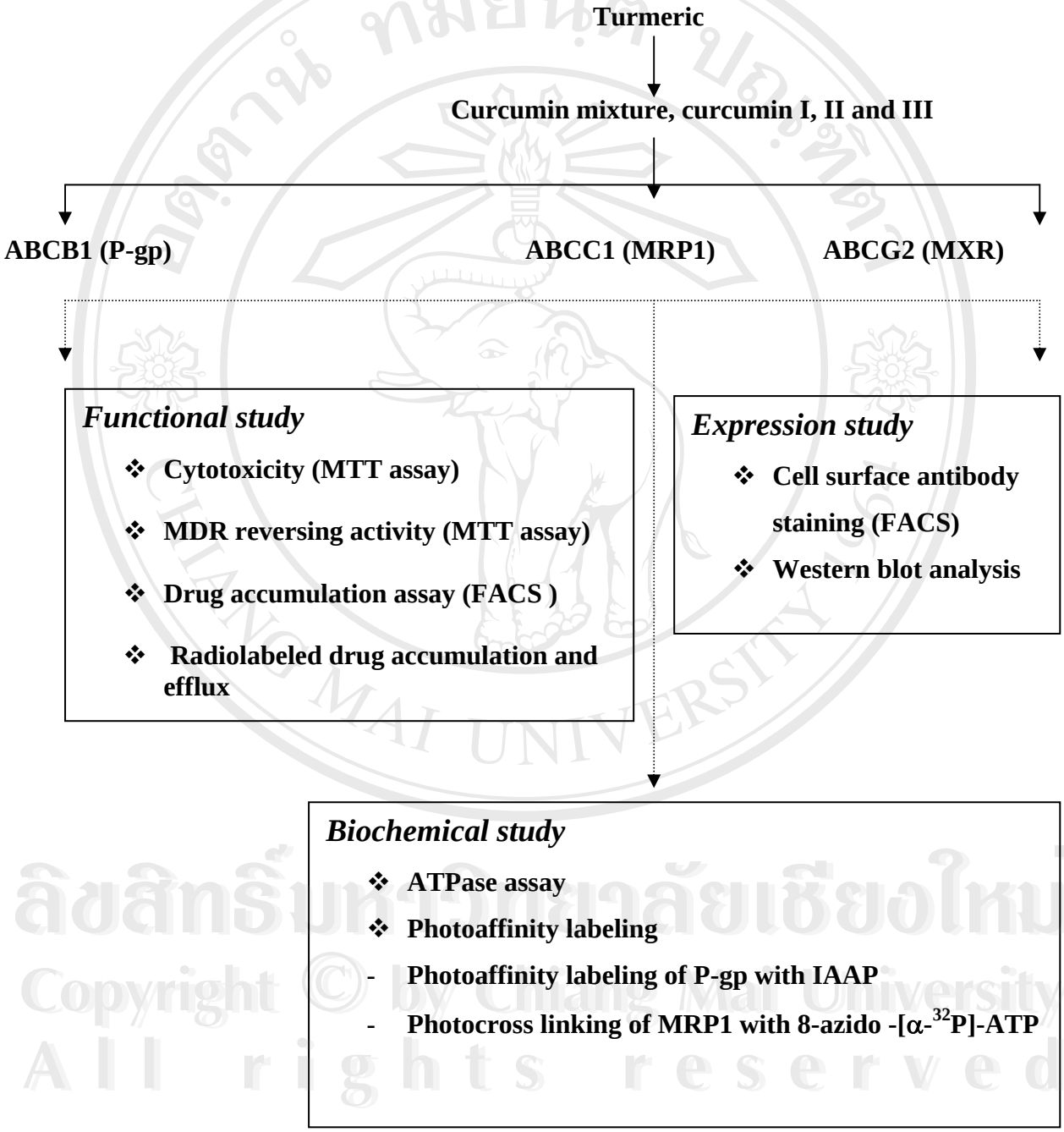


Figure 10. Outline of the experimental plan.