Review

Advances in Studies of P-Glycoprotein and Its Expression Regulators

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This review deals with recent advances in studies on P-glycoprotein (P-gp) and its expression regulators, focusing especially on our own research. Firstly, we describe findings demonstrating that the distribution of P-gp along the small intestine is heterogeneous, which explains why orally administered P-gp substrate drugs often show bimodal changes of plasma concentration. Secondly, we discuss the post-translational regulation of P-gp localization and function by the scaffold proteins ezrin, radixin and moesin (ERM proteins), together with recent reports indicating that tissue-specific differences in regulation by ERM proteins in normal tissues might be retained in corresponding cancerous tissues. Thirdly, we review evidence that P-gp activity is enhanced in the process of epithelial-to-mesenchymal transition (EMT), which is associated with cancer progression, without any increase in expression of P-gp mRNA. Finally, we describe two examples in which P-gp critically influences the brain distribution of drugs, i.e., oseltamivir, where low levels of P-gp associated with early development allow oseltamivir to enter the brain, potentially resulting in neuropsychiatric side effects in children, and cilnidipine, where impairment of P-gp function in ischemia allows cilnidipine to enter the ischemic brain, where it exerts a neuroprotective action.

Key words P-glycoprotein; bimodal change; membrane localization; ezrin, radixin and moesin (ERM) protein; epithelial-to-mesenchymal transition; brain distribution

1. INTRODUCTION

Studies on transporters that influence the dynamics and kinetics of drugs as well as endogenous biological compounds have expanded enormously in the last decade. Indeed, the number of articles hit by searching the keyword “transporter” on PubMed reached 26000 in the year 2015, compared with around 16000 per year at the end of the last century. Early work was focused on the roles of individual transporters in regulating endogenous compounds, but in recent years, attention has shifted to transporters that influence the pharmacokinetics of clinical medicines. Influx transporters are involved in the absorption, distribution and excretion of drugs by importing them into cells. On the other hand, efflux transporters help to block absorption and distribution of drugs by mediating their export from cells, organs, and ultimately from the body.

P-glycoprotein (P-gp) was first identified as an efflux transporter related to multidrug resistance (MDR) in cancerous cells, including adenocarcinoma and leukemia. Subsequently, it was found that this transporter is expressed not only in tumor cells, but also in normal tissues such as the intestine, liver, kidney, brain, adrenal gland and so on. P-gp serves to protect these organs by mediating efflux transport of exogenous compounds. In particular, this transporter is considered to serve as a barrier to drug absorption in the intestine and also to drug distribution to the brain and to tumor cells. Thus, P-gp function, gene expression and substrate specificity have been extensively investigated to aid drug development.

This review focuses on recent findings on P-gp and its expression regulators, especially our own research, in the following areas.

1) Relationship between P-gp expression pattern in the intestine and the bimodal pharmacokinetic profile of many drugs.
2) Tissue-selective regulation of P-gp localization and function by scaffold proteins.
3) Involvement of P-gp in cancer progression, and its association with epithelial-to-mesenchymal transition.
4) Role of P-gp in the neuropsychiatric side effects of oseltamivir in children.
5) Role of P-gp in the neuroprotective effects of cilnidipine under ischemic conditions.

2. RELATIONSHIP OF P-GP EXPRESSIO Pattern in the Intestine to Bimodal Phamacokinetic Profile of Orally Administered Substrate Drugs

When P-gp substrate drugs are administered orally to patients or to animals, the plasma concentration often shows a bimodal change, which is characterized by a second peak that is higher than the first. We were able to show that this bimodality can be explained, at least in part, by regional differences in P-gp expression along the intestine in rats and mice. Conflicting findings have been reported on segmental differences in expression and activity of P-gp within human and animal intestine. To resolve the issue, we measured P-gp protein levels within nine segments of rat small intestine, using the serial intestinal non-everted sac method. P-gp activity in each segment was also evaluated by measuring the permeability of rhodamine123 (Rho123), a typical P-gp substrate. Our results indicated that the P-gp protein level is maximum in the middle ileum. P-gp activity was also highest in the middle ileum, and was well correlated to P-gp protein levels. These results were in marked contrast to the conventional idea...
that P-gp increases monotonically towards the downstream end of the small intestine. We also showed that the bimodal plasma concentration profile observed after oral intake of Rho123 in rats could be accurately reproduced by an intestinal compartmental kinetic model incorporating inter-segmental differences in absorption and excretion rate constants (Fig. 1). Moreover, the bimodality of the Rho123 plasma concentration profile disappeared after oral co-administration of verapamil, a typical P-gp substrate/inhibitor. Also, the bimodal plasma concentration profile seen after oral administration of vinblastine, another P-gp substrate, was lost in P-gp knockout (KO) mice (mdr1a−/−). Thus, our results indicated that the heterogeneous distribution of P-gp along the small intestine can account for bimodal plasma concentration profiles of P-gp substrates following oral administration.

However, not all P-gp substrates show bimodal plasma concentration profiles after oral administration. Therefore, we examined this issue. In a membrane permeation study using Caco-2 cells, we found that the influx permeation rate of drugs that showed a bimodal pharmacokinetic profile was extremely low (absolute standard, \(P_{app, \text{Influx}} < 1.0 \times 10^{-6} \text{cm/s}\), being overwhelmingly smaller than the efflux permeation rate (relative standard, \(P_{app, \text{Influx}} / P_{app, \text{efflux}} < 0.1\)).

3. TISSUE-SELECTIVE REGULATION OF P-GP FUNCTION BY SCAFFOLD PROTEINS

Many researchers have used mRNA levels of P-gp as an indicator of its transport activity. However, P-gp mRNA levels are not always correlated with transport activity levels. Therefore, in this section we focus on the post-translational regulation of P-gp in normal and cancerous tissues.

**Fig. 1. Bimodal Plasma Concentration Profile Observed after Oral Intake of Rho123 in Rats**

Observed plasma concentrations of Rho123 are shown (open circle), together with simulated values calculated by a compartment model based on nine intestinal compartments (solid line). The model incorporates differential absorption and excretion at all segments. Y: central compartment, Xg: gastric compartment, Xn (n=1–9): intestinal compartment, \(k_n\) (n=1–10): GI transit rate constant, \(k_{xy}\): absorption rate constant from XNA to Y, keen (n=1–9): intestinal excretion rate constant from Y to XNA. The figure was prepared by reference to Wada et al. The role of inter-segmental differences in P-glycoprotein expression and activity along the rat small intestine in causing the double-peak phenomenon of substrate plasma concentration. Drug. Metab. Pharmacokinet., 28, 98–103 (2013) with permission from Elsevier.

**Biography**

Dr. Takuo Ogihara was born in Yokohama city in 1959. He studied organic synthetic chemistry under Prof. Oyo Mitsunobu at the Graduate School of Science and Engineering at Aoyama Gakuin University and completed his degree in 1983. He then worked as a researcher on kinetics and toxicity at a pharmaceutical company, and subsequently received a Ph.D. in pharmacy from Kanazawa University under the supervision of Prof. Akira Tsuji in 2000. In 2006, he became professor of the Laboratory of Biopharmaceutics, Department of Pharmacology, Faculty of Pharmacy, Takasaki University of Health and Welfare (http://www.takasaki-u.ac.jp/p_yaku/1581/). Currently, he is studying transporters, focusing on P-glycoprotein. He is also interested in drug discovery and related clinical trials, and launched the KENDAI Translational Research Center in 2014 (http://www.takasaki-u.ac.jp/wp-content/uploads/2017/01/602903316c0354646e492dbd522e11f3.pdf). Recent research activity: http://www.takasaki-u.ac.jp/wp-content/uploads/2017/04/d026d0ca3f1ee976f1be4a929b77d4f.txt.
It is reported that P-gp mRNA levels in the small intestine increase from the upper region, the duodenum, to the lower region, the ileum. On the other hand, P-gp activity is highest in the central ileum, where the maximum efflux of Rho123 is observed. Moreover, P-gp substrates such as vinblastine are absorbed from the duodenum and the ileum, but not the middle area of the small intestine, the jejunum. Therefore, it is clear that mRNA levels are not always correlated with P-gp activity. One of the reasons for this is the intracellular distribution of P-gp. This glycoprotein is localized in plasma or organelle membranes, including endoplasmic reticulum, Golgi, mitochondria and nucleus. ATP binding cassette (ABC) transporters, as exemplified by P-gp, have an eflux transport function at the plasma membrane, serving to prevent intracellular accumulation of cytotoxic compounds by pumping drugs from cells. Such action of P-gp, which leads to a reduction of intestinal absorption in healthy intestine and the development of cancer chemoresistance, requires the localization of P-gp in the apical membrane. Several researchers have investigated regulatory factors determining the intracellular localization of P-gp. It has been shown that N-linked glycosylation of asparagine residues in P-gp acts as a signal for trafficking to the plasma membrane, nuclear envelope, endosomes and so on. Also, P-gp protein levels at the apical membrane of cancerous cells might be increased by N-glycosylation. However, the mechanism of P-gp glycosylation-mediated transfer to the apical membrane remains unclear, although three sites of glycosylation have been identified in the first transmembrane domain of P-gp. On the other hand, this modification is increased in tumors during malignant transformation. When ribophorin II (RPN2), protein glycosyltransferase subunit 2, is silenced by small interfering RNA (siRNA) in docetaxel-resistant breast cancer cells, the membrane localization of P-gp is decreased concomitantly with a reduction in glycosylation. P-gp membrane localization is also decreased by tunicamycin, an N-glycosylation inhibitor, and tunicamycin treatment caused human primary ovarian cancer cell lines (WIPR and WITR cells) and human colon adenocarcinoma cell lines (Lovo and clone A cells) to recover sensitivity to cytotoxic drugs. However, it is not clear whether N-glycosylation is required to retain P-gp in the apical membrane. Furthermore, another report indicates that tunicamycin treatment induces only P-gp deglycosylation without a decrease in membrane expression or efflux activity in mouse leukemia L1210 cells. This discrepancy might arise from differences in regulation of the maintenance of P-gp localization by some factor(s) in each cell type. Such regulators include molecules anchoring P-gp at the plasma membrane. For example, ezrin, radixin and moesin (ERM proteins) are distributed as membrane scaffold proteins throughout the whole body and are composed of a 4.1-band ERM (FERM) domain at the amino-terminus (N-terminus), an α-helix region which is bracketed by and connected to the carboxy-terminal (C-terminus) and N-terminus regions, and an F-actin-binding site at the C-terminus. The FERM, F-actin-binding domain and α-helix region consist of about 300, 100 and 200 amino acids, respectively. Both the FERM domain and the F-actin-binding site share more than 75% homology of amino acid sequence in these proteins. The FERM domain normally binds the intramolecular C-terminus in the cytosol, causing these proteins to take an inactive state that cannot bind other proteins. The intramolecular binding is dissociated by phosphorylation of threonine in the C-terminus, generating the active structure. The activated ERM proteins play important roles in cell adhesion, cortical morphogenesis, signal transduction, and apoptosis, as well as in regulating drug transporters on the plasma membrane, and so on. Among these functions, regulation of drug transport is dependent on membrane sorting of the transporter via direct or indirect binding to membrane proteins. Accordingly, P-gp efflux activity is dependent upon post-translational regulators that determine sorting levels to the plasma membrane. Among apical membrane transporters, multidrug resistance associated protein (MRP/Mrp) 2 is well known to be linked to the canalicular membrane by radixin (Rdx), which serves as a post-translational regulator in the liver. Indeed, Rdx KO eliminated mouse Mrp2 from the canalicular membrane, resulting in conjugated hyperbilirubinemia. Moreover, the apical localization of human MRP2 is regulated by Rdx and ezrin (Ezr) in human colon adenocarcinoma Caco-2 cells. On the other hand, it is assumed that membrane localization of P-gp may be regulated by all of the ERM proteins, since P-gp can bind to all of them. Overall, it appears that individual ERM proteins regulating P-gp function may differ among different tissues, but are unchanged between normal and cancerous conditions in the same organ. So far, the relationship between P-gp and ERM proteins in the small intestine has been most intensively studied. We found that in mouse small intestine, P-gp mRNA and protein expression levels tend to increase from the upper region to the lower region. In addition, Rdx protein expression shows a similar trend. However, P-gp protein expression in plasma membrane fractions of Rdx KO mice was decreased in the middle and lower parts of the small intestine and the segmental variation was lost. Thus, the absorption rate and area under the blood concentration–time curve (AUC) of Rho123 in Rdx KO mice are significantly increased compared with those of wild-type mice. Furthermore, other researchers have observed that membrane localization of P-gp in the small intestine is upregulated by repeated etoposide treatment, resulting in resistance to the analgesic action of oral morphine (a P-gp substrate). As one of the mechanisms of this P-gp membrane sorting, it is suggested that etoposide administration induces phosphorylation of Rdx by activating signaling via the Ras homolog gene family, member A (RhoA) and Rho-associated, coiled-coil containing protein kinase 1 (ROCK). As a result, phosphorylated Rdx regulates P-gp membrane localization in the intestinal brush border membrane. These findings suggest that Rdx serves as the main cross linker between P-gp and actin in the small intestinal membrane. Furthermore, we reported that the knockdown of Rdx by siRNA significantly decreased P-gp transport function in human colon adenocarcinoma Caco-2 cells. Moreover, it was suggested that Rdx silencing did not impair the cell membrane, since passive transport was not affected, in accordance with our previous findings in Rdx KO mice. Consequently, regulation in Caco-2 cells is likely to be the same as in healthy small intestine. Knockdown of Ezr and moesin (Msn) had no effect on the function of P-gp. On the other hand, in the kidney, the excretion phase of Rho123 is the same in wild-type and Rdx KO mice. Since Rho123 is mainly excreted from the kidney, it is suggested that renal P-gp is not regu-
lated by Rdx. Surprisingly, in human renal carcinoma Caki-1 cells, knockdown of each ERM had no effect on P-gp activity, suggesting that P-gp is not regulated by the ERM proteins.\(^\text{49}\) Thus, renal P-gp seems not to be regulated by Rdx in either healthy or cancerous kidney. A possible explanation is that the kidney is routinely exposed to undesirable substances, such as metabolites or toxins, and therefore P-gp might be constantly present in the renal plasma membrane to eliminate such substances from the body. Furthermore, Rdx reduction in a rat liver inflammation model caused a decrease in P-gp expression at the membrane.\(^\text{51}\) This might imply that Rdx is the dominant protein among ERM in the liver. However, Rdx KO mice retained P-gp localization in the canalicular membrane, although some decline of P-gp protein levels was observed.\(^\text{51}\) Thus, there is a discrepancy between these reports. Although it is unclear whether Rdx regulates P-gp in hepatocytes, it might depend on the precise conditions of the experimental model, including adjuvant-induced arthritis or cholestasis. We found that Rdx plays a significant role in the post-translational regulation of plasma membrane expression of P-gp, as well as MRP2, in HepG2 cells.\(^\text{52}\) On the other hand, when Ezr or Msn was silenced by siRNA, the P-gp intracellular localization showed no change. Moreover, Ezr might be related to the transcriptional regulation of P-gp, since P-gp mRNA levels were reduced in Ezr knockdown cells. As in healthy liver, P-gp may also be regulated by Rdx in liver cancer.

Regulation of P-gp function at the blood–brain barrier (BBB) is also very important in vivo, serving to control the entry of drugs into the brain in conjunction with other efflux transporters and the tight junctions, which restrict passive permeation. The membrane localization and function of P-gp in the brain capillary endothelial cells are regulated by Msn in morphine-treated mice and/or Ezr in sphingomyelin synthase-deficient mice.\(^\text{53,54}\) In summary, it appears that P-gp localization and function could be regulated by different ERM proteins in different normal tissues (Fig. 2, Table 1).

Moreover, tissue-specific differences in the regulation of P-gp localization and activity by ERM proteins might be retained in cancerous tissues of the same organs.

A recent study has shown that P-gp is transferred from drug-resistant human breast cancer-derived MCF-7 cells to drug-sensitive parental cells via small membrane vesicles (SMVs).\(^\text{55}\) In particular, when Ezr and Msn are silenced by siRNA in parental cells (P-gp-recipient cells), the expression of P-gp is significantly increased by the co-incubation with SMVs compared to that in the case of control and Rdx-silenced parental cells.\(^\text{56}\) It has also been reported that extracellular vesicles including not only breast cancer resistance protein (BCRP), but also P-gp are involved in anticancer drug sequestration in drug-resistant MCF-7 cells and provide protection from cell death. As a part of the sequestration mechanism, P-gp is localized with ERM protein complex at the extracellular vesicles.\(^\text{57}\) Therefore, ERM proteins might also contribute at least in part to anticancer drug resistance via the sorting of P-gp into extracellular vesicles.

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**Table 1. Contribution of ERM Proteins to P-gp Activity in Several Tissues of Human and Rodents**

<table>
<thead>
<tr>
<th></th>
<th>Liver</th>
<th>Intestine</th>
<th>Kidney</th>
<th>Brain</th>
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<tbody>
<tr>
<td>Normal</td>
<td>Rdx</td>
<td>Rdx</td>
<td>Not Rdx</td>
<td>Ezr and/or Msn</td>
</tr>
<tr>
<td>Cancer</td>
<td>Rdx</td>
<td>Rdx</td>
<td>Not ERM</td>
<td>Unknown</td>
</tr>
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Ezr: ezrin, Rdx: radixin, Msn: moesin. Not Rdx indicates that P-gp was not regulated by radixin. Not ERM indicates that P-gp was not regulated by any ERM proteins. The Table was prepared by reference to Yano et al., Different regulation of P-glycoprotein function between Caco-2 and Caki-1 cells by ezrin, radixin and moesin proteins. J. Pharm. Pharmacol., 68, 361–367 (2016)\(^\text{49}\) with permission from Wiley.

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4. RELATIONSHIP BETWEEN P-GP AND CANCER PROGRESSION, AND THE ROLE OF EPITHELIAL-TO-MESENCHYMAL TRANSITION

Several reports have shown that P-gp overexpression causes cancer MDR\(^{58-60}\) which leads to a poor prognosis for many cancer patients.\(^{61-67}\) However, it is still unclear whether malignant evolution of cancer causes P-gp overexpression or vice versa. In this section, we discuss this issue.

Epithelial-to-mesenchymal transition (EMT) is considered to be the first step of cancer metastasis, in which epithelial cancer cells in the primary organ are converted to mesenchymal cells. These cells acquire high mobility and migration potential, enabling metastasis to distant organs. Li \textit{et al.} showed that breast cancer MCF-7 cells with EMT induced by Snail readily acquire doxorubicin resistance. This resistance is associated with both P-gp mRNA and protein up-regulation in the cells.\(^{68}\) Another report indicated that transcriptional factor Twist induces both EMT and P-gp expression in human colorectal cancer cells.\(^{69}\) Lu \textit{et al.} have shown that P-gp expression and efflux function in colorectal cancer HCT116 cells are increased in response to CCL21 treatment.\(^{70}\) CCL21 is a chemoattractant cytokine protein that induces stem cell properties and promotes chemoresistance of cancer cells. They showed that CCL21 treatment also induced overexpression of Snail, a transcriptional factor considered to be a master regulator of EMT. Recently, various reports have shown that microRNAs (miRNAs) induce cancer EMT and P-gp up-regulation. It is reported that overexpression of miR-181a is observed in both chemoresistant ovarian cancer tissues and paclitaxel-resistant human ovarian cancer cell line SKOV3. MiR-181a overexpression leads to EMT in SKOV3 cells and increasing paclitaxel resistance accompanied with P-gp up-regulation. Moreover, anti-miR-181a enhances paclitaxel cytotoxicity.\(^{71}\) On the other hand, miR-30a is down-regulated in chemoresistant gastric cancer tissues and cisplatin (DDP)-resistant variant SGC-7901/DDP gastric cancer cell lines. MiR-30a expression suppresses EMT and P-gp expression in SGC-7901/DDP.\(^{72}\) These data suggest that miRNAs that control EMT contribute to P-gp alteration.

Our experiments revealed that during the EMT process, lung cancer cells acquire MDR without alteration of P-gp expression level, but with an increase of P-gp efflux activity.\(^{73}\) Thus, the outcome of cellular uptake experiments using Rho123 and paclitaxel indicated that P-gp is activated in Snail-overexpressing cells, even though Western blot analysis revealed P-gp expression levels in the overexpressing cells were similar to those in Mock cells. In addition, the overexpressing cells showed greater viability than Mock cells in the presence of paclitaxel. Caveolin-1 dephosphorylation and reduced GRB2 expression in Snail-overexpressing cells were also noted. These results uncovered a novel pathway leading to cancer MDR, in which Snail induces EMT with an attendant decrease of GRB2-mediated caveolin-1 phosphorylation, resulting in P-gp activation (Fig. 3).

The cancer-related EMT process might be closely associated with alteration of P-gp, as mentioned above. But, on the other hand, some researchers have suggested that P-gp is an inducer of malignant evolution. Zhang \textit{et al.} revealed that P-gp knockdown decreases the invasiveness and migration proper-

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**Fig. 3.** Overview of EMT-Induced P-gp Activation in Cancer Cells (Upper Panel) and Mechanism of P-gp Activation in EMT-Induced Cancer Cells (Lower Panel)

P-gp expression is enhanced in EMT cells (1). Activity related to posttranslational modification is also enhanced, such as alteration of phosphocaveolin-1 expression (2).
ties of adriamycin-resistant MCF-7 cells (MCF-7/ADR). They also revealed that P-gp promotes tyrosine (Tyr) phosphorylation of Anxa2, an inducer of cancer metastasis, via Src Tyr kinase. This interaction was also supported by identification of Rack1 as a scaffold protein of P-gp. It was also reported that P-gp mRNA expression levels were positively correlated with breast cancer lymph node status. These studies indicate that P-gp might also work as a signal transducer.

5. ROLE OF P-GP IN THE NEUROPSYCHIATRIC SIDE EFFECTS OF OSELTAMIVIR IN CHILDREN

Oseltamivir (Tamiflu) is an ester-type prodrug of the neuraminidase inhibitor Ro 64-0802. It is widely used for treatment of influenza in Japan, but is known to induce neuropsychiatric side effects, especially among the young. Studies of toxicity and brain distribution of oseltamivir in experimental animals led us to hypothesize that the low level of distribution of oseltamivir and/or Ro 64-0802 in adult brain was due to the presence of a specific efflux transporter at the BBB.

We examined the role of P-gp as a regulator of brain distribution of oseltamivir and Ro 64-0802 in vitro using cells over-expressing human MDR1 P-gp on the apical membrane, and in vivo using mdrla/b KO mice. The permeability of oseltamivir in the basal-to-apical direction was notably higher than that in the opposite direction. The addition of cyclosporin A, a P-gp inhibitor, caused the directional transport to disappear. The brain distribution of oseltamivir was enhanced in mdrla/b KO mice compared with wild-type mice. In contrast, transport of Ro 64-0802 by P-gp was minimal in both in vitro and in vivo experiments. These results indicate that oseltamivir, a substrate of P-gp, but Ro 64-0802 is not. Moreover, we examined developmental changes in the brain distribution of oseltamivir and Ro 64-0802, and in the expression of P-gp at the BBB in rats. The brain concentration and Kpapp,brain (brain-to-plasma concentration ratio) value of oseltamivir were highest in 2-week-old rats, and were negatively correlated with both age and P-gp expression at the BBB. Oseltamivir concentration in the brain was 70-fold and 0.7-fold higher than that of Ro 64-0802 in 2-week-old and 8-week-old rats, respectively. These results suggest that the adverse effects on the central nervous system (CNS) observed in some patients treated with oseltamivir, especially young children, might be explained by low levels of P-gp activity, or by drug–drug interactions at P-gp, resulting in enhanced brain accumulation of oseltamivir. If such developmental changes of prodrug/drug concentration ratio also occur in humans, they may provide a tenable explanation for the putative CNS side effects in young patients.

Recently, it has been suggested that P-gp polymorphism affects the pharmacokinetics of oseltamivir in humans, based on the findings that the plasma concentrations of oseltamivir and Ro 64-0802 in a P-gp mutant group were higher than those in the wild-type and heterozygous groups after administration of oseltamivir. Further large-scale studies will be needed to validate these findings and establish their clinical relevance.

6. ROLE OF P-GP IN THE NEUROPROTECTIVE EFFECTS OF CILNIDIPINE UNDER ISCHEMIC CONDITIONS

Cilnidipine is known to show an antihypertensive effect with neuroprotective action in rat brain ischemia models, but shows minimal distribution to normal brain, suggesting that its brain uptake is restricted by efflux transporter(s). We therefore examined whether P-gp regulates the distribution of cilnidipine to the brain. Intracellular build up cilnidipine was reduced in P-gp-overexpressing porcine kidney epithelial cells (LLC-GA5-COL150 cells) compared to control LLC-PK1 cells, and the decrease was notably inhibited by a P-gp inhibitor, verapamil. Furthermore, cilnidipine concentration in the brain of P-gp KO mice was significantly increased after its administration, compared with that in wild-type mice. In addition, when cilnidipine was administered to male spontaneously hypertensive rats (SHR) with tandem occlusion of the distal middle cerebral and ipsilateral common carotid artery, its concentration in the ischemic hemisphere was 1.6-fold higher than that in the contralateral hemisphere. These findings were supported by visual representation of cilnidipine distribution using matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF)/MS imaging (Fig. 4). These results suggested that cilnidipine would usually be excluded from the brain by P-gp, but that P-gp function is impaired in the ischemic brain and thus cilnidipine is distributed to the ischemic region, manifesting a neuroprotective action. If this is also the case in humans, cilnidipine may be useful for management of hypertension in patients with a history and/or a potential risk of cerebral ischemia.

In conclusion, in this paper, we have reviewed the factors controlling P-gp expression and activity, focusing especially on work in our laboratory. We show that the bimodal changes of plasma concentration often observed after oral administration of P-gp substrate drugs can be well explained by the heterogeneous distribution of P-gp along the small intestine. In addition, P-gp localization and function are influenced by the scaffold proteins ezrin, radixin and moesin (ERM proteins), which fix P-gp to the cell membrane. There are tissue-specific differences in regulation by ERM proteins in normal tissues, and these appear to be retained in corresponding cancerous

![Fig. 4. MALDI-TOF/MS Imaging of Cilnidipine Distribution in the Intact Right Hemisphere and Ischemic Left Hemisphere of Rat Brain](Image)

The white dotted circle indicates the ischemic area. Blue and pink colors represent the absence and presence of cilnidipine in the brain, respectively. Colors corresponding to various cilnidipine concentrations are indicated in the dotted circles (right panel). The figure was prepared by reference to Yano et al. Role of P-glycoprotein in regulating cilnidipine distribution to intact and ischemic brain. Drug Metab. Pharmacokinet., 29, 254–258 (2014) with permission from Elsevier.
tissues. Furthermore, P-gp function may change depending on factors such as age and disease status (cancer metastasis, cerebral ischemia, infection). For example, P-gp activity is enhanced during EMT, which is associated with cancer progression, without any increase of P-gp mRNA expression. Finally, we describe how developmental changes of P-gp can explain the neuropsychiatric side effects of oseltamivir in children, and conversely how ischemia-related changes of P-gp can explain the neuroprotective effect of cilnidipine in patients with cerebral ischemia. Thus, numerous factors that control the expression and activity of P-gp have been clarified. On the other hand, less information is available on other excretory transporters, and this will be an important area for further research.

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Conflict of Interest The authors declare no conflict of interest.

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