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Interaction of multidrug resistance reversal agents with P-glycoprotein ATPase activity on blood-brain barrier[1]

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KEY WORDS P-glycoprotein; adenosine triphosphatase; blood-brain barrier; multiple drug resistance; isoquinolines

ABSTRACT

AIM: To gain further insights into the mechanism of the ATP-dependent interaction of P-glycoprotein (P-gp) with various multidrug resistance (MDR) reversal agents. **METHODS**: Bovine brain capillary endothelial cells (BCEC) were isolated from cerebral gray matter using modifications of the mechanical homogenization technique. Plasma membranes were prepared from BCEC. The P-gp adenosine triphosphatase (ATPase) activity of the isolated BCEC membranes was estimated by measuring inorganic phosphate liberation. **RESULTS**: The basal P-gp ATPase activity was increased by verapamil (Ver), vincristine (VCR), doxorubicin (Dox), tetrandrine (Tet), dauricine (DRC), berbamine (BBM), and daurisoline (DRS), with respective half-maximal activity concentrations K_m of about 17, 5.9, 41, 2.3, 11, 23, and 22 μmol/L. Berberine (BBR) produced a relatively slight activation. *dl*-Tetrahydropalmatine (*dl*-THP) and *l*-tetrahydropalmatine (*l*-THP) does not alter the basal P-gp ATPase activity. Cyclosporin A (CsA) inhibited both the basal and the drug-stimulated ATPase activity of P-gp with high affinity. Kinetic analysis indicated a competitive inhibition of Ver- or VCR-stimulated ATPase activity and a noncompetitive inhibition of Dox- or Tet-activated ATPase activity by CsA. Moreover, Dox inhibited Tet-activated P-gp ATPase activity in a noncompetitive manner. **CONCLUSION**: Various MDR reversal agents could interact with P-gp and alter its ATPase activity in different manners. This is the result of the broad molecular recognition specificity of P-gp. CsA, Ver, and VCR could bind P-gp either on overlapping sites or distant but interacting sites, while CsA, Dox, and Tet could independently bind P-gp on separated sites on blood-brain barrier.

INTRODUCTION

P-glycoprotein (P-gp) is a plasma membrane glycoprotein that confers multidrug resistance (MDR) phenotype by virtue of its ability to exclude a wide range of chemotherapeutic drugs and other hydrophobic compounds from cells in an adenosine triphosphate (ATP)-dependent manner[1]. It is also expressed at high levels in non-cancerous tissues, such as the endothelial cells of the blood-brain barrier (BBB) capillaries of human as well as animals. P-gp may be involved in the exclusion of various drugs from the capillary endothelial cells, blocking their entry into the brain[2,3].

Numerous compounds with no structural analogy or common pharmacological properties have been identified, such as verapamil (Ver), cyclosporin A (CsA), vincristine (VCR), and doxorubicin (Dox), *etc*, which inhibit the function of P-gp, thus reversing MDR in cultured cells, termed chemosensitisers, P-gp antagonists, or MDR reversal agents. Some isoquinoline alkaloids such as tetrandrine (Tet), dauricine (DRC), berbamine (BBM), *etc*, have been reported also to be capable of reversing MDR[4-6].

P-gp itself exhibits *in vitro* ATPase activity, the mechanism of its drug-efflux action and the mechanism of MDR reversal agents action in the modulation of P-gp function are not yet well understood. The most widely-discussed current hypothesis is that P-gp uses energy of ATP hydrolysis to export drugs from the cell. P-gp in plasma membranes shows a basal rate of hydrolysis of ATP, which is stimulated by several fold on addition of drugs[7]. The aim of this paper was to gain further

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insights into the mechanism of the ATP-dependent interaction of P-gp with its various MDR reversal agents and investigate the binding sites which were involved in the modulation of P-gp function on BBB.

MATERIALS AND METHODS

Materials Cyclosporin A, ouabain, and sodium azide were gifts of Dr LIU Xiao-Dong (China Pharmaceutical University). Doxorubicin was a gift of Prof GUO Qing-Long (China Pharmaceutical University). Isoquinoline alkaloids were kindly donated by Prof HUA Wei-Yi (China Pharmaceutical University). Verapamil and vincristine were purchased from Sigma Co. All other chemicals were of analytical grade and commercially available.

Isolation of bovine brain capillaries Bovine brain capillaries were isolated from cerebral gray matter using modifications of the mechanical homogenization technique of Dallaire *et al*[8]. Briefly, bovine brain cortex was stripped of the pial membrane and minced in phosphate-buffered saline (PBS) containing edetic acid 5 mmol/L, pH 7.4. Samples were homogenized with a tissue homogenizer run at 6000 r/min for 15 s. The combined homogenates were diluted with PBS and passed through a 127-μm nylon mesh. Then the filtrate was passed through a 72-μm nylon mesh and washed. The material retained on top of the 72-μm meshes was obtained and centrifuged at 1000 × g for 10 min. The pellet was the brain capillary preparation. The purity of the capillary preparation was examined by microscopy and assaying the relative specific activity of the marker enzyme γ-glutamyltransferase (γ-GTase) for the BBB-specific endothelial cell. The results showed that the preparation was composed of capillary segments with less than 2 % of contamination cells and enriched 14.7-fold in γ-GTase specific activity over the brain homogenate. The capillary preparation was resuspended in 10 volumes of 0.1 % (w/v) collagenase Ⅱ in PBS and incubated at 37 ℃ for 30 min. The suspension was centrifuged at 1000 × g for 10 min at 4 ℃ and the pellet was washed twice with PBS, then the brain capillary endothelial cells (BCEC) were obtained[9].

Preparation of plasma membranes Plasma membranes were prepared from BCEC as previously described[10]. Isolated cells were suspended in hypotonic lysis buffer (in mmol/L: Tris-HCl 10, pH 7.8, KCl 10, $MgCl_2$ 2, dithiothreitol 1, egtazic acid 1) and allowed to swell for 20 min at 4 ℃. Swollen cells were disrupted by sonication for 10 s at 20 % maximum power (Sonicator W-225R Heat System Ultrasonics) and the resulting homogenate was centrifuged (1400 × g, 10 min, 4 ℃). The supernatant was then layered on a 46 % sucrose cushion in lysis buffer and centrifuged (7000 × g, 20 min, 4 ℃). The layer at the sucrose interface was removed, diluted twice with lysis buffer, and sedimented (135 000 × g, 15 min, 4 ℃). The pellet of total membranes was washed twice in lysis buffer and finally resuspended in lysis buffer supplemented with NaCl 100 mmol/L at a total membrane protein concentration of 2.1 g/L. The protein concentration was determined by the Lowry method, with bovine serum albumin as standard[11].

P-gp ATPase activity measurement The ATPase activity of the isolated BCEC membranes was estimated by measuring inorganic phosphate liberation[12]. Membrane suspensions (about 20 μg of membrane protein, as determined by a modified Lowry method) were incubated at 37 ℃ in 0.1 mL of a medium containing (in mmol/L): Tris-HCl 50 (pH 6.8), dithiothreitol 2, $MgCl_2$ 5, ouabain 2 (to eliminate Na^+, K^+-ATPase activity), egtazic acid 2 (to eliminate Ca^{2+}-ATPase activity), sodium azide 5 (to eliminate F_1-F_0-ATPase activity), and the ATPase reaction was started by the addition of MgATP 5 mmol/L. Inorganic phosphate (P_i) was measured by a modification of the sensitive colorimetric reaction described previously. The samples were supplemented with 0.4 mL of reagent containing H_2SO_4 2.5 mol/L, 1 % ammonium molybdate, 0.014 % antimony potassium tartrate, and 1 mL of distilled water. For the reduction of the complex, 0.2 mL of 1 % ascorbic acid (freshly prepared) was added and the optical density read at 880 nm. Activity were calculated from the initial linear rate of P_i production and ATPase activity was estimated by difference obtained in P_i levels between 0 min (reaction stopped immediately) and 30 min incubation periods.

Kinetic analysis[13] Data from experiments measuring ATPase activity were fitted to the Michaelis-Menten equation by nonlinear least square regression analysis (Eq 1). V_{max} and K_m values with standard errors were derived from these curves (Tab 1) and K_i values were calculated using the equation for the competitive (Eq 2) or noncompetitive (Eq 3) inhibition.

$$V = V_{max} \cdot C/(K_m + C) \quad \text{(Eq 1)}$$

$$V = V_{max} \cdot C/[K_m(1 + I/K_i) + C] \quad \text{(Eq 2)}$$

Tab 1. Effects of MDR reversal agents on basal P-gp ATPase activity in BCEC membranes. K_m and V_{max} values were determined by fitting data to the Michaelis-Menten equation. $n = 4$. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ vs no drug.

Drugs/ μmol·L^{-1}		Total P-gp ATPase activity /μmol·min^{-1}·g^{-1}	Drug-stimulated ATPase activity /μmol·min^{-1}·g^{-1}	Michaelis-Menten equation parameters K_m	V_{max}
No drug		117 ± 18	0 ± 19		
Verapamil	1	134 ± 22	17 ± 4		
	2.5	158 ± 28^b	40 ± 9		
	5	189 ± 33^c	71 ± 11	17 ± 7	313 ± 101
	10	230 ± 32^c	113 ± 26		
	20	281 ± 99^c	164 ± 48		
	50	342 ± 105^c	225 ± 51		
	100	277 ± 56^c	160 ± 35		
	200	180 ± 77^c	63 ± 23		
Vincristine	1	134 ± 28	17 ± 7		
	2	146 ± 41	29 ± 6		
	5	171 ± 37^b	54 ± 21	5.9 ± 2.5	117 ± 38
	10	191 ± 39^c	74 ± 13		
	20	208 ± 44^c	91 ± 22		
	50	163 ± 33^b	46 ± 18		
	75	139 ± 42	22 ± 5		
Doxorubicin	5	126 ± 29	9 ± 3		
	10	133 ± 25	16 ± 5		
	20	143 ± 31	26 ± 7	41 ± 16	80 ± 29
	40	157 ± 27^b	40 ± 8		
	80	172 ± 36^b	53 ± 11		
	100	148 ± 31	31 ± 9		
	150	123 ± 24	6 ± 2		
Tetrandrine	1	203 ± 41^c	86 ± 23		
	2.5	265 ± 43^c	148 ± 29	2.3 ± 0.9	284 ± 92
	5	312 ± 31^c	195 ± 37		
	10	348 ± 49^c	231 ± 54		
	20	306 ± 39^c	189 ± 33		
	40	224 ± 40^c	107 ± 41		
Dauricine	1	148 ± 27	31 ± 11		
	2.5	186 ± 31^b	69 ± 10		
	5	235 ± 18^c	117 ± 23	11 ± 5	374 ± 133
	10	296 ± 22^c	178 ± 19		
	20	359 ± 50^c	242 ± 38		
	50	273 ± 45^c	156 ± 30		
	100	197 ± 56^b	80 ± 28		
Berbamine	5	172 ± 22^b	55 ± 19		
	10	210 ± 28^c	93 ± 17		
	20	260 ± 39^c	143 ± 22	23 ± 8	308 ± 95
	50	328 ± 20^c	211 ± 35		
	80	356 ± 47^c	239 ± 45		
	100	341 ± 32^c	224 ± 20		
	150	301 ± 36^c	184 ± 40		
Daurisoline	5	174 ± 30^b	57 ± 11		
	10	213 ± 28^c	95 ± 18		
	20	262 ± 25^c	145 ± 30	22 ± 10	297 ± 111
	50	327 ± 37^c	209 ± 38		
	80	352 ± 33^c	235 ± 41		
	100	296 ± 36^c	179 ± 31		
	200	204 ± 23^c	87 ± 21		
Berberine	5	127 ± 24	11 ± 5		
	10	136 ± 26	19 ± 5		
	20	147 ± 19	30 ± 11	32 ± 12	78 ± 30
	50	164 ± 31^b	47 ± 23		
	100	176 ± 29^b	59 ± 25		
	200	158 ± 17^b	41 ± 19		
	250	141 ± 32	24 ± 5		
CsA	0.01	103 ± 19	−14 ± 3		
	0.05	78 ± 21^b	−39 ± 5		
	0.25	57 ± 15^c	−59 ± 9	−	−
	0.5	41 ± 12^c	−76 ± 13		
	1	30 ± 9^c	−87 ± 23		
	5	21 ± 8^c	−96 ± 37		
dl-THP	5	118 ± 25	1 ± 3		
	10	116 ± 18	−1 ± 4		
	20	117 ± 20	0 ± 3	−	−
	50	120 ± 17	3 ± 1		
	100	111 ± 24	−6 ± 3		
l-THP	5	120 ± 38	3 ± 1		
	10	127 ± 23	10 ± 6	−	−
	20	123 ± 30	6 ± 2		
	50	132 ± 40	15 ± 4		
	100	124 ± 31	7 ± 3		

$$V = V_{max} \cdot C / [K_m(1 + I/K_i) + C(1 + I/K_i)] \quad \text{(Eq 3)}$$

Double-reciprocal treatment:

$$1/V = 1/V_{max} + (K_m/V_{max}) \cdot 1/C \quad \text{(Eq 1)}$$

$$1/V = 1/V_{max} + [K_m(1 + I/K_i)/V_{max}] \cdot 1/C \quad \text{(Eq 2)}$$

$$1/V = (1 + I/K_i)/V_{max} + [K_m(1 + I/K_i)/V_{max}] \cdot 1/C \quad \text{(Eq 3)}$$

Where V and V_{max} are the drug-stimulated ATPase activity and the maximum velocity, respectively; K_m and K_i are the half-maximal activity concentration (Michaelis

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constant) and inhibition constant, respectively; C and I are the concentrations of stimulator and inhibitor, respectively.

RESULTS

Effects of Ver, VCR, Dox, and CsA on the basal P-gp ATPase activity We used a plasma membrane preparation from the BCEC, which constitutively high expressed P-gp. The plasma membranes contained 30 % P-gp as percentage of total membrane protein. In the presence of three ionic pump inhibitors, sodium azide, ouabain, and egtazic acid, the extra ATPase activity measured for the P-gp-containing membranes can be attributed to P-gp. This so-called basal ATPase activity, measured in the absence of any added drug, is about (117 ± 18) $\mu mol \cdot min^{-1} \cdot g^{-1}$ protein, depending on cell batch and membrane preparation.

Ver, VCR, and Dox stimulated P-gp ATPase activities in a concentration-dependent manner. All three drugs displayed biphasic effects, as drug concentration increased beyond the maximum value of the activities, then the activity decreased. The same measurements were made using membranes devoid of P-gp from human umbilical vein endothelial cells (HUVEC) for checking the specificity of observed ATPase activity variations. In this case, increasing concentrations of any of the tested molecules do not induce any significant change in the P-gp independent ATPase activity (data not shown).

CsA inhibited the P-gp ATPase activity in a concentration-dependent manner, the concentration producing 50 % inhibition was (0.23 ± 0.07) μmol/L. An almost complete inhibition (< 20 % of the control activity) of the basal ATPase activity was observed with CsA 5 μmol/L.

Fitting the data by nonlinear least square regression analysis and assuming simple Michaelis-Menten kinetics, the apparent K_m and V_{max} values of each test drug were calculated (Tab 1).

Effects of seven isoquinolines on the basal P-gp ATPase activity Seven isoquinolines displayed diverse effects on P-gp ATPase activity. Tet, DRC, BBM, and daurisoline (DRS) markedly induced bell-shaped concentration dependence curves for the P-gp ATPase activity stimulation. BBR produced a relatively slight activation, while *dl*-tetrahydropalmatine (*dl*-THP) and *l*-tetrahydropalmatine (*l*-THP) virtually did not alter the basal P-gp ATPase activity.

Effects of CsA on Ver- or VCR-stimulated P-gp ATPase activity The influence of MDR reversal agents, with diverse structure, on the P-gp ATPase activity were further explored by studying their combined effects, ie, the concentration-dependent effect of one of them on the P-gp ATPase activity due to another one. The P-gp ATPase activity stimulation curves were shifted toward higher concentrations by the initial addition of CsA. A kinetic analysis revealed that in the presence of CsA 0.2 and 0.4 μmol/L, the apparent K_m (Ver) was increased from (17 ± 7) to (25 ± 9) and (32 ± 12) μmol/L, respectively and K_m (VCR) was increased from (5.9 ± 2.5) to (8.2 ± 3.2) and (10 ± 4) μmol/L respectively, while the V_{max} values remained unchanged. It indicated that CsA inhibited Ver- or VCR-activated P-gp ATPase activities in a competitive manner with a calculated inhibition constant K_i of (0.46 ± 0.18) μmol/L (Fig 1A) or (0.51 ± 0.23) μmol/L (Fig 1B).

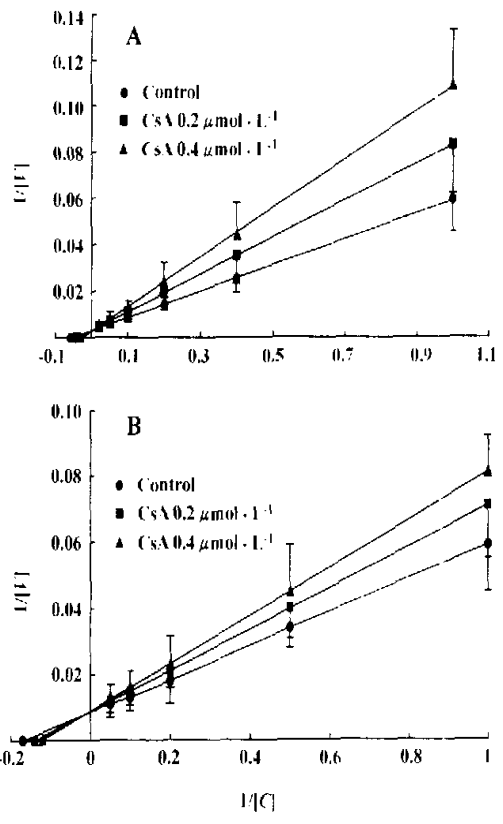

Fig 1. Kinetics of the inhibitions of verapamil (A)- and vincristine (B)-stimulated P-gp ATPase activity by CsA. K_m, K_i, and V_{max} values were determined by fitting data to the Michaelis-Menten equation. [C]: drug concentration (μmol/L); [V]: ATPase activity (μmol·min^{-1}·g^{-1} protein). $n = 4$. $x \pm s$.

Effects of CsA on Dox- or Tet-stimulated P-gp ATPase activity In the presence of CsA 0.2 and 0.4 μmol/L, P-gp ATPase activity stimulation curves displayed a progressively decreasing maximal activation with no noticeable shift for half-maximal activating concentrations. The kinetic analysis revealed that in the presence of CsA 0.2 and 0.4 μmol/L, the apparent K_m (Dox) or K_m (Tet) was essentially unchanged, while V_{max} (Dox) was reduced from (80 ± 29) to (50 ± 14) and (36 ± 12) μmol·min^{-1}·g^{-1}, V_{max} (Tet) was reduced from (284 ± 92) to (207 ± 83) and (163 ± 51) μmol·min^{-1}·g^{-1}, respectively. This indicated that CsA inhibited Dox- or Tet-stimulated P-gp ATPase activity in a non-competitive manner with a calculated inhibition constant K_i of (0.35 ± 0.19) μmol/L (Fig 2A) or (0.54 ± 0.26) μmol/L (Fig 2B).

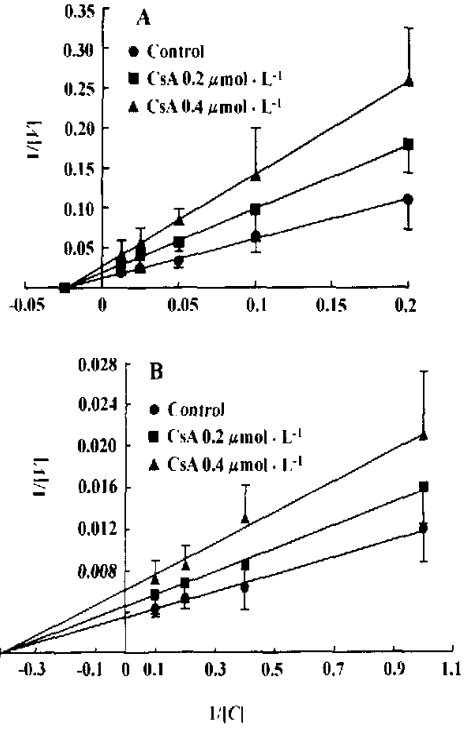

Fig 2. Kinetics of inhibitions of the doxorubicin (A)- and tetrandrine (B)-stimulated P-gp ATPase activity by CsA. K_m, K_i, and V_{max} values were determined by fitting data to the Michaelis-Menten equation. [C]: drug concentration (μmol/L); [V]: ATPase activity (μmol·min^{-1}·g^{-1} protein). $n = 4$. $\bar{x} \pm s$.

Effect of Dox on the Tet-stimulated P-gp ATPase activity P-gp ATPase activity stimulated by Tet was measured in the presence and absence of Dox. Dox 5 and 10 μmol/L reduced V_{max} (Tet) from (284 ± 92) to (164 ± 53) and (115 ± 42) μmol·min^{-1}·g^{-1}, respectively, but the K_m (Tet) was unchanged. It was shown that Dox inhibited the Tet-activated P-gp ATPase activity non-competitively with a calculated K_i of (6.8 ± 2.7) μmol/L (Fig 3).

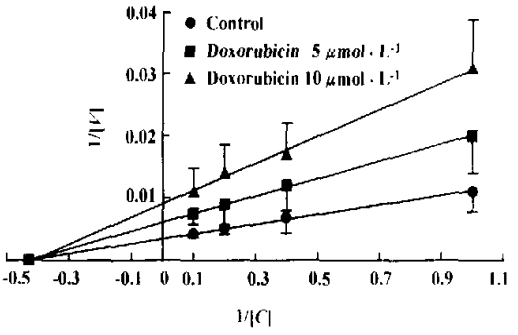

Fig 3. Kinetics of inhibition of the tetrandrine-stimulated P-gp ATPase activity by doxorubicin. K_m, K_i, and V_{max} values were determined by fitting data to the Michaelis-Menten equation. [C]: drug concentration (μmol/L); [V]: ATPase activity (μmol·min^{-1}·g^{-1} protein). $n = 4$. $\bar{x} \pm s$.

Effect of MgATP on P-gp ATPase activity in the presence or absence of Tet Since Tet stimulated P-gp ATPase activity with a very high affinity according to its calculated K_m, we examined whether Tet interfered with ATP binding to P-gp by measuring ATPase activity at a serial concentrations of MgATP in the absence and presence of fixed concentration of Tet. In absence of Tet, with the increase of MgATP concentrations, the basal ATPase activity of P-gp showed Michaelian behavior with a Michaelis constant K_m (MgATP) of (2.1 ± 0.7) μmol/L and V_{max} of (171 ± 48) μmol·min^{-1}·g^{-1}. In the presence of Tet 5 and 10 μmol/L, the K_m (MgATP) was (2.6 ± 0.9) and (2.4 ± 0.8) μmol/L, respectively and the V_{max} (MgATP) was increased to (463 ± 151) and (509 ± 137) μmol·min^{-1}·g^{-1}, respectively. In this case, V_{max} (MgATP) was markedly increased by Tet, but the apparent K_m (MgATP) was essentially unchanged. It suggested that P-gp ATPase modulation by Tet could not be attributed to the effect on ATP binding to P-gp (Fig 4).

DISCUSSION

P-gp is an adenosine in 5′-triphosphate (ATP)-

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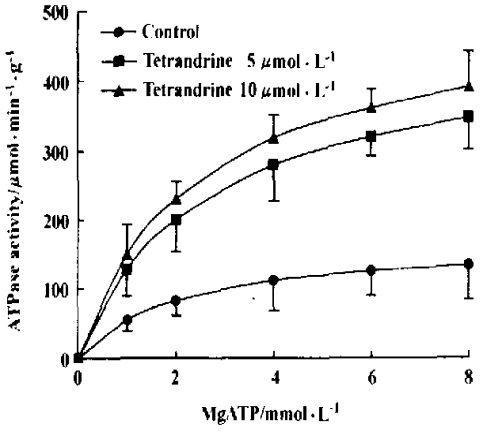

Fig 4. Effect of MgATP on the P-gp ATPase activity in the presence or absence of tetrandrine. K_m(MgATP) and V_{max} values were determined by fitting data to the Michaelis-Menten equation. $n=4$. $\bar{x} \pm s$.

dependent drug transporter which pumps out several anticancer agents from multidrug-resistant tumor cells and then reduces the intracellular concentration of drugs, thereby conferring drug resistance. It was also found to be expressed at the BBB of human as well as animals. The movement of compounds from the circulating blood into brain is strictly regulated by the BCEC, which are connected to each other by well developed tight junctions without fenestrations and form a lipoidal membrane barrier, ie, the BBB. Clearly, an understanding of the efflux mechanisms would give us much useful information regarding drug delivery to the brain[7]. P-gp has 12 transmembrane domains contained in two homologous halves and there are two ATP-binding cassette domains in each of the halves that catalyse ATP hydrolysis. However, comparatively little is known about the site(s) or nature of the molecular interactions between drugs and P-gp or how drug binding is coupled to ATP hydrolysis to elicit transport[14]. We have therefore used a sensitive *in vitro* P-gp ATPase assay to investigate the mechanism of P-gp interaction with various compounds.

Since only those compounds that stimulating ATP hydrolysis are transported by the P-gp[15], in this paper, the results suggested that there is more than one type of interaction with P-gp. There is two classes of MDR reversal agents interacting with P-gp: one activated ATPase activity, implying that these drugs were transported by P-gp and also appeared to be substrates of P-gp, such as Ver, VCR, Dox, Tet, DRC, BBM, DRS, and BBR, *etc*. The other failed to activate ATPase activity, including *dl*-THP and *l*-THP, virtually did not alter the basal P-gp ATPase activity, while CsA interacts with P-gp with high affinity but fails to elicit ATP hydrolysis. These agents may only be able to bind to P-gp without being effluxed out of the BCEC

According to the K_m given in Tab 1, the rank order of affinity was CsA > Tet > VCR > DRC > Ver > DRS ≈ BBM > BBR > Dox. These data consisted with the potency of their MDR reversing action[2,5,6]. The MDR reversal agents which interact with P-gp with a higher affinity and a tighter binding exhibited a greater potency of P-gp antagonism.

In addition to the well-defined ATP-binding site, P-gp presents another two types of binding sites, one for transport (drug binding site) and one for modulation (modulator site). The various reversal agents would be able to interact with one or both sites. Reversal agents may block therapeutic drug efflux by acting as competitive or non-competitive inhibitors, perhaps by binding to similar drug substrate binding sites or to other modulator sites which cause allosteric changes resulting in inhibition of therapeutic drug binding or transport[4,15]. However, there is little known about whether various MDR reversal agents function similarly as inhibitors of drug efflux, where and how many binding sites exist for reversal agents on P-gp or the possible communication between them. Our enzymatic data demonstrated that CsA inhibited Ver- or VCR-stimulated P-gp ATPase activity in a competitive manner. It indicated that CsA with Ver or VCR were mutually exclusive for their P-gp ATPase modulation. The binding sites on P-gp of these three drugs could be either identical or partially overlaping or distant and subject to negative allosteric heterotrope interaction. Moreover, our results suggested that Tet modulate P-gp ATPase without interfering with the binding of ATP, it seemed that Tet was not acting by binding to the ATP-binding site in the P-gp membrane domains. We also have shown that CsA noncompetitively inhibited Dox- or Tet-activated P-gp ATPase activity and Dox noncompetitively inhibited Tet-activated P-gp ATPase activity. This indicated that CsA, Dox, and Tet were non-exclusive for their modulating effects on P-gp ATPase. We could thus conclude that there are three different separate sites for CsA, Dox, and Tet on P-gp, and that each of these drugs can bind to its own site dependently in the presence of others. This suggested that at least three modulatory sites exist on P-gp in the BBB.

In summary, numerous MDR reversal agents with no structural analogy or common pharmacological pro-

perties modulated P-gp ATPase in different manner. There was more than one type of interaction with P-gp and multisites (more than three) rather than a universal site involved in the modulation of P-gp function in BBB. This may be related to the broad molecular recognition specificity of P-gp.

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多药耐药逆转剂与血脑屏障上 P-糖蛋白 ATP 酶活性间的相互作用[1]

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关键词 P-糖蛋白; 腺苷三磷酸酶; 血脑屏障; 多种抗药性; 异喹啉类

目的: 进一步探讨 P-糖蛋白(P-gp)与其多种耐药逆转剂间 ATP 依赖性相互作用的机制. **方法**: 从牛脑灰质中分离得到微血管内皮细胞(BCEC), 制成细胞膜, 定磷法测定 BCEC 膜上 P-gp ATPase 活性. **结果**: 维拉帕米(Ver)、长春新碱(VCR)、阿霉素(Dox)、粉防己碱(Tet)、蝙蝠葛碱(DRC)、小檗胺(BBM)以及蝙蝠葛苏林碱(DRS)增加基础 P-gp ATPase 活性, 其 K_m 值分别约为 17、5.9、41、2.3、11、23 和 22 μmol/L. 小檗碱(BBR)仅有轻微的激活作用, 延胡索乙素(*dl*-THP)和左旋四氢巴马汀(*l*-THP)不改变基础P-gp ATPase 活性. 环孢素 A(CsA)抑制基础P-gp ATPase 活性; 竞争性抑制 Ver 或 VCR 激活的P-gp ATPase 活性; 非竞争性抑制 Dox 或 Tet 激活的P-gp ATPase 活性. Dox 非竞争性抑制 Tet-激活的P-gp ATPase 活性. **结论**: 各种多药耐药逆转剂与P-gp相互作用的机制及其对P-gp ATPase 活性的影响各不相同. CsA、Ver 和 VCR 在血脑屏障P-gp上的结合部位可能是重叠的或者是有相互联系的, 而 CsA、Dox 和 Tet 与P-gp的结合是相互独立的, 并存在各自不同的结合部位.

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