BIOCHEMICAL PHARMACOLOGY 75 (2008) 973–980

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/biochempharm

Role of P-glycoprotein in accumulation and cytotoxicity of amrubicin and amrubicinol in MDR1 gene-transfected LLC-PK1 cells and human A549 lung adenocarcinoma cells

Asumi Hira [a], *Hiroshi Watanabe* [a], *Yukiko Maeda* [a], *Koji Yokoo* [a], *Emiko Sanematsu* [a], *Junko Fujii* [a], *Ji-ichiro Sasaki* [b], *Akinobu Hamada* [a], *Hideyuki Saito* [a,*]

[a] *Department of Pharmacy, Kumamoto University Hospital, 1-1-1 Honjo, Kumamoto 860-8556, Japan*
[b] *Department of Respiratory Medicine, Graduate School of Medical Science, Kumamoto University, 1-1-1 Honjo, Kumamoto 860-8556, Japan*

ARTICLE INFO

Article history:
Received 1 September 2007
Accepted 22 October 2007

Keywords:
Amrubicin
Amrubicinol
P-glycoprotein
Transporter

ABSTRACT

Amrubicin is a completely synthetic 9-aminoanthracycline agent for the treatment of lung cancer in Japan. The cytotoxicity of C-13 hydroxy metabolite, amrubicinol, is 10 to 100 times greater than that of amrubicin. The transporters responsible for the intracellular pharmacokinetics of amrubicin and amrubicinol remains unclear. This study was aimed to determine whether P-glycoprotein (P-gp) plays functional and preventive role in cellular accumulation and cytotoxicity of amrubicin and its active metabolite amrubicinol by *in vitro* transport and toxicity experiments. Cytotoxicity and intracellular accumulation of amrubicin and amrubicinol were evaluated by LLC-PK1 cells, MDR1 gene-transfected LLC-PK1 (L-MDR1) cells overexpressing P-gp, and human A549 lung adenocarcinoma cells. L-MDR1 cells showed 6- and 12-fold greater resistance to amrubicin and amrubicinol, respectively, than the parental LLC-PK1 cells. The intracellular accumulation of both drugs in L-MDR1 cells was significantly reduced compared to the LLC-PK1 cells. The basal-to-apical transepithelial transport of both drugs markedly exceeded, whereas the apical-to-basal transport of both drugs was significantly lower in L-MDR1 cells than LLC-PK1 cells. Cyclosporin A (CyA) restored the sensitivity, intracellular accumulation and transport activity for both drugs in L-MDR1 cells. In A549 cells, CyA significantly increased the intracellular accumulation and cytotoxicity of both drugs. These findings indicated that P-gp is responsible for cellular accumulation and cytotoxicity of both amrubicin and amrubicinol, therefore suggesting that the antitumor effect of amrubicin could be affected by the expression level of P-gp in lung cancer cells in chemotherapeutic treatments.

1. Introduction

Amrubicin is a completely synthetic 9-aminoanthracycline agent for the treatment of small-cell lung cancer (SCLC) and non-small-cell lung cancer (NSCLC) in Japan [1]. Its antitumor activity was found to be more potent than that of doxorubicin in nude mice transplanted with various human tumor xenografts [2]. In a phase II clinical trial of amrubicin, the response rates were 75.8% and 18.3% against SCLC and NSCLC, respectively [3,4]. In general, anthracyclines are inactivated

* *Corresponding author*. Tel.: +81 96 373 5820; fax: +81 96 373 5820.
E-mail address: saitohide@fc.kuh.kumamoto-u.ac.jp (H. Saito).

Abbreviations: MDR, multidrug resistance; P-gp, P-glycoprotein; CyA, cyclosporine A; MRP, multidrug-resistance-associated protein.

0006-2952/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2007.10.023

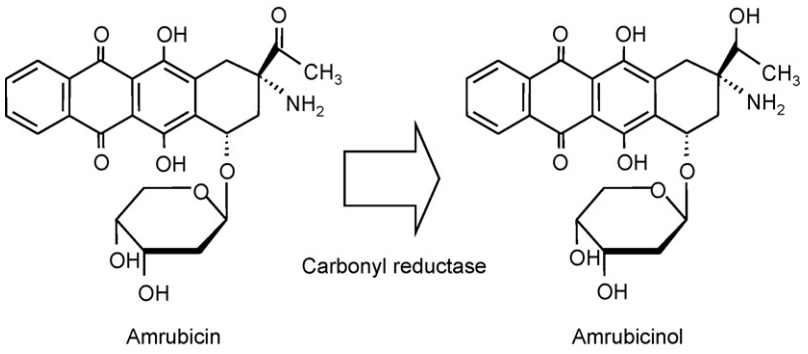

Fig. 1 – Chemical structures of amrubicin and amrubicinol.

following conversion to the C-13 hydroxy metabolite by carbonyl reductase [5,6]. As with other anthracyclines, amrubicin is also reduced to its C-13 hydroxy metabolite, amrubicinol (Fig. 1). However, the cytotoxicity of amrubicinol is 10–100 times greater than that of amrubicin *in vitro* [7]. The major toxicity of amrubicin is severe myelosupression, whereas the cardiotoxicity caused by amrubicin is relatively moderate [8,9]. Meanwhile, in a clinical pharmacokinetic study of amrubicin, we found that the apparent total clearance of amrubicin showed a large interindividual variability [10]. Although there may be many potential factors that contribute to pharmacokinetic variation, the interindividual variability of enzymatic conversion of amrubicin to amrubicinol appeared to be small in the previous clinical study [10].

ATP-binding cassette (ABC) transporters are ATP-binding site containing membrane proteins that function as drug efflux pumps. Many ABC transporters have been identified, including P-glycoprotein (P-gp), multidrug-resistance-associated proteins (MRPs) and breast cancer resistance protein (BCRP). P-gp, encoded by the MDR1 gene, is a broad-spectrum multidrug efflux pump that recognizes various compounds, including antitumor drugs [11]. P-gp is also present in normal tissues, such as the gastrointestinal tract, kidney, liver and at the blood–brain barrier [12]. Studies have shown that P-gp affects the pharmacokinetics of many drugs by limiting their absorption from the gastrointestinal tract and by secreting them into the bile and urine [13,14]. Moreover, P-gp expressed in tumor tissue exerts an effect upon the efficacy of certain chemotherapeutic agents. Most cancers derived from tissues that express P-gp show resistance to chemotherapy [15], and P-gp expression is thought to be associated with a poor prognosis in leukemia and breast cancer [16,17]. Therefore, it is important to clarify the involvement of P-gp on both the pharmacokinetics and antitumor efficacy of such drugs.

Most anthracyclines have been shown to serve as substrates for multidrug efflux pumps, such as P-gp, MRP1, MRP2 and other ABC transporters [18,19]. In a previous study, the intracellular accumulation of amrubicin and amrubicinol was studied using murine leukemia P388 cells and P388/ADR cells, which are resistant to doxorubicin. The intracellular accumulation of amrubicinol appeared to be decreased in P388/ADR cells compared with the parental P388 cells, suggesting that amrubicinol is a substrate for efflux transporters [20]. However, the transporters responsible for the efflux of amrubicin and amrubicinol have not been precisely determined. The present study was aimed to determine whether P-gp plays functional and preventive roles in cellular accumulation and cytotoxic effects of amrubicin and amrubicinol by using P-gp-overexpressing renal epithelial cells and human lung adenocarcinoma cells.

2. Material and methods

2.1. Materials

Amrubicin and amrubicinol were provided by Dainippon Sumitomo Pharma Co. Ltd. (Osaka, Japan). Cyclosporine A (CyA) was provided by Novartis Pharma (Basal, Switzerland). All other chemicals were of the highest purity available.

2.2. Cell culture

Porcine kidney epithelial LLC-PK1 and L-MDR1 cells transfected with human MDR1 cDNA (generous gifts from Dr. Erin G. Schuetz, St. Jude Children's Research Hospital, Memphis, TN, USA) were cultured in complete medium consisting of Medium 199 supplemented with 10% fetal bovine serum (FBS). Human lung A549 cells were provided by Institute of Development, Aging and Cancer, Tohoku University. The cells were maintained in RPMI 1640 medium supplemented with 10% FBS. All cells were grown under an atmosphere of 5% CO_2–95% air at 37 °C.

2.3. Cytotoxicity assay

The sensitivity of the cells was determined using the Alamar Blue assay [21]. Briefly, cells (1×10^4 cells/mL) were seeded in 96-well plates and allowed to attach and grow for 24 h prior to drug treatment. Subsequently, the cells were exposed to various concentrations of amrubicin or amrubicinol with or without 5 μM or 10 μM CyA. After 72 h, 11 μL of Alamar blue was added to each well of the plates, which were then incubated under the culture conditions for an additional 3.5 h. Fluorescence was measured using a fluorescence plate reader (Fluoroscan Ascent; Labsystem, Helsinki, Finland) with excitation at 540 nm and emission at 590 nm.

2.4. Intracellular drug accumulation

The cells (1×10^5 cells/mL) were seeded on 60 mm dishes and grown for 3 days. The culture medium was replaced with fresh medium 10 min before drug accumulation experiments. At the end of pre-incubation, the cells were exposed to medium containing 2 μM of amrubicin or amrubicinol for 20 min. In the inhibition study, 1 μM or 5 μM CyA was added to the medium. After the drug treatments, the medium containing drugs was aspirated and the cells were washed twice with ice-cold phospate-buffered saline (PBS). To extract the intracellular drugs, the cells were immersed in 1 mL of 50% methanol for 1 h at room temperature. The extract solutions were centrifuged at 13,000 rpm for 5 min and the supernatants were analyzed by HPLC. The cell monolayers were solubilized in 1 mL of 1 N NaOH and the protein content was determined using a Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA, USA). The drug accumulation and retention studies in A549 cells were carried out similarly. For uptake studies, medium containing 2 μM amrubicin or amrubicinol was removed after 5, 15, 30 and 60 min of drug treatment. For retention studies, cells were exposed to drugs for up to 60 min, washed with ice-cold PBS and re-incubated in warm culture medium in the absence of amrubicin and amrubicinol. At certain intervals, the cells were examined for their intracellular drug concentrations by HPLC.

2.5. Transepithelial transport assay

The transport assays of amrubicin and amrubicinol were performed as described previously [22]. The cells were seeded at a density of 1×10^6 cells/mL in polycarbonate membrane Transwell® plates and grown for 3 days in complete medium with one medium replacement. In the drug transport experiments, after removal of the culture medium from both sides of the cell monolayers, the cells were pre-incubated for 1 h with fresh medium. After the pre-incubation, the experiment was started by replacing the medium with 2 mL of complete medium containing 2 μM of amrubicin or amrubicinol at either the apical or the basal side of the cell layer. In the inhibition study, CyA was added to the medium of both sides. The cells were incubated at 37 °C in 5% CO_2, and 250 μL aliquots were taken from the opposite compartment at 10, 30, 60, 90, 180 and 240 min. The samples were centrifuged at 13,000 rpm for 5 min and the supernatants were measured by HPLC.

2.6. HPLC analysis

The concentrations of amrubicin and amrubicinol were determined by HPLC as described previously (10). Briefly, the sample was combined with a mixture of acetonitrile and 50 mM sodium phosphate (pH 3.0) containing 2% of tetramethylammonium chloride (27: 73, v/v). A portion (200 μL) of the solution was injected into the HPLC system. Separation was performed on a C_{18} reversed-phase column (Sumipax ODS A-212, 150 by 6 mm, with a particle size of 4 μm; Sumika Chemical Analysis Service, Osaka, Japan) at 40 °C. The mobile phase was a 1:30:80 (v/v/v) mixture of tetrahydrofuran, acetonitrile, and 10 mM sodium phosphate buffer (pH 2.6). The fluorescence detector was set at excitation and emission wavelengths of 465 nm and 560 nm, respectively.

2.7. Statistical analysis

Statistical analysis was performed by Student's *t*-test, or by the one-way analysis of variance (ANOVA) followed by Tukey test, where multiple comparisons against the control were necessary.

3. Results

3.1. Cytotoxicity of amrubicin or amrubicinol in LLC-PK1 and L-MDR1 cells

We examined whether expression of P-gp confers resistance to cytotoxicity of amrubicin and amrubicinol using MDR1 cDNA-transfected LLC-PK1 (L-MDR1) cells. As shown in Table 1, the IC_{50} values for LLC-PK1 and L-MDR1 cells treated with amrubicin were 112 nM and 630 nM, respectively. Thus, L-MDR1 cells were observed to be 5.6-fold more resistant to amrubicin than were LLC-PK1 cells. For amrubicinol, the IC_{50} value in L-MDR1 cells was 79 nM, which represents an 11.8-fold greater degree of resistance than observed in LLC-PK1 cells (7 nM). Additionally, the effect of CyA, a typical inhibitor of P-gp, on the cytotoxicity of the drugs was also evaluated. The cytotoxicity of amrubicin and amrubicinol in L-MDR1 cell carrying P-gp was restored by CyA.

3.2. Intracellular accumulation of amrubicin and amrubicinol

The involvement of P-gp on the intracellular accumulation of amrubicin and amrubicinol was next examined. The cells were incubated with 2 μM amrubicin or amrubicinol for 20 min. The intracellular accumulation of amrubicin or amrubicinol in L-MDR1 cells was reduced to 30–70% of the accumulation found in the parental LLC-PK1 cells (Fig. 2). In addition, CyA treatment enhanced the intracellular accumulation of both amrubicin and amrubicinol in L-MDR1 cells in a dose-dependent manner. Significant differences of intracel-

Table 1 – The IC_{50} values for amrubicin or amrubicinol in LLC-PK1 and L-MDR1 cells

	IC_{50} (nM)		
	LLC-PK1	L-MDR1	Fold resistance
Amrubicin	112 ± 31	630 ± 72	5.63
Amrubicin + CyA (5 μM)	232 ± 32	197 ± 39	0.85
Amrubicinol	7 ± 1	79 ± 24	11.8
Amrubicinol + CyA (5 μM)	7 ± 1	7 ± 1	0.97

Fold resistance, the ratio of IC_{50} in L-MDR1 cells to IC_{50} in LLC-PK1 cells. All values represent the means ± S.D. for at least three separate experiments.

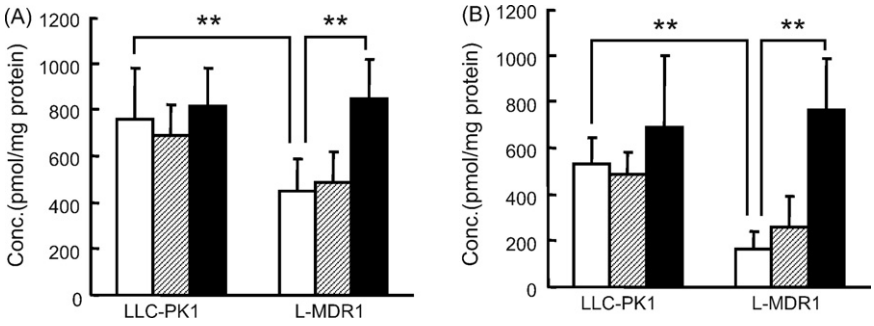

Fig. 2 – Intracellular accumulation of amrubicin (A) and amrubicinol (B) in LLC-PK1 cells and L-MDR1 cells. Cells were incubated in the absence (open column) or presence of 1 μM (shaded column) or 5 μM (closed column) CyA in combination with 2 μM amrubicin or amrubicinol. Cellular drug content was measured by HPLC. Each point represents the mean ± S.D. of four independent measurements. The significance differences was determined by Tukey test following ANOVA. $^{**}p < 0.01$, compared to L-MDR1 cells without CyA.

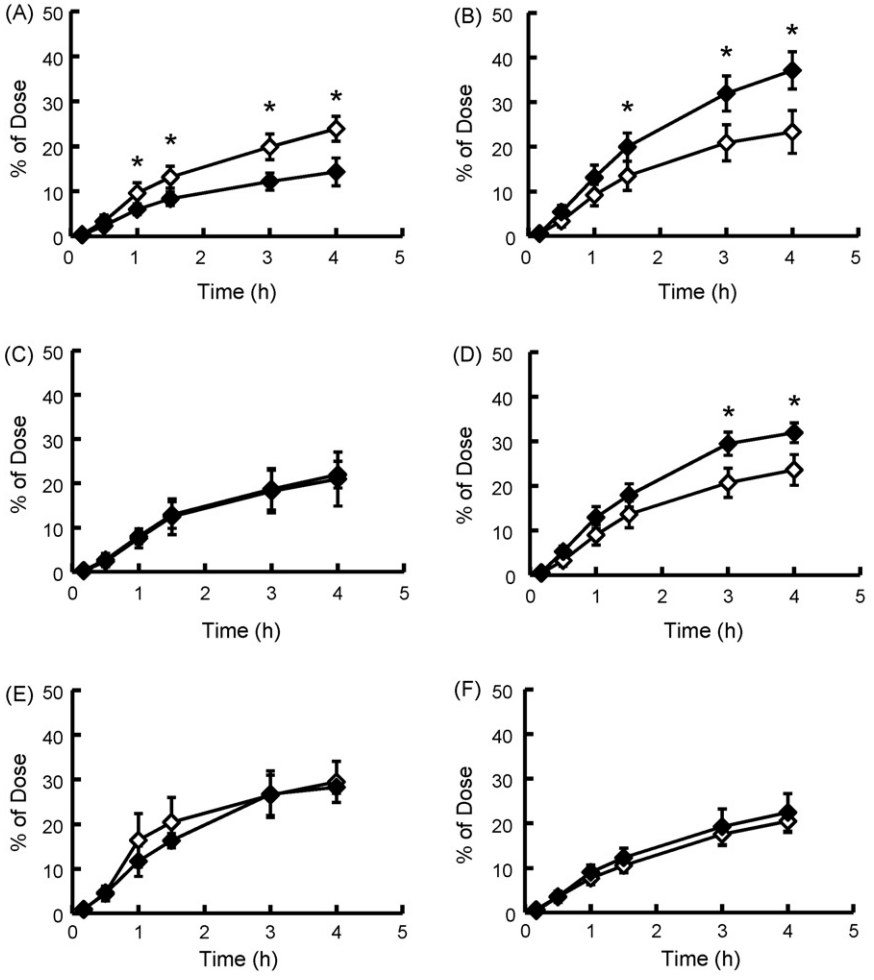

Fig. 3 – Transcellular transport of amrubicin in LLC-PK1 cells and L-MDR1 cells. The medium containing 2 μM of amrubicin was added to either apical (A, C, and E) or basal (B, D, and F) side of the cell monolayer in LLC-PK1 cells (open symbol) or L-MDR1 cells (closed symbol). Samples were taken from the opposite side at each time. In the inhibition study, 1 μM (C and D) or 5 μM (E and F) of CyA was added to both apical and basal sides. Each data point represents the mean ± S.D. of 3–4 independent measurements. $^{*}p < 0.05$, compared to LLC-PK1 cells.

lular accumulation were observed when the cells were treated with 5 μM CyA.

3.3. Transepithelial transport of amrubicin and amrubicinol

LLC-PK1 and L-MDR1 cells were cultured in Transwell® dish, and compartment-to-compartment transport of drugs was compared in these two cell lines. Figs. 3 and 4 show the time profiles of transepithelial transport of amrubicin or amrubicinol across LLC-PK1 and L-MDR1 monolayers. For amrubicin, the basal-to-apical transport in L-MDR1 cells significantly exceeded that observed in LLC-PK1 cells, whereas the apical-to-basal transport was lower in L-MDR1 cells (Fig. 3). The transport profiles of amrubicinol were similar to that of amrubicin (Fig. 4). The ratio of basal-to-apical transport versus apical-to-basal transport of amrubicin and amrubicinol at 4 h was 2.4 and 13.2 in L-MDR1 cells, respectively. Furthermore, CyA did not affect the basal-to-apical or apical-to-basal transport of amrubicin in LLC-PK1 cells, but decreased the basal-to-apical transport of amrubicin in L-MDR1 cells. In contrast, the basal-to-apical transport of amrubicinol in L-MDR1 cells showed a tendency to decrease by the treatment with CyA in a dose-dependent manner.

3.4. Intracellular accumulation and cytotoxicity of amrubicin and amrubicinol in lung carcinoma cells

We next investigated the effects of CyA treatment on the cytotoxicity and intracellular accumulation of amrubicin and amrubicinol using human lung carcinoma A549 cells. Intracellular drug accumulation was measured at various times during a 60 min incubation in the presence of amrubicin and amrubicinol, and at various times during a further 60 min incubation after removal of the drugs (Fig. 5). CyA increased the intracellular accumulation and retention of amrubicin and amrubicinol. In particular, the accumulation of amrubicinol generated by the intracellular metabolism of amrubicin was markedly increased (Fig. 5B). Furthermore, the cytotoxicities of amrubicin and amrubicinol in A549 cells were also enhanced

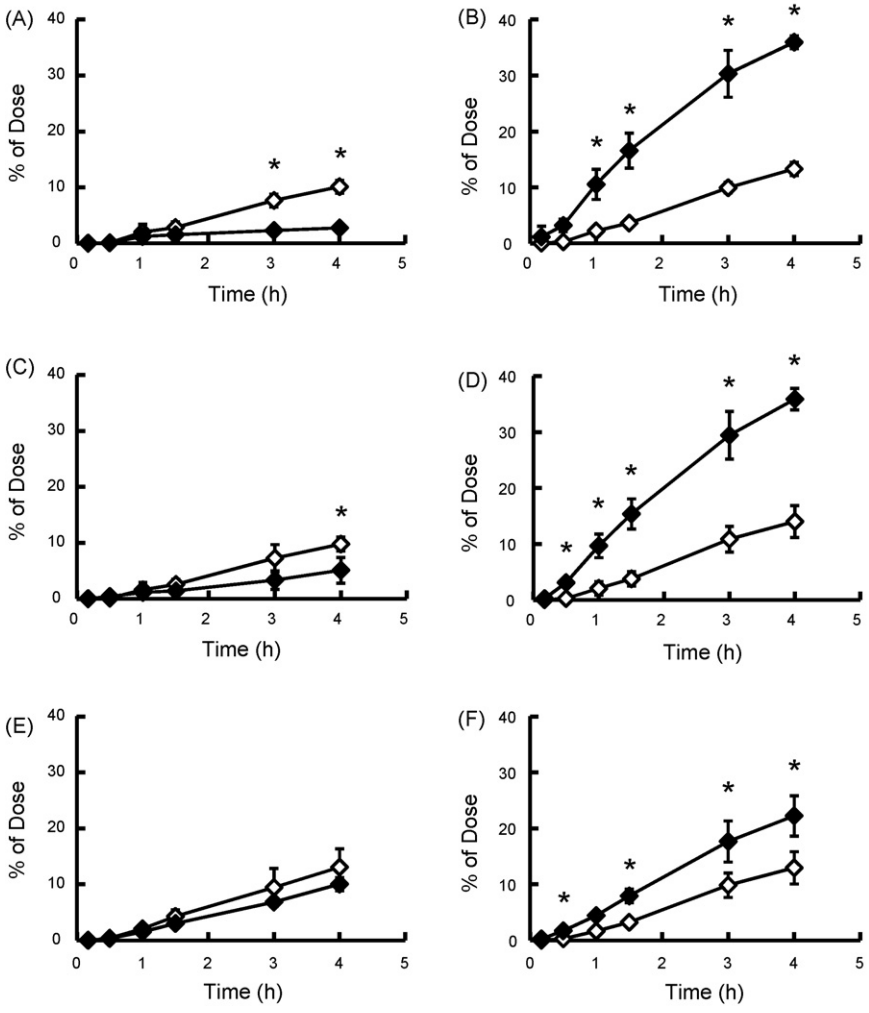

Fig. 4 – Transcellular transport of amrubicinol in LLC-PK1 cells and L-MDR1 cells. The medium containing 2 μM of amrubicinol was added to either apical (A, C, and E) or basal (B, D, and F) side of the cell monolayer in LLC-PK1 cells (open symbol) or L-MDR1 cells (closed symbol). Samples were taken from the opposite side at each time. In the inhibition study, 1 μM (C and D) or 5 μM (E and F) of CyA was added to both sides. Each data point represents the mean ± S.D. of 3–4 independent measurements. *$p < 0.05$, compared to LLC-PK1 cells.

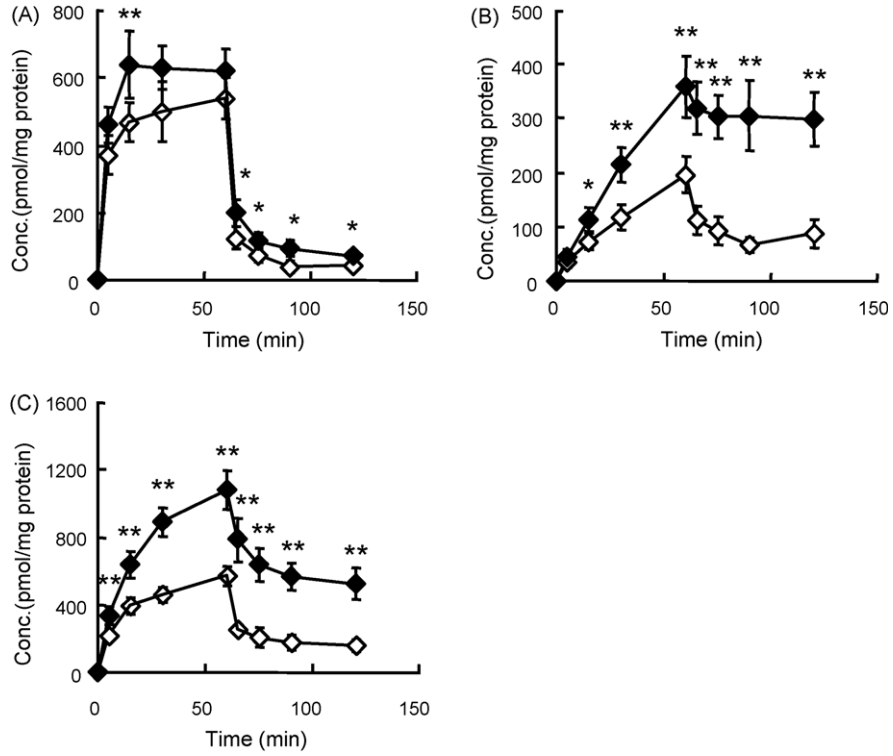

Fig. 5 – Accumulation and efflux of amrubicin (A), amrubicinol formed from amrubicin (B) and amrubicinol (C) in A549 cells. Cells were incubated with 2 μM of amrubicin or amrubicinol in absence (open symbol) or presence (closed symbol) of 10 μM CyA. Each point represents the mean ± S.D. of four independent measurements. $^{*}p < 0.05$, $^{**}p < 0.01$, compared to the treatment of amrubicin or amrubicinol without CyA.

by treatment with 5 μM CyA (Table 2). CyA reduced the IC50 values by 50% and 70% for amrubicin and amrubicinol, respectively.

4. Discussion

The dosage of most anticancer drugs including amrubicin is generally determined on the basis of body surface area of patients [23]. On the other hand, a previous study showed that body surface area-based dosing did not lead to a decrease in interindividual variability in the clearance of 28 out of 33 potential anticancer agents [24]. In fact, a large interindividual variability in the apparent clearance of amrubicin was observed in clinical pharmacokinetic study of amrubicin, suggesting that factors other than body surface area could be important determinants of its systemic clearance [10]. In this study, we focused on drug efflux pumps such as P-gp as a potential pharmacokinetic and toxicity determinant factor, and we thus investigated P-gp-mediated transport of amrubicin and amrubicinol and their cytotoxicity.

Table 2 – The IC_{50} values for amrubicin and amrubicinol in A549 cells

	IC_{50} (nM)		
	Without CyA	With CyA (10 μM)	Fold resistance
Amrubicin	2360 ± 162	1230 ± 142	1.92
Amrubicinol	130 ± 18	40 ± 9	3.25

Fold resistance, the ratio of IC_{50} without CyA to IC_{50} with CyA. All values represent the means ± S.D. for at least three separate experiments.

The cytotoxicity and intracellular accumulation of amrubicin in P-gp overexpressed L-MDR1 cells were significantly decreased compared to those in LLC-PK1 cells (Table 1 and Fig. 2). On the other hand, 5 μM CyA increased the cytotoxicity of amrubicin and amrubicinol in L-MDR1 cells. In addition, transepithelial transport studies showed that the basal-to-apical transport of amrubicin was increased in L-MDR1 cells, and that it was inhibited by treatment with CyA (Fig. 3). Similar results for amrubicinol were obtained (Fig. 4). These results clearly indicated that both amrubicin and amrubicinol served as substrates for P-gp. In addition, the fold resistance and the decrease of intracellular accumulation of amrubicinol in L-MDR1 cells were greater than those of amrubicin (Table 1, Fig. 2). Furthermore, the transport ratio of amrubicinol at 4 h in L-MDR1 cells was 6 times greater than that of amrubicin, suggesting that cellular accumulation of amrubicinol might be more affected by P-gp (Figs. 3 and 4).

Ross et al. reported the effects of P-gp on the cytotoxicity and intracellular accumulation of idarubicin [25], daunorubicin (DNR) and the corresponding C-13 alcohol metabolites, idarubicinol (IDAol) and daunorubicinol (DNRol) using HL-60 human

leukemia cells and a vicristine-resistant subline, HL-60/Vinc cells, overexpressing P-gp [25]. They found that the diminution of intracellular accumulation of each drug in HL-60/Vinc cells in the order of susceptibility to P-gp was DNRol ≫ DNR > IDAol > IDA. Our results were consistent with this study in terms of greater susceptibility to alcohol metabolites.

We next investigated the effect of CyA on the cytotoxicity and intracellular accumulation of amrubicin and amrubicinol using human lung carcinoma A549 cells. The intracellular accumulation of amrubicin or amrubicinol was increased by CyA, suggesting the involvement of P-gp in the transport of amrubicin. However, as CyA is known to inhibit not only P-gp but also other ABC transporters such as MRP1 and MRP2, we need to take the effect of other efflux transporters on the transport of amrubicin and amrubicinol in A549 cells into consideration. In a previous clinical study, P-gp levels in lung cancer cells and the antitumor effects of drugs have been reported: the levels of P-gp expression in lung cancer cells from 17 chemo-naive patients were found to be inversely correlated with cisplatin/etoposide chemotherapy response [26]. A high expression of P-gp as well as MRP1 could be associated with a poor clinical outcome in chemotherapeutic treatment of lung cancer. Moreover, another study reported that P-gp polymorphisms and their haplotypes were associated with a response to chemotherapy in SCLC patients [27]. Although the variation in expression or activity of P-gp in lung cancer could affect the sensitivity to amrubicin, a detailed in vivo study would be required to predict the clinical outcome of amrubicin. On the other hand, since expression of MRP, BCRP and other ABC-transporters were also reported in A549 cells [28,29]. In particular, MRP1 is known to be expressed ubiquitously, especially in lung cancer. Furthermore, CyA is not only a potent inhibitor of P-gp but also inhibits other transporters including MRP1 [30]. We need to examine whether amrubicin is a substrate of other transporters involved in multidrug resistance, and to determine whether these transporters have an impact on the cytotoxity and/or intracellular accumulation of amrubicin in A549 cells. Further study is required to elucidate the contribution of P-gp to the pharmacokinetics of amrubicin. Greater than 70% of amrubicin is eliminated by biliary excretion. The variation of biliary excretion of amrubicin could be an important factor affecting its pharmacokinetics. However, it is yet unclear whether P-gp mediates the biliary excretion of amrubicin. The determination of the effects of P-gp on the pharmacokinetics of amrubicin should lead to a better understanding of the factors contributing to interindividual variability observed in our previous clinical study [10].

In conclusion, P-gp is responsible for cellular accumulation and cytotoxicity of amrubicin and amrubicinol, suggesting that the antitumor efficacy of amrubicin is affected by the expression level of P-gp in lung cancer cells in chemotherapy. Furthermore, P-gp could play a pharmacokinetic role in disposition of both amrubicin and amrubicinol in patients.

Acknowledgments

This work was supported in part by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (JSPS) for Hideyuki Saito (KAKENHI 17390158) and Akinobu Hamada (KAKENHI 19590149). We would like to thank Mr. Yusuke Matsunaga for the helpful technical suggestion for HPLC assay.

REFERENCES

[1] Ogawa M. Novel anticancer drugs in Japan. J Cancer Res Clin Oncol 1999;125:134–40.
[2] Hanada M, Noguchi T, Murayama T. Profile of the antitumor effects of amrubicin, a completely synthetic anthracycline. Nippon Yakurigaku Zasshi 2003;122:141–50.
[3] Takeda K, Takifuji N, Negoro S, Furuse K, Nakamura S, Takada Y, et al. Phase II study of amrubicin, 9-aminoanthracycline, in patients with advanced non-small-cell lung cancer: a West Japan Thoracic Oncology Group (WJTOG) study. Invest New Drugs 2007;25:377–83.
[4] Yana T, Negoro S, Takada M, Yokota S, Takada Y, Sugiura T, et al. Phase II study of amrubicin in previously untreated patients with extensive-disease small cell lung cancer: West Japan Thoracic Oncology Group (WJTOG) study. Invest New Drugs 2007;25:253–8.
[5] Bernardini N, Giannessi F, Bianchi F, Dolfi A, Lupetti M, Zaccaro L, et al. Comparative activity of doxorubicin and its major metabolite, doxorubicinol, on V79/AP4 fibroblasts: a morphofunctional study. Exp Mol Pathol 1991;55:238–50.
[6] Kuffel MJ, Reid JM, Ames MM. Anthracyclines and their C-13 alcohol metabolites: growth inhibition and DNA damage following incubation with human tumor cells in culture. Cancer Chemother Pharmacol 1992;30:51–7.
[7] Yamaoka T, Hanada M, Ichii S, Morisada S, Noguchi T, Yanagi Y. Cytotoxicity of amrubicin, a novel 9-aminoanthracycline, and its active metabolite amrubicinol on human tumor cells. Jpn J Cancer Res 1998;89:1067–73.
[8] Suzuki T, Minamide S, Iwasaki T, Yamamoto H, Kanda H. Cardiotoxicity of a new anthracycline derivative (SM-5887) following intravenous administration to rabbits: comparative study with doxorubicin. Invest New Drugs 1997;15:219–25.
[9] Noda T, Watanabe T, Kohda A, Hosokawa S, Suzuki T. Chronic effects of a novel synthetic anthracycline derivative (SM-5887) on normal heart and doxorubicin-induced cardiomyopathy in beagle dogs. Invest New Drugs 1998;16:121–8.
[10] Matsunaga Y, Hamada A, Okamoto I, Sasaki J, Moriyama E, Kishi H, et al. Pharmacokinetics of amrubicin and its active metabolite amrubicinol in lung cancer patients. Ther Drug Monit 2006;28:76–82.
[11] van Helvoort A, Smith AJ, Sprong H, Fritzsche I, Schinkel AH, Borst P, et al. MDR1 P-glycoprotein is a lipid translocase of broad specificity, while MDR3 P-glycoprotein specifically translocates phosphatidylcholine. Cell 1996;87:507–17.
[12] Sai Y, Tsuji A. Transporter-mediated drug delivery: recent progress and experimental approaches. Drug Discov Today 2004;9:712–20.
[13] Sparreboom A, van Asperen J, Mayer U, Schinkel AH, Smit JW, Meijer DK, et al. Limited oral bioavailability and active epithelial excretion of paclitaxel (Taxol) caused by P-glycoprotein in the intestine. Proc Natl Acad Sci USA 1997;94:2031–5.
[14] Schinkel AH, Mayer U, Wagenaar E, Mol CA, van Deemter L, Smit JJ, et al. Normal viability and altered pharmacokinetics in mice lacking mdr1-type (drug-transporting) P-glycoproteins. Proc Natl Acad Sci USA 1997;94:4028–33.

[15] Fojo AT, Ueda K, Slamon DJ, Poplack DG, Gottesman MM. Pastan I. Expression of a multidrug-resistance gene in human tumors and tissues. Proc Natl Acad Sci USA 1987;84:265–9.
[16] van der Kolk DM, de Vries EG, van Putten WJ, Verdonck LF, Ossenkoppele GJ, Verhoef GE, et al. P-glycoprotein and multidrug resistance protein activities in relation to treatment outcome in acute myeloid leukemia. Clin Cancer Res 2000;6:3205–14.
[17] Mechetner E, Kyshtoobayeva A, Zonis S, Kim H, Stroup R, Garcia R, et al. Levels of multidrug resistance (MDR1) P-glycoprotein expression by human breast cancer correlate with in vitro resistance to taxol and doxorubicin. Clin Cancer Res 1998;4:389–98.
[18] Tanaka K, Hirai M, Tanigawara Y, Ueda K, Takano M, Hori R, et al. Relationship between expression level of P-glycoprotein and daunorubicin transport in LLC-PK1 cells transfected with human MDR1 gene. Biochem Pharmacol 1997;53:741–6.
[19] Tada Y, Wada M, Migita T, Nagayama J, Hinoshita E, Mochida Y, et al. Increased expression of multidrug resistance-associated proteins in bladder cancer during clinical course and drug resistance to doxorubicin. Int J Cancer 2002;98:630–5.
[20] Yamaoka T, Hanada M, Ichii S, Morisada S, Noguchi T, Yanagi Y. Uptake and intracellular distribution of amrubicin, a novel 9-amino-anthracycline, and its active metabolite amrubicinol in P388 murine leukemia cells. Jpn J Cancer Res 1999;90:685–90.
[21] Back SA, Khan R, Gan X, Rosenberg PA, Volpe JJ. A new Alamar Blue viability assay to rapidly quantify oligodendrocyte death. J Neurosci Methods 1999;91:47–54.
[22] Schinkel AH, Wagenaar E, van Deemter L, Mol CA, Borst P. Absence of the mdr1a P-glycoprotein in mice affects tissue distribution and pharmacokinetics of dexamethasone, digoxin, and cyclosporin A. J Clin Invest 1995;96:1698–705.
[23] Sawyer M, Ratain MJ. Body surface area as a determinant of pharmacokinetics and drug dosing. Invest New Drugs 2001;19:171–7.
[24] Baker SD, Verweij J, Rowinsky EK, Donehower RC, Schellens JH, Grochow LB, et al. Role of body surface area in dosing of investigational anticancer agents in adults, 1991–2001. J Natl Cancer Inst 2002;94:1883–8.
[25] Ross DD, Doyle LA, Yang W, Tong Y, Cornblatt B. Susceptibility of idarubicin, daunorubicin, and their C-13 alcohol metabolites to transport-mediated multidrug resistance. Biochem Pharmacol 1995;50:1673–83.
[26] Triller N, Korosec P, Kern I, Kosnik M, Debeljak A. Multidrug resistance in small cell lung cancer: expression of P-glycoprotein, multidrug resistance protein 1 and lung resistance protein in chemo-naive patients and in relapsed disease. Lung Cancer 2006;54:235–40.
[27] Sohn JW, Lee SY, Lee SJ, Kim EJ, Cha SI, Kim CH, et al. MDR1 polymorphisms predict the response to etoposide-cisplatin combination chemotherapy in small cell lung cancer. Jpn J Clin Oncol 2006;36:137–41.
[28] Kang HK, Lee E, Pyo H, Lim SJ. Cyclooxygenase-independent down-regulation of multidrug resistance-associated protein-1 expression by celecoxib in human lung cancer cells. Mol Cancer Ther 2005;4:1358–63.
[29] Imai Y, Nakane M, Kage K, Tsukahara S, Ishikawa E, Tsuruo T, et al. C421A polymorphism in the human breast cancer resistance protein gene is associated with low expression of Q141K protein and low-level drug resistance. Mol Cancer Ther 2002;1:611–6.
[30] Qadir M, O'Loughlin KL, Fricke SM, Williamson NA, Greco WR, Minderman H, et al. Cyclosporin A is a broad-spectrum multidrug resistance modulator. Clin Cancer Res 2005;11:2320–6.