Biochemical Pharmacology, Vol. 53, pp. 741–746, 1997.

ISSN 0006-2952/97/$17.00 + 0.00
PII S0006-2952(96)00810-6

Relationship between Expression Level of P-Glycoprotein and Daunorubicin Transport in LLC-PK$_1$ Cells Transfected with Human *MDR1* Gene

Kumiko Tanaka, Midori Hirai,*† Yusuke Tanigawara,*‡*
Kazumitsu Ueda,§ Mikihisa Takano, Ryohei Hori*‖ and Ken-ichi Inui*¶*

*Department of Pharmacy, Faulty of Medicine, Kyoto University Hospital; and §Laboratory of Biochemistry, Department of Agricultural Chemistry, Faculty of Agriculture, Kyoto University, Kyoto 606-01, Japan

ABSTRACT. P-Glycoprotein-mediated transcellular transport and intracellular accumulation of [^{3}H]daunorubicin were examined in cell monolayers with different levels of P-glycoprotein. The porcine kidney epithelial cell line LLC-PK$_1$ was transfected with MDR1 cDNA, and four sublines, LLC-GA5, LLC-GA5-VLB4, LLC-GA5-COL10, and LLC-GA5-COL150, were obtained by culturing the cells in the absence or in the presence of 4 ng/mL vinblastine, 10 ng/mL colchicine, and 150 ng/mL colchicine, respectively. Western blot analysis showed a large difference in P-glycoprotein expression within these sublines. The degree of drug resistance was dependent on the expression level of P-glycoprotein. The amount of the unidirectional transport of [^{3}H]daunorubicin by P-glycoprotein corresponded to the expression level of P-glycoprotein, which was followed by the decrease in intracellular accumulation of the agent. The concentration of cyclosporin A required for the inhibition of P-glycoprotein-mediated transport of [^{3}H]daunorubicin was higher in cells with a high expression of P-glycoprotein. These findings suggest that the transport of daunorubicin by P-glycoprotein and its inhibition by cyclosporin A correspond to the expression level of P-glycoprotein. BIOCHEM PHARMACOL 53;5:741–746, 1997.

KEY WORDS. P-glycoprotein; multidrug resistance; daunorubicin; cyclosporin A; transcellular transport

Drug resistance is a major obstacle in the chemotherapy of human cancer. P-Glycoprotein is considered an important factor in multidrug resistance; this membrane-bound protein pumps a wide variety of anticancer agents out of the cells and lowers their intracellular levels [1, 2]. Some studies *in vitro* show that the expression levels of P-glycoprotein correlate with the level of resistance [3–6]. Furthermore, the concentration of cyclosporin A required for overcoming multidrug resistance is dependent on the expression level of P-glycoprotein [6]. However, these studies were focused on the relationship between P-glycoprotein expression and drug resistance, but not on the transporting activity of P-glycoprotein. We have demonstrated previously that several compounds including daunorubicin are unidirectionally transported by P-glycoprotein in the transcellular transport system with cell monolayers that hyperexpress P-glycoprotein on the apical side [7, 8]. In the present study, the cell monolayers with different levels of P-glycoprotein were utilized to elucidate the relationship between P-glycoprotein expression level and P-glycoprotein-mediated transport of [^{3}H]daunorubicin, and to examine the inhibitory effect of cyclosporin A on [^{3}H]daunorubicin transport.

MATERIALS AND METHODS

Materials

[^{3}H]Daunorubicin (162.8 GBq/mmol) and [*methoxy*-^{14}C]inulin (0.61 GBq/mmol) were purchased from Du Pont-New England Nuclear (Boston, MA, U.S.A.). [*Mebmt*-β-^{3}H]cyclosporin A (433 GBq/mmol) was purchased from Amersham International plc (Buckinghamshire, U.K.). Cyclosporin A was supplied by Sandoz Pharmaceutical (Basel, Switzerland). Daunorubicin hydrochloride was purchased from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). Vinblastine sulfate and colchicine were purchased from Nacalai Tesque (Kyoto, Japan). All other chemicals were of the highest purity available.

† Present address: Department of Pharmaceutics, Kobe Pharmaceutical University, Higashinada-ku, Kobe 658, Japan.
‡ Present address: Department of Pharmacy, Kobe University Hospital, Kobe 650, Japan.
‖ Present address: Pharmaceutical Research and Technology Institute, Kinki University, 3-4-1 Kowakae, Higashi-osaka 577, Japan.
¶ Corresponding author: Professor Ken-ichi Inui, Department of Pharmacy, Kyoto University Hospital, Sakyo-ku, Kyoto 606-01, Japan. Tel. 81-75-751-3577; FAX 81-75-751-4207.
Received 10 June 1996; accepted 4 September 1996.

Cell Lines and Cell Culture

LLC-PK_1 cells, derived from porcine kidney, were transfected with human MDR1 cDNA by calcium phosphate precipitation, and a stable clone designated LLC-GA5 was obtained [9]. This clone was cultured in the absence or in the presence of agents; LLC-GA5 was cultured in the absence of agents, LLC-GA5-VLB4 was cultured in the presence of 4 ng/mL vinblastine, LLC-GA5-COL10 in the presence of 10 ng/mL colchicine, and LLC-GA5-COL150 in the presence of 150 ng/mL colchicine. These cells were propagated in medium 199 (Flow Laboratories, Rockville, MD, U.S.A.) supplemented with 10% fetal bovine serum, and subcultured every 4–5 days for LLC-PK_1, LLC-GA5, LLC-GA5-VLB4, and LLC-GA5-COL10, and 6–8 days for LLC-GA5-COL150. KB8-5, a resistant subline of human epidermal carcinoma [10], was cultured in Dulbecco's modified Eagle's medium (GIBCO, Life Technologies, Grand Island, NY, U.S.A.) supplemented with 10% fetal bovine serum, and subcultured every 4–5 days.

Resistance to Anticancer Agents

Drug sensitivity of the cells was examined by MTT* assay [11]. After 4 days of cell culture in the logarithmic growth period in the absence or presence of anticancer agents, MTT was added to each well, followed by incubation for 4 hr. Then the medium was discarded; blue formazan, a reduced form of MTT, was dissolved in dimethyl sulfoxide; and the UV absorption was measured at 540 nm.

Preparation of Plasma Membrane and Western Blotting

Cells were disrupted in isotonic buffer by nitrogen cavitation, and membrane fractions were obtained by sucrose gradient centrifugation [12]. Aliquots (20 μg) of the membrane fraction from LLC-PK_1, LLC-GA5, LLC-GA5-VLB4, LLC-GA5-COL10, and LLC-GA5-COL150 cells were run in 7% SDS–PAGE, were electroblotted onto nitrocellulose, and were reacted with anti-P-glycoprotein antibody C219. ECL western blotting detection reagents (Amersham International plc) were used in the detection procedure.

Transcellular Transport and Intracellular Accumulation Study

Transcellular transport study was done as previously reported [7]. Briefly, LLC-PK_1, LLC-GA5, LLC-GA5-VLB4, LLC-GA5-COL10, and LLC-GA5-COL150 cells were seeded on microporous polycarbonate membrane filters (3 μm pore size, 4.71 cm^2 growth area) inside Transwell™ cell chambers (Costar, Cambridge, MA, U.S.A.). For LLC-GA5-VLB4, vinblastine was added at a final concentration of 4 ng/mL, and for LLC-GA5-COL10 and LLC-GA5-COL150 cells, colchicine was added at a final concentration of 10 and 150 ng/mL, respectively. Cells were incubated for 3 nights, and medium was replaced by drug-free medium 6 hr before the experiment. To measure the transcellular transport, the medium on either the basal or the apical side of the monolayer was replaced with medium containing [^{3}H]daunorubicin together with [^{14}C]inulin, and the medium on the opposite side was taken at 1, 2, and 3 hr. In either experiment, the paracellular flux monitored by the transcellular transport of [^{14}C]inulin in both directions was less than 5% in 3 hr. After the last sampling, the cells were washed with 4 mL of ice-cold medium and lysed in 1 mL of 0.3 N NaOH, and the intracellular accumulation of the radiolabeled agents was also measured. To examine the effect of cyclosporin A on the transcellular transport and the intracellular accumulation of [^{3}H]daunorubicin, cyclosporin A was added on both sides of the monolayers 1 hr before the start of the experiments.

To evaluate the intracellular level of cyclosporin A, [*mebmt*-^{3}H]cyclosporin A was added at various concentrations on both sides of the monolayers and, after incubation for 1 hr, the radioactivity of [*mebmt*-^{3}H]cyclosporin A accumulated in the cells was examined.

RESULTS

Western blotting was performed to examine the expression of P-glycoprotein in LLC-PK_1 and the transfectant cells (Fig. 1). In LLC-PK_1, LLC-GA5, and LLC-GA5-VLB4 cells, P-glycoprotein was not clearly detectable. On the other hand, P-glycoprotein was detected clearly at 170 kDa in LLC-GA5-COL10 and LLC-GA5-COL150 cells, and also in KB8-5 cells. The amount of P-glycoprotein detected in LLC-GA5-COL10 cells was less, while the amount of P-glycoprotein in LLC-GA5-COL150 cells was more, than that in KB8-5 cells.

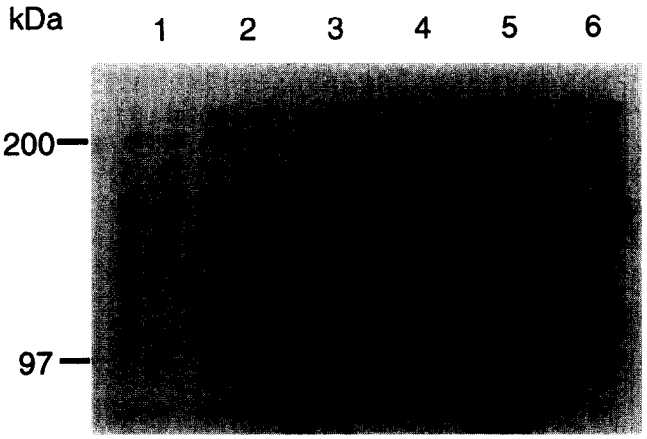

FIG. 1. Western blot analysis of membrane fractions from LLC-PK_1 (lane 1), LLC-GA5 (lane 2), LLC-GA5-VLB4 (lane 3), LLC-GA5-COL10 (lane 4), LLC-GA5-COL150 (lane 5), and KB8-5 (lane 6). Membrane fractions from the cells were added to the lanes and reacted with monoclonal antibody C219 as a probe for P-glycoprotein. Molecular size standards are indicated in kilodaltons on the left.

* *Abbreviations:* MTT, 3-(4,5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide; and ECL, enhanced chemiluminescence.

Growth inhibitory effects of three anticancer agents, daunorubicin, vinblastine, and colchicine, were examined by MTT assay in the five cell lines (Table 1). The IC_{50} values of daunorubicin, vinblastine, and colchicine for LLC-GA5 cells were similar to those for LLC-PK_1 cells. However, for LLC-GA5-VLB4 and LLC-GA5-COL10 cells, the IC_{50} values of these agents were 3- to 15-fold and 5- to 23-fold higher than for LLC-PK_1 cells, respectively. The IC_{50} for LLC-GA5-COL150 cells was 31- to 160-fold higher.

As we reported previously, P-glycoprotein is specifically expressed on the apical membrane in LLC-GA5-COL150 cells, and the monolayers of this subline showed unidirectional transport of daunorubicin from the basal to the apical side [7]. In the present study, the transcellular transport of [^{3}H]daunorubicin was examined in the five sublines (Fig. 2, A–E). In LLC-PK_1 and LLC-GA5 cells, the transcellular transport in both directions, from the basal to apical side and from the apical to basal side, was similar. On the other hand, unidirectional transport was observed in LLC-GA5-VLB4, LLC-GA5-COL10, and LLC-GA5-COL150 cells. In these cell lines, the transport from the basal to apical side increased and the transport in the opposite direction decreased, compared with those in LLC-PK_1 cells. The amount of the unidirectional transport was comparable to the level of P-glycoprotein examined by western blotting [LLC-GA5-COL150 (Fig. 1, lane 5) > LLC-GA5-COL10 (Fig. 1, lane 4) > LLC-GA5-VLB4 (Fig. 1, lane 3)].

The effect of 1 μM cyclosporin A on transcellular transport of [^{3}H]daunorubicin was examined in cells with P-glycoprotein expression (Fig. 3). As an index for the P-glycoprotein-mediated transport, net basal-to-apical transport was calculated by subtracting the apical-to-basal transport from the basal-to-apical transport, and was shown in the percentage of [^{3}H]daunorubicin applied. In LLC-PK_1 and LLC-GA5 cells, the net basal-to-apical transport of [^{3}H]daunorubicin was small in the absence and presence of cyclosporin A. In LLC-GA5-VLB4 cells, the net basal-to-apical transport of [^{3}H]daunorubicin in the presence of cyclosporin A was reduced by 85% relative to that in the absence of cyclosporin A. In LLC-GA5-COL10 cells, the net basal-to-apical transport was reduced by 70% by cyclosporin A, indicating a weaker effect compared with that in LLC-GA5-VLB4 cells. In LLC-GA5-COL150 cells, the effect of cyclosporin A was much smaller; transport was decreased by only 9%.

The effect of 1 μM cyclosporin A on the intracellular accumulation of [^{3}H]daunorubicin was also examined simultaneously within these cells (Fig. 4). In the absence of cyclosporin A, the intracellular accumulation in LLC-PK_1 and LLC-GA5 cells was the highest within the cells examined. In LLC-GA5-VLB4, the intracellular accumulation was 67% of that in LLC-PK_1 cells. [^{3}H]Daunorubicin accumulation in LLC-GA5-COL10 and LLC-GA5-COL150 cells decreased to 29 and 6% of that in LLC-PK_1 cells, respectively. In the presence of cyclosporin A, the intracellular accumulation of [^{3}H]daunorubicin in LLC-PK_1 and LLC-GA5 cells did not decrease. The intracellular accumulation of [^{3}H]daunorubicin in LLC-GA5-VLB4 cells in the presence of 1 μM cyclosporin A was the same as the level in the LLC-PK_1 cells, and in LLC-GA5-COL10 cells, it was also similar to that in the LLC-PK_1 cells. However, in LLC-GA5-COL150 cells, the accumulation of [^{3}H]daunorubicin was less than one-fifth of that in LLC-PK_1 cells, although the amount of the intracellular accumulation itself was 2.7-fold higher than that in LLC-GA5-COL150 cells in the absence of cyclosporin A.

The concentration dependence of the inhibitory effect of cyclosporin A on [^{3}H]daunorubicin transport was examined in LLC-GA5-COL150 cells. With 1 μM cyclosporin A, the directional transport of [^{3}H]daunorubicin was affected only slightly, but with 3 μM cyclosporin A, the transport was decreased to 35%, and with 5 μM cyclosporin A, it was decreased to 24% of that without cyclosporin A (Fig. 5A). The intracellular accumulation was increased slightly with 1 μM cyclosporin A, while it was increased by 12-fold with 3 or 5 μM cyclosporin A (Fig. 5B).

The intracellular accumulation of cyclosporin A was examined in LLC-GA5-VLB4, LLC-GA5-COL10, and LLC-GA5-COL150 cells (Fig. 6A). When 1 μM cyclosporin A was added on both sides of the monolayers, the accumulation was in the decreasing order of LLC-GA5-VLB4 > LLC-GA5-COL10 > LLC-GA5-COL150, which was inversely related with the expression level of P-glycoprotein. The intracellular accumulation of various concentrations of cyclosporin A was also investigated in LLC-GA5-COL150 cells (Fig. 6B). The concentration-dependence of cyclosporin A accumulation in LLC-GA5-COL150 cells was ob-

TABLE 1. Resistance against anticancer agents

	IC_{50} (ng/mL)		
Cells	**Daunorubicin**	**Vinblastine**	**Colchicine**
LLC-PK_1	4.0 ± 1.9	2.8 ± 0.5	7.9 ± 1.1
LLC-GA5	8.5 ± 6.6	3.5 ± 1.0	6.1 ± 0.4
LLC-GA5-VLB4	28.1 ± 11.7	40.9 ± 7.6	28.2 ± 1.3
LLC-GA5-COL10	77.0 ± 17.0	63.8 ± 9.0	42.8 ± 4.2
LLC-GA5-COL150	578.4 ± 163.1	373.4 ± 163.3	238.9 ± 51.2

Values represent the means ± SEM of four independent experiments.

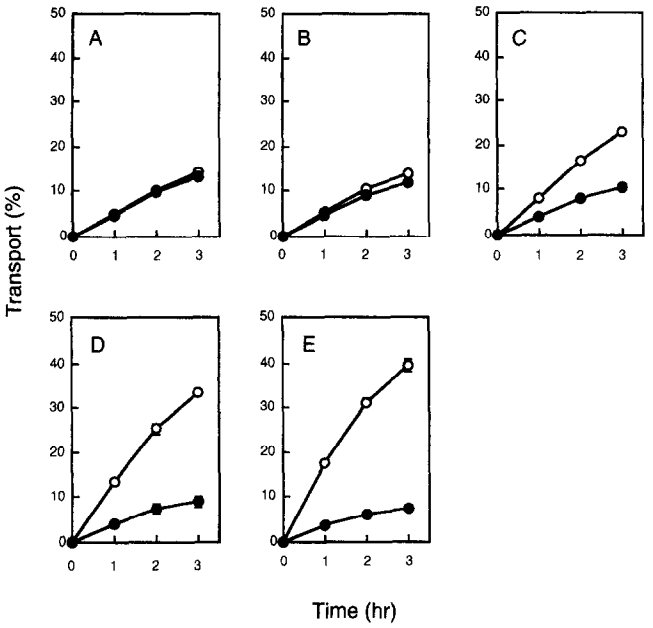

FIG. 2. Transcellular transport of [^{3}H]daunorubicin in LLC-PK$_1$ (A), LLC-GA5 (B), LLC-GA5-VLB4 (C), LLC-GA5-COL10 (D), and LLC-GA5-COL150 (E) cells. [^{3}H]Daunorubicin (100 nM, 2 mL) was added to either the basal or the apical side of the monolayers. The open circles (○) represent the transport from basal to apical side, and the solid circles (●) represent the transport from apical to basal side. Each point represents the mean ± SEM of at least three independent experiments.

served; the accumulation increased as the concentration increased.

DISCUSSION

We have examined the relationship between P-glycoprotein expression and P-glycoprotein-mediated transport of anticancer agents utilizing the transcellular transport system. The porcine kidney epithelial cell line LLC-PK$_1$ forms a highly polarized monolayer with microvilli and tight junctions. Moreover, P-glycoprotein, introduced externally as MDR1 cDNA, is localized specifically in the apical membrane, resulting in a unidirectional transport from basal to apical side of the agents that are transported by P-glycoprotein [9]. In this study, the P-glycoprotein-mediated transport was comparable to the expression level of P-

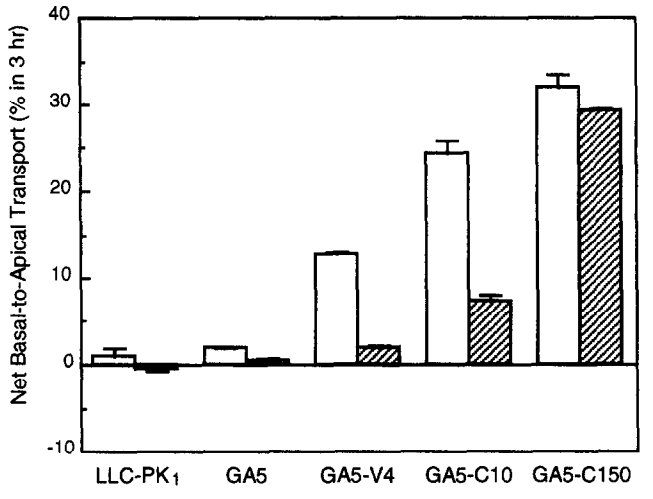

FIG. 3. Net basal-to-apical transport of [^{3}H]daunorubicin and the effect of 1 μM cyclosporin A in LLC-PK$_1$, LLC-GA5, LLC-GA5-VLB4, LLC-GA5-COL10, and LLC-GA5-COL150 cells. [^{3}H]Daunorubicin (100 nM, 2 mL) was added to either the basal or the apical side of the monolayers. Net basal-to-apical transport was calculated by subtracting [^{3}H]daunorubicin transported from apical to basal side from [^{3}H]daunorubicin transported from basal to apical side, and was shown as the percentage of total [^{3}H]daunorubicin applied in each well. Open columns and hatched columns represent [^{3}H]daunorubicin transport in the absence and in the presence of cyclosporin A, respectively. Key: (GA5) LLC-GA5; (GA5-V4) LLC-GA5-VLB4; (GA5-C10) LLC-GA5-COL10; and (GA5-C150) LLC-GA5-COL150. Each column represents the mean ± SEM of at least three independent experiments.

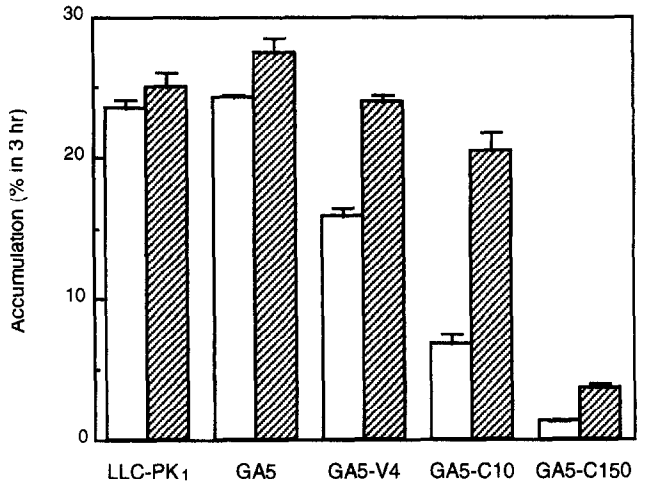

FIG. 4. Intracellular accumulation of [^{3}H]daunorubicin and the effect of 1 μM cyclosporin A in LLC-PK$_1$, LLC-GA5, LLC-GA5-VLB4, LLC-GA5-COL10, and LLC-GA5-COL150 cells. [^{3}H]Daunorubicin (100 nM, 2 mL) was added to the basal side of the monolayers, and the intracellular accumulation at 3 hr was measured. Open columns and hatched columns represent the accumulation of [^{3}H]daunorubicin in the absence and in the presence of cyclosporin A, respectively. Key: (GA5) LLC-GA5; (GA5-V4) LLC-GA5-VLB4; (GA5-C10) LLC-GA5-COL10; and (GA5-C150) LLC-GA5-COL150. Each column represents the mean ± SEM of at least three independent experiments.

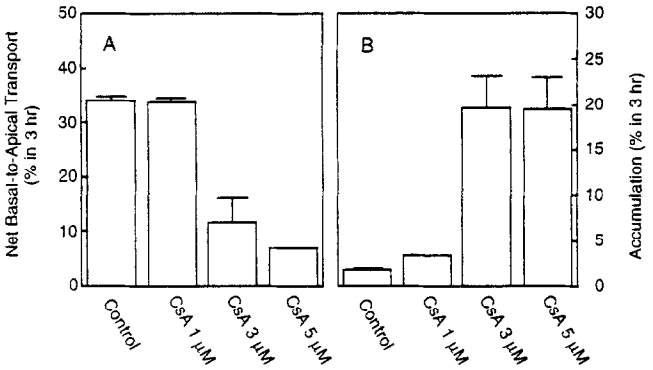

FIG. 5. Concentration dependence of the effect of cyclosporin A on transport and accumulation of [^{3}H]daunorubicin in LLC-GA5-COL150 cells. (A) Effect of cyclosporin A on the net basal-to-apical transport of [^{3}H]daunorubicin. Net basal-to-apical transport was calculated as shown in Fig. 3. (B) Effect of cyclosporin A on the intracellular accumulation of [^{3}H]daunorubicin. [^{3}H]Daunorubicin (100 nM, 2 mL) was added to the basal side of the monolayers, and the intracellular accumulation at 3 hr was measured. Each column represents the mean ± SEM of at least three independent experiments.

glycoprotein; sublines with high levels of P-glycoprotein showed a large increase in basal-to-apical transport of daunorubicin, accompanied by a large decrease in intracellular accumulation. Since the degree of resistance to anticancer agents was also dependent on the expression level of P-glycoprotein in these sublines, these findings suggest that the expression level of P-glycoprotein determines the degree of resistance by regulating the intracellular level of anticancer agents.

We have also examined the effect of cyclosporin A on the P-glycoprotein-mediated transport of [^{3}H]daunorubicin in these sublines with different levels of P-glycoprotein expression. Our findings show that in cells with a higher level of P-glycoprotein expression, the inhibitory effect on P-glycoprotein-mediated transport of [^{3}H]daunorubicin was smaller than that in the cells with lower expression. Besides the potent activity to modulate multidrug resistance, cyclosporin A is a substrate of P-glycoprotein and is excreted into the extracellular space, resulting in a lower intracellular level in P-glycoprotein expressing cells [13]. In this study, the intracellular level of cyclosporin A was also comparable to the expression level of P-glycoprotein. Moreover, the increase in the intracellular level of cyclosporin A by raising its concentration resulted in a potent inhibition of the transcellular transport of [^{3}H]daunorubicin in LLC-GA5-COL150 cells. Therefore, the intracellular level of cyclosporin A seemed to determine the inhibitory effect of P-glycoprotein-mediated transport, again implying the importance of the P-glycoprotein-mediated efflux of the agents.

Although P-glycoprotein-mediated resistance to anticancer agents has been well characterized in preclinical models, its expression in clinical tumors is not as well characterized. Interindividual differences of MDR1 mRNA levels are often large within tumors [14, 15]. It is also suggested that heterogeneity within a tumor may exist at the cellular level, that is, there may exist populations of cells that may or may not express the *MDR1* gene [16, 17]. Hence, when the modulators of P-glycoprotein for reversal of resistance are used, the effect of modulators that are transported by P-glycoprotein may vary among individuals or even within the cellular level, according to the expression levels of P-glycoprotein. The modulators that are not transported by P-glycoprotein would be more appropriate for clinical use.

The cells utilized in this report were transfected with a vector that contained MDR1 cDNA and the adenosine deaminase gene as a selectable marker [9]. Although the clones in which the vector was introduced stably were selected with 9-β-D-xylofuranosyl adenine and 2′-deoxycoformycin, the presence of colchicine or vinblastine in the culture medium was required for the expression of P-glycoprotein. Moreover, the concentration of the anticancer agents affected the expression level of P-glycoprotein [2]. There are three possibilities to explain this phenomenon. The first possibility is that the expression of the protein itself was heterogeneous within the cells and only those possessing a certain amount of P-glycoprotein were able to survive in the presence of either agent. The second possibility is that P-glycoprotein was induced or positively regulated by the agents coexisting in the culture medium. Although anticancer agents can activate the promoter region of the *MDR1* gene [18], this is unlikely in the present study, since the vector introduced does not contain the promoter region of the *MDR1* gene [19]. Some events that occur at or after transcription may have been influenced. The third possibility is that duplication or amplification of the expression vector has occurred under the selective pressure of the cytotoxic agents.

Since we utilized the cells transfected with human P-

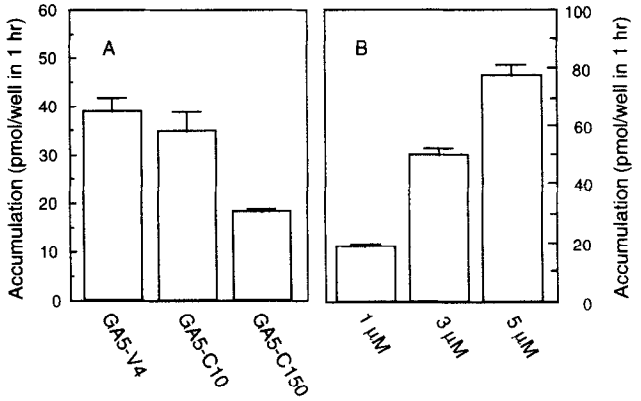

FIG. 6. Intracellular accumulation of [*mebmt*-β-^{3}H]cyclosporin A. (A) Intracellular accumulation of 1 μM [*mebmt*-β-^{3}H]cyclosporin A in LLC-GA5-VLB4, LLC-GA5-COL10, and LLC-GA5-COL150 cells. Key: (GA5-V4) LLC-GA5-VLB4; (GA5-C10) LLC-GA5-COL10; and (GA5-C150) LLC-GA5-COL150. (B) Intracellular accumulation of various concentrations of [*mebmt*-β-^{3}H]cyclosporin A in LLC-GA5-COL150 cells. Each column represents the mean ± SEM of at least three independent experiments.

glycoprotein, we consider that the resultant drug-resistant cell lines were expressing human P-glycoprotein from the result of western blotting (Fig. 1). However, we cannot rule out the possibility that porcine P-glycoprotein was induced under the selective pressure of anticancer agents, because the monoclonal antibody C219 utilized in this study might cross-react with porcine P-glycoprotein. Similarly, it may be possible that other transporters that participate in drug resistance, such as multidrug resistance-associated protein, were induced in resistant sublines, although we did not examine this in the present study.

In conclusion, the transport of daunorubicin by P-glycoprotein and its inhibition by cyclosporin A correspond to the expression of P-glycoprotein.

This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan, and by a Grant-in-Aid from the Tokyo Biochemical Research Foundation.

References

1. Roninson IB (Ed.), *Molecular and Cellular Biology of Multidrug Resistance in Tumor Cells*. Plenum Press, New York, 1991.
2. Gottesman MM and Pastan I, Biochemistry of multidrug resistance mediated by the multidrug transporter. *Annu Rev Biochem* **62:** 385–427, 1993.
3. Ganapathi R, Kuo T, Teeter L, Grabowski D and Ford J, Relationship between expression of P-glycoprotein and efficacy of trifluoperazine in multidrug resistance. *Mol Pharmacol* **39:** 1–8, 1991.
4. Choi K, Frommel TO, Stern RK, Perez CF, Kriegler M, Tsuruo T and Roninson IB, Multidrug resistance after retroviral transfer of the human *MDR1* gene correlates with P-glycoprotein density in the plasma membrane and is not affected by cytotoxic selection. *Proc Natl Acad Sci USA* **88:** 7386–7390, 1991.
5. Nielsen D, Maare C and Skovsgaard T, Kinetics of daunorubicin transport in Ehrlich ascites tumor cells with different expression of P-glycoprotein: Influence of verapamil. *Biochem Pharmacol* **47:** 2125–2135, 1994.
6. Ishida Y, Shimada Y and Shinoyama M, Synergistic effect of cyclosporin A and verapamil in overcoming vincristine resistance of multidrug resistant cultured human leukemia cells. *Jpn J Cancer Res* **81:** 834–841, 1990.
7. Hirai M, Tanaka K, Shimizu T, Tanigawara Y, Yasuhara M, Hori R, Kakehi Y, Yoshida O, Ueda K, Komano T and Inui K, Cepharathin, a multidrug resistant modifier, is a substrate for P-glycoprotein. *J Pharmacol Exp Ther* **275:** 73–78, 1995.
8. Tanaka K, Hirai M, Tanigawara Y, Yasuhara M, Hori R, Ueda K and Inui K, Effect of cyclosporin analogues and FK506 on transcellular transport of daunorubicin and vinblastine via P-glycoprotein. *Pharm Res* **13:** 1081–1085, 1996.
9. Ueda K, Okamura N, Hirai M, Tanigawara Y, Saeki T, Kioka N, Komano T and Hori R, Human P-glycoprotein transports cortisol, aldosterone, and dexamethasone, but not progesterone. *J Biol Chem* **267:** 24248–24252, 1992.
10. Akiyama S, Fojo A, Hanover JA, Pastan I and Gottesman MM, Isolation and genetic characterization of human KB cell lines resistant to multiple drugs. *Somat Cell Mol Genet* **11:** 117–126, 1985.
11. Carmichael J, DeGraff WG, Gazdar AF, Minna JD and Mitchell JB, Evaluation of a tetrazolium-based semiautomated colorimetric assay: Assessment of chemosensitivity testing. *Cancer Res* **47:** 936–942, 1987.
12. Cornwell MM, Gottesman MM and Pastan I, Increased vinblastine binding to membrane vesicles from multidrug-resistant KB cells. *J Biol Chem* **261:** 7921–7928, 1986.
13. Saeki T, Ueda K, Tanigawara Y, Hori R and Komano T, Human P-glycoprotein transports cyclosporin A and FK506. *J Biol Chem* **268:** 6077–6080, 1993.
14. Goldstein LJ, Galski H, Fojo A, Willingham M, Lai S-L, Gazdar A, Pirker R, Green A, Crist W, Brodeur GM, Lieber M, Cossman J, Gottesman MM and Pastan I, Expression of a multidrug resistance gene in human cancers. *J Natl Cancer Inst* **81:** 116–124, 1989.
15. Noonan KE, Beck C, Holzmayer TA, Chin JE, Wunder JS, Andrulis IL, Gazdar AF, Willman CL, Griffith B, Von Hoff DD and Roninson IB, Quantitative analysis of *MDR1* (multidrug resistance) gene expression in human tumors by polymerase chain reaction. *Proc Natl Acad Sci USA* **87:** 7160–7164, 1990.
16. Nooter K and Herweijer H, Multidrug resistance (*mdr*) genes in human cancer. *Br J Cancer* **63:** 663–669, 1991.
17. Goldstein L, Clinical reversal of drug resistance. *Curr Probl Cancer* **19:** 67–124, 1995.
18. Kohno K, Sato S, Takano H, Matsuo K and Kuwano M, The direct activation of human multidrug resistance gene (*MDR1*) by anticancer agents. *Biochem Biophys Res Commun* **165:** 1415–1421, 1989.
19. Kane SE, Reinhard DH, Fordis CM, Pastan I and Gottesman MM, A new vector using the multidrug resistance gene as a selective marker enables overexpression of foreign genes in eukaryotic cells. *Gene* **84:** 439–446, 1989.