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Correlation of expression levels of P-glycoprotein with resistance to adriamycin in a renal adenocarcinoma cell line

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Abstract We have demonstrated that low-level expression of P-glycoprotein (PGP), detectable by reverse transcriptase–polymerase chain reaction (RT-PCR) assay and flow cytometric assay, is an important factor in multidrug resistance (MDR) in ACHN cancer cells. In this study, we established a subline highly resistant to adriamycin (ACHN/ADM) from ACHN cells, and determined the correlation between PGP levels and MDR levels using ACHN/ADM cells and their parent ACHN cells. The ACHN/ADM cells showed overexpression of PGP, and sensitivity to antitumor agents was lower than that found in ACHN cells. Intracellular accumulation of ADM in ACHN/ADM cells was approximately half the amount of its accumulation in ACHN cells. Sensitivity to ADM in ACHN/ADM cells was enhanced by chemosensitizers with an increase in intracellular ADM accumulation. These results indicate that PGP levels correlate with MDR levels and suggest that chemotherapy using chemosensitizers might be effective in the treatment of renal cancers with overexpression of PGP.

Key words Renal adenocarcinoma · ACHN · P-glycoprotein · Multidrug resistance

Introduction

Resistance to multiple chemotherapeutic agents is a major problem in the treatment of human renal cancers [17]. One possible mechanism of multidrug resistance (MDR) is the expression of P-glycoprotein (PGP), which causes a decrease in intracellular accumulation of antitumor agents [4, 6, 9]. Many attempts have been made to detect PGP in cell lines and clinical specimens resistant to several agents, and overexpression of PGP has frequently been demonstrated [3, 7, 10].

The ACHN human renal adenocarcinoma cell line [2] is one of the intrinsic multidrug-resistant cells in which sensitivity to antitumor agents is enhanced by chemosensitizers. However, ACHN cells have been considered to be PGP negative because PGP expression has not been detected by immunohistochemical staining [12, 15]. Recently, we have successfully detected low-level expression of PGP in the ACHN cell line by means of a highly sensitive reverse transcriptase–polymerase chain reaction (RT-PCR) and flow cytometric analysis [11]. This low-level expression of PGP, detectable by sensitive methods, might contribute to creating cells resistant to antitumor agents. However, an assessment explaining the association between PGP level and MDR level was lacking in our previous study. In this current study, we established a subline highly resistant to adriamycin (ACHN/ADM) from ACHN cells and clarified the correlation between PGP level and MDR level.

Materials and methods

Cells and cell culture

ACHN human renal adenocarcinoma cell line [2] was obtained from the American Type Culture Collection (Rockville, Md.). Cells were grown in a monolayer in minimum essential medium (MEM; Nissui Seiyaku, Tokyo, Japan) containing 10% fetal calf serum and kanamycin. Cultures were grown in 5% CO_2 under 100% humidity at 37°C, and the medium was renewed daily starting on day 7 after seeding. A subline highly resistant to adriamycin (ACHN/ADM) was isolated from the ACHN cells by culturing in progressively higher concentrations of ADM. Finally, the concentration of ADM in the medium reached to 0.64 μg/ml, and the subline was exposed continuously to this concentration of ADM.

Drugs and chemicals

The drugs were from the following sources: adriamycin (ADM), 5-fluorouracil (5-FU), mitomycin C (MMC) and medroxyproges-

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terone acetate (MPA) from Kyowa Hakko, Tokyo, Japan; etoposide (VP-16) and cisplatin (CDDP) from Nihon Kayaku, Tokyo, Japan; vinblastine sulfate (VLB) from Shionogi, Tokyo, Japan; verapamil hydrochloride from Eisai, Tokyo, Japan; cyclosporin A (CYA) from Sandoz, Basel, Switzerland; SN-38 from Yakult, Tokyo, Japan.

Determination of growth characteristics of ACHN and ACHN/ADM cells

Cells were seeded in fresh medium at a density of 10^4 cells per well in 24-well tissue culture plates. The population doubling time was judged by performing serial cell counts in triplicate.

Assay of cytotoxic effect of drugs

The cytotoxicity of antitumor agents was determined by the bioluminescence assay for ATP we have described previously [11]. Cytotoxicity was expressed as the IC50 value, defined as the concentration of drug causing a 50% reduction in control cells from the dose-response curves. Chemosensitizers were used to attempt to enhance the sensitivity to antitumor agents. The maximum concentration of chemosensitizer that showed no cytotoxicity in either type of cell (verapamil 2.5 μg/ml, CYA 0.5 μg/ml and MPA 1.0 μg/ml) was previously determined by the ATP assay.

Detection of PGP expression by Western blot hybridization

Immunoblot analysis with an antibody specific for PGP was performed as previously described [11]. ACHN/ADM cells in the exponential growth phase (10^7 cells per flask) were extracted from the solution of 50 mM TRIS-HCl, pH 8.0 and 1% NP-40. The fractions were centrifuged at 1500 *g* for 5 min and 100-μg aliquots of protein were electrophoresed, then transferred to nitrocellulose with an electroblotting system. The nitrocellulose membrane was blocked with a blocking buffer [0.01 M phosphate buffered saline, pH 7.2 (PBS) containing 3% bovine serum albumin] at 4°C overnight. Then the membrane was incubated with 1 μg/ml of C-219 anti-PGP antibody (Centocor, Malvern, Pa.) in fresh blocking buffer for 1 h at room temperature. After a 15-min wash with a washing buffer (PBS containing 0.05% Tween 20), the membrane was incubated with peroxidase-conjugated rabbit anti-mouse IgG (DAKO, Copenhagen, Denmark) in the blocking buffer for 1 h at room temperature. After another 15-min wash with washing buffer, the membrane was incubated in ECL solution (Amersham International, Bucks., UK) for 1 min at room temperature. Finally, the membrane was exposed to Polaroid 667 film.

Detection of PGP mRNA expression by RT-PCR-based assay

The mRNA was isolated from ACHN/ADM cells by Quick Prep Micro mRNA Purification Kit (Pharmacia, Uppsala, Sweden) according to the manufacturer's instructions. The mRNA from the final elution was precipitated with ethanol, dried and resuspended in 20 μl of 10 mM TRIS-HCl, pH 7.4, with 1 mM EDTA. To detect PGP mRNA and β-actin mRNA, 0.2 μg of mRNA was used for RT-PCR. Synthesis of cDNA from the isolated mRNA was performed with a First-Strand cDNA Synthesis Kit (Pharmacia), and PCR was performed using PGP-specific primers and internal control primers for β-actin as previously described [11]. The PCR with PGP-specific primers was expected to produce a DNA fragment of 327 bp.

A PCR mixture consisted of 10 μl of 10 × PCR buffer [500 mM KCl, 100 mM TRIS-HCl (pH 8.3), 15 mM $MgCl_2$, 0.01% gelatin], 1 μl of deoxynucleotide triphosphate mixture (20 mM concentrations each of dATP, dCTP, dGTP and dTTP), 5 μl of each oligonucleotide primer (4 μM), 0.5 μl of AmpliTaq DNA polymerase (5 units/μl Perkin Elmer Cetus, Norwalk, Conn.), 1/2 of the cDNA synthesis solution (7.5 μl) including 0.1 μg of the mRNA and 71 μl of distilled water. Finally, 100 μl of paraffin oil was added to prevent evaporation. Thirty of the following incubation cycles were performed: 1 min at 95°C, 30 s at 55°C and 30 s at 72°C. PCR products were electrophoresed on 3% NuSieve 3:1 agarose gel (FMC BioProducts, Rockland, Meo.) with *Hae*III-digested ϕ X 174 DNA molecular weight standards and visualized by ethidium bromide staining.

Detection of PGP expression by flow cytometry

For flow cytometric determination of PGP, monoclonal antibodies (C-219 and JSB-1; Funakoshi, Tokyo, Japan) and isotype control mouse monoclonal immunoglobulins [IgG2a (DAKO) for C-219 and IgG1 (DAKO) for JSB-1] were used. Cells in the exponential growth phase were washed and resuspended in PBS. Cell suspensions were fixed in 100% methanol at −20°C for 15 min; washed with 0.05% Tween 20 in PBS (TPBS); and incubated with C-219 (1.0 μg/ml), JSB-1 (1.0 μg/ml) or an isotype control (IgG2a, 1.0 μg/ml; IgG1, 1.0 μg/ml) at 4°C for 30 min. After incubation, the cells were washed with ice-cold TPBS, then incubated with 1.0 μg/ml fluorescein isothiocyanate (FITC)-labeled goat anti-mouse antibody (MONOSAN, Uden, The Netherlands) for 30 min. Next they were passed through a 50-μm nylon mesh and resuspended. Using a FACScan flow cytometer (Becton Dickinson, Mountain View, Calif.), the cells were excited with a 488 nm argon ion laser beam, and green fluorescence (FITC) was collected by a combination of a 560 dichroic mirror and 530 BP filters. From the FITC fluorescence histograms based on 10 000 gated cells, the mean channel numbers were calculated as a measure of the expression of PGP.

Uptake experiment of ADM

The intracellular ADM uptake experiment was performed by flow cytometric measurements as previously described [11]. ACHN and ACHN/ADM cells in the exponential growth phase (10^6 cells per flask) were used in this experiment. The medium was removed and fresh medium containing 10 μg/ml ADM was added with or without a chemosensitizer that showed no cytotoxicity in either type of cell. The cells in the medium were incubated for 30 min, 1 h and 2 h at 37°C. Following exposure to the drugs, the medium was removed and the monolayer washed twice with PBS. The cells were then trypsinized, the cell suspensions filtered on a 50 μm nylon mesh and resuspended in PBS to a concentration of 5×10^5 cells/ml. Using a FACScan flow cytometer, cells were excited with a 488 nm argon ion laser beam, and fluorescence intensity between 530 and 640 nm was evaluated. From the fluorescence histograms based on 10 000 gated cells, the mean channel numbers were calculated as a measure of the intracellular accumulation of ADM.

Statistical methods

Statistical analysis was conducted using a two-sample *t*-test with Welch's correction. All statistical comparisons were two-tailed and performed with significance set at $P < 0.05$.

Results

Growth characteristics of ACHN and ACHN/ADM cells

The growth characteristics of the two cell lines are shown in Fig. 1. While the population doubling time of ACHN cells was 32.4 h, ACHN/ADM cells exhibited a longer doubling time (49.2 h).

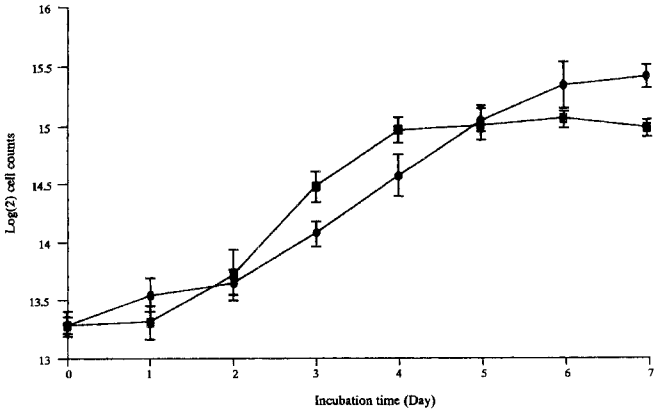

Fig. 1 Time course of cell growth of ACHN cells (*squares*) and ACHN/ADM cells (*circles*). While the population doubling time of ACHN cells was 32.4 h, ACHN/ADM cells exhibited a longer doubling time (49.2 h). The *bars* indicate SD values

Table 1 IC50 and relative resistance in ACHN and ACHN/ADM cells (*ADM* adriamycin, *VLB* vinblastine sulfate, *VP-16* etoposide, *CDDP* cisplatin, *mmc* mitomycin C, *5-FU* 5-fluorouracil)

Drugs	IC50 (μg/ml)[a]		Relative resistance[b]
	ACHN	ACHN/ADM	
ADM	0.12746	5.47017	42.92
VLB	0.01610	0.45561	28.30
VP-16	6.14954	69.48843	11.30
CDDP	1.14799	4.34191	3.78
MMC	0.73883	0.90748	1.23
5-FU	27.62393	33.66313	1.22
SN-38	0.00017	0.00039	2.29

[a] Assessed in triplicate by the ATP assay
[b] mean ratio or IC50 ACHN/ADM to IC50 ACHN

Drug sensitivity of ACHN and ACHN/ADM cells

The susceptibility of the two cell lines to the cytotoxic effects of antitumor agents, as determined by ATP assay, is shown in Table 1. In terms of IC50 values, ACHN/ADM cells were 43 times more resistant to ADM than ACHN cells. In addition, marked cross-resistance was found to VLB and VP-16 in ACHN/ADM cells.

P-glycoprotein expression detected by Western blot hybridization

The immunoblotting analysis is shown in Fig. 2. While no signal was detected from ACHN cells in our previous study, bands of PGP were detected at quantities as low as 1/8 × 100 μg protein prepared from ACHN/ADM cells.

PGP mRNA expression detected by RT-PCR

Detection of PGP mRNA by RT-PCR from ACHN/ADM cells is shown in Fig. 3. Various concentrations of

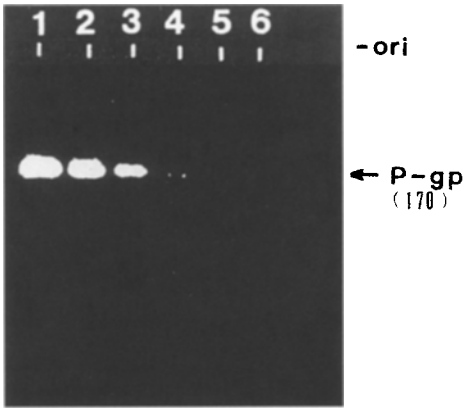

Fig. 2 Western blot hybridization analysis of P-glycoprotein (PGP) in ACHN/ADM cells. Lanes show the analyses for 100 μg (*lane 1*), 1/2 × 100 μg (*lane 2*), 1/4 × 100 μg (*lane 3*), 1/8 × 100 μg (*lane 4*), 1/16 × 100 μg (*lane 5*), 1/32 × 100 μg (*lane 6*) the fractions extracted from ACHN/ADM cells. Bands of PGP were detected at quantities as low as 1/8 × 100 μg of protein prepared from ACHN/ADM cells

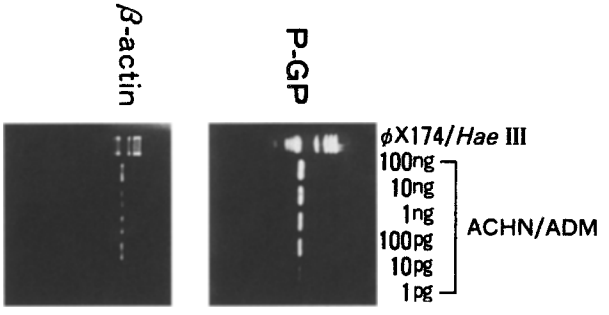

Fig. 3 Detection of PGP mRNA from ACHN/ADM cells. The marker lane was loaded with *Hae*III-digested φ X 174 DNA. Bands of PGP and β-actin were detected in quantities as low as 10 pg of mRNA prepared from ACHN/ADM cells

mRNA prepared from ACHN cells were loaded using primers specific for PGP mRNA or for β-actin. While bands of PGP and β-actin were detected in quantities as low as 100 pg of mRNA prepared from ACHN cells in our previous study, the ACHN/ADM cells in this current study gave a stronger signal for PGP mRNA, as low as 10 pg of mRNA.

P-glycoprotein expression detected by flow cytometry

Figure 4 illustrates the frequency histograms of fluorescence intensity for both cell lines after incubation with PGP-specific antibodies (C-219 and JSB-1) or isotype control immunoglobulins. Both cell lines expressed PGP with more than one logarithm difference in peak channel values between the isotype control and the sample treated with C-219 or JSB-1 antibody. ACHN/ADM cells showed a significant increase in reactivity with C-219 or JSB-1 antibody in comparison with ACHN cells ($P < 0.05$).

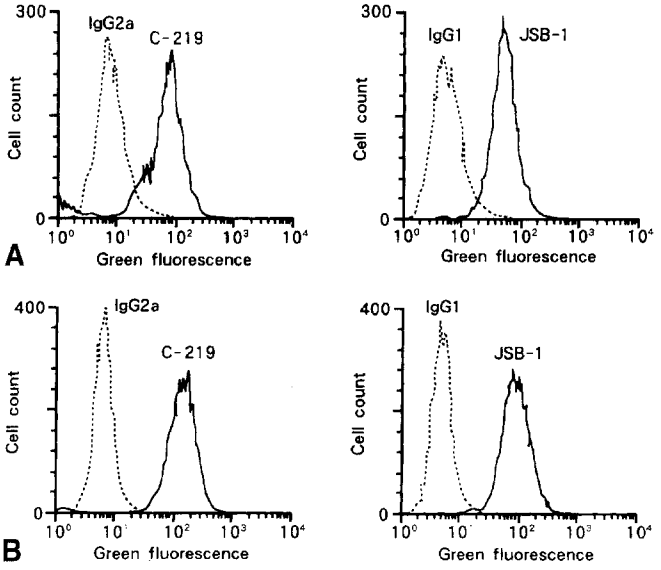

Fig. 4 Expression of PGP in ACHN cells (**A**) and ACHN/ADM cells (**B**) was analyzed by flow cytometry. A *continuous line* represents a fluorescence histogram of PGP-specific antibody (C-219 or JSB-1), and a *dotted line* represents a fluorescence histogram of control antibody (IgG 2a antibody for C-219 or IgG 1 antibody for JSB-1). ACHN/ADM cells showed a significant increase in reactivity with C-219 or JSB-1 antibody in comparison with ACHN cells ($P < 0.05$)

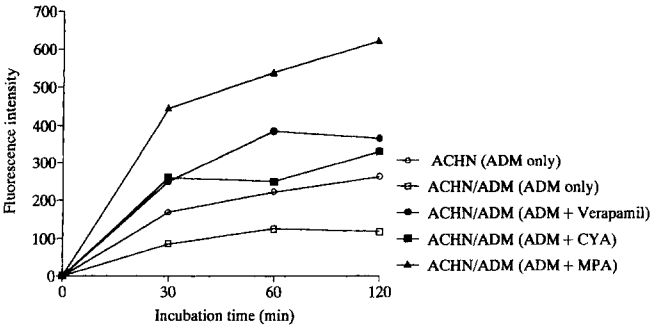

Fig. 5 Intracellular adriamycin (ADM) accumulation in ACHN/ADM cells. ACHN/ADM cells were incubated in medium containing 10 μg/ml of ADM in the absence of chemosensitizers (*open squares*) or the presence of 2.5 μg/ml verapamil (*filled circles*), 0.5 μg/ml cyclosporin A (CYA; *filled squares*) or 1.0 μg/ml medroxyprogesterone acetate (mPA; *filled triangles*). ACHN cells were incubated in minimum essential medium (MEM) containing 10 μg/ml of ADM in the absence of chemosensitizers (*open circles*). The intracellular ADM accumulation in ACHN/ADM cells was significantly increased by the addition of chemosensitizers ($P < 0.05$)

Intracellular ADM uptake

The intracellular ADM accumulation in both types of cells after exposure to 10 μg/ml of ADM with or without chemosensitizers is shown in Fig. 5. An initial rapid uptake of the drug was observed for the first 30-min period, followed by a slow uptake that continued up to 120 min or reached a plateau before the end of the 120 min. When no chemosensitizers were added, intracellular ADM accumulation over 120 min in ACHN/

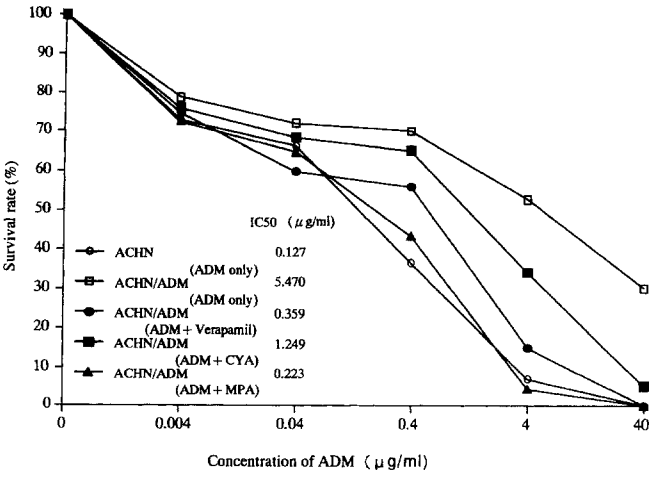

Fig. 6 Modulation of the cytotoxic effect of ADM. ACHN/ADM cells were incubated in MEM containing appropriate concentrations of ADM in the absence of chemosensitizers (*open squares*) or the presence of 2.5 μg/ml verapamil (*filled circles*), 0.5 μg/ml CYA (*filled squares*) or 1.0 μg/ml MPA (*filled triangles*). ACHN cells were incubated in MEM containing ADM in the absence of chemosensitizers (*open circles*). The sensitivity to ADM was assessed in triplicate experiments of the ATP assay

ADM cells was significantly lower than that in ACHN cells ($P < 0.05$). The addition of noncytotoxic concentrations of verapamil (2.5 μg/ml), CYA (0.5 μg/ml) or MPA (1.0 μg/ml) enhanced the initial uptake of the drug and steadily increased the intracellular ADM concentrations in ACHN/ADM cells. The ADM concentrations in these resistant cells were increased 3.1 times, 2.8 times and 5.3 times by verapamil, CYA and MPA, respectively; in all instances the accumulation was higher than in the parent ACHN cells ($P < 0.05$).

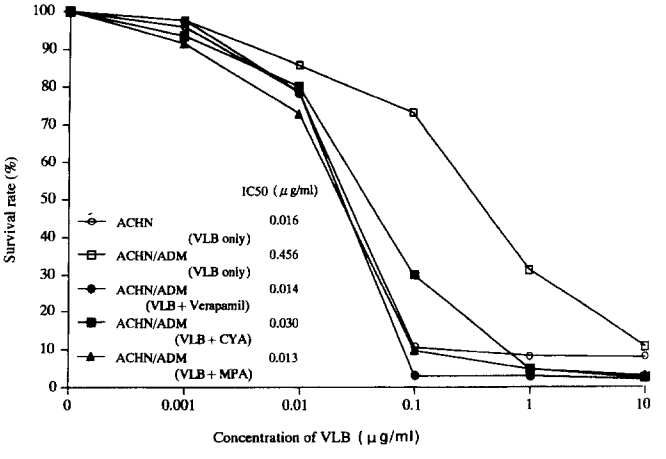

Fig. 7 Modulation of the cytotoxic effect of vinblastine sulfate (VLB). ACHN/ADM cells were incubated in MEM containing appropriate concentrations of VLB in the absence of chemosensitizers (*open squares*) or the presence of 2.5 μg/ml verapamil (*filled circles*), 0.5 μg/ml CYA (*filled squares*) or 1.0 μg/ml MPA (*filled triangles*). ACHN cells were incubated in MEM containing VLB in the absence of chemosensitizers (*open circles*). The sensitivity to VLB was assessed in triplicate experiments of the ATP assay

Modulation of cytotoxic effects by antitumor agents

Dose-response curves of ACHN/ADM cells with or without a chemosensitizer are shown in Fig. 6 for ADM and Fig. 7 for VLB. In terms of the IC50, ACHN/ADM cells were sensitized 15 times, 4 times and 24 times to ADM by addition of verapamil, CYA and MPA, respectively (Fig. 6). MPA reversed the ADM resistance of ACHN/ADM cells to the point where it was equivalent to that of ACHN cells. The sensitivity to VLB was enhanced 33 times, 15 times and 36 times by verapamil, CYA and MPA, respectively (Fig. 7). Verapamil and MPA also overcame the VLB resistance of ACHN/ADM cells, reducing it to a level equivalent to that of ACHN cells.

Discussion

A main goal of this study was to clarify the correlation between the expression level of PGP and the level of MDR. To obtain cells that expressed a high level of PGP, ACHN cells with low-level expression of PGP were continuously exposed to ADM. These established ACHN/ADM cells showed overexpression of PGP at both the mRNA level and the protein level. In RT-PCR analysis, ACHN/ADM cells gave a signal for PGP mRNA as low as 10 pg of mRNA, while no signal was detected in the same amount of mRNA prepared from ACHN cells. In flow cytometric analysis, ACHN/ADM cells showed approximately a 2-fold increase in reactivity with C-219 or JSB-1 antibody in comparison with ACHN cells. As regards the resistance levels to antitumor agents, the IC50 value of ADM in ACHN/ADM cells was approximately 43 times higher than that in the parent cells, and cross-resistance to VLB and VP-16 were demonstrated in the subline. These findings suggest that resistance levels in both types of cells might correlate with PGP levels. The quantitative data on PGP expression would be important information for evaluating intrinsic or acquired MDR levels.

Previous studies have demonstrated that chemosensitizers show competitive inhibition in binding antitumor agents to PGP [14, 20]. In this study, the intracellular ADM accumulation in ACHN/ADM cells was approximately half the amount found in the parent cells when both cell lines were treated with the same concentration of ADM. Our previous report demonstrated that intracellular ADM uptake in ACHN cells over 120 min was increased 1.8 times, 1.9 times and 2.6 times by verapamil, CYA and MPA, respectively [11]. This study demonstrated a contrasting intracellular ADM uptake in ACHN/ADM cells over 120 min, with levels increasing 3.1 times, 2.8 times and 5.3 times on addition of the same concentrations of chemosensitizers. The increases in ADM uptake were greater in the cells with high-level expression of PGP than in those with low-level expression of PGP. These findings suggest that resistance levels in both cell lines might be regulated by PGP levels. In addition, chemosensitizers enhanced the cytotoxicity of ADM and VLB in ACHN/ADM cells. In our experience, MPA was the most effective agent for overcoming drug resistance. By treatment with MPA, the IC50 value of ADM in ACHN/ADM cells could be reversed to a level equivalent to the value found in ACHN cells. Additionally, by treatment with verapamil and MPA, the sensitivity of the subline to VLB could be reversed to a level equivalent to that of the parent cells. These results suggest that chemosensitizers would be effective agents in helping to overcome drug resistance in tumor cells with overexpression of PGP.

However, our findings include a discrepancy that can not be explained simply by the action of PGP. While chemosensitizers enhanced intracellular ADM accumulation in ACHN/ADM cells much more than they did in ACHN cells, the efficacy in sensitizing to ADM in the subline was limited by the sensitivity level of the parent cells. This result leads to the speculation that ACHN/ADM cells might be affected by other factors contributing to MDR, such as glutathione and metallothionein levels, topoisomerase activities, and multidrug-resistance-associated protein [1, 5, 8, 18].

Certainly we are aware that limitations of this study include the absence of any assessment of other mechanisms of MDR in ACHN and ACHN/ADM cells, and that inhibition of PGP by itself will not be sufficient to overcome MDR. However, this study indicates significant correlation between the expression level of PGP and the MDR level. Additionally, acquired MDR caused by the enhancement of PGP expression was reversed to the intrinsic MDR level by chemosensitizers. In previous studies using renal cancer cell lines with intrinsic overexpression of PGP and MDR phenotype, chemosensitizers such as verapamil have been shown to enhance sensitivity to antitumor agents [13]. From these findings, we propose that chemosensitizers can be useful in clinical cases as agents for overcoming MDR caused by the intrinsic overexpression of PGP or by the enhancement of PGP expression by antitumor agents. However, the clinical usefulness of the chemosensitizers will depend not only on their ability to reverse drug resistance but also on their in vivo tolerance. Previous study using high doses of MPA in patients with advanced breast cancer reported that severe side effects were not observed [16]. Although such high concentrations of MPA appears to be currently usable in the treatment of advanced renal cancers, further clinical studies will be required to clarify to what extent this chemosensitizer may be useful as a therapeutic agent. In addition, the development of more potent and/or broader-acting sensitizing agents will be needed. These agents will then offer an attractive therapeutic option for patients with multidrug-resistant renal cancers.

References

1. Ahn H, Lee E, Kim K, Lee C (1994) Effect of glutathione and its related enzymes on chemosensitivity of renal cell carcinoma and bladder carcinoma cell lines. J Urol 151:263
2. Borden EC, Hogan TF, Voelkel JG (1982) Comparative antiproliferative activity in vitro of natural interferons α and β for diploid and transformed human cells. Cancer Res 42:4948
3. Bradley G, Juranka PF, Ling V (1988) Mechanism of multidrug resistance. Biochim Biophys Acta 948:87
4. Chen CJ, Chin JE, Ueda K, Clark DP, Pastan I, Gottesman MM, Roninson IB (1986) Internal duplication and homology with bacterial transport proteins in the mdr1 (P-glycoprotein) gene from multidrug-resistant human cells. Cell 47:381
5. Efferth T, Mattern J, Volm M (1992) Immunohistochemical detection of P-glycoprotein, glutathione S transferase and DNA topoisomerase II in human tumors. Oncology 49:368
6. Fojo AT, Shen DW, Mickley LA, Pastan I, Gottesman MM (1987) Intrinsic drug resistance in human kidney cancer is associated with expression of a human multidrug-resistance gene. J Clin Oncol 5:1922
7. Fojo AT, Ueda K, Slamon DJ, Poplack DG, Gottesman MM, Pastan I (1987) Expression of a multidrug-resistance gene in human tumors and tissues. Proc Natl Acad Sci USA 84:265
8. Grant EC, Valdimarsson G, Hipfner DR, Almquist KC, Cole SPC, Deeley RG (1994) Overexpression of multidrug resistance-associated protein (MRP) increases resistance to natural product drugs. Cancer Res 54:357
9. Horio M, Gottesman MM, Pastan I (1988) ATP-dependent transport of vinblastine in vesicles from human multidrug resistant cells. Proc Natl Acad Sci USA 85:3580
10. Kakehi Y, Kanamaru H, Yoshida O, Ohkubo H, Nakanishi S, Gottesman MM (1988) Measurement of multidrug-resistance messenger RNA in urogenital cancers: elevated expression in renal cell carcinoma is associated with intrinsic drug resistance. J Urol 139:862
11. Kawamoto S, Deguchi T, Nezasa S, Yamada S, Okano M, Kawada Y (1996) Detection of low-level expression of P-glycoprotein in ACHN renal adenocarcinoma cells. Jpn J Cancer Res 87:475
12. Kikuchi H, Nishiyama K, Sumizawa T, Furukawa T, Yoshimura A, Akiyama S (1991) Verapamil potentiates the effect of adriamycin and vincristine on human renal adenocarcinoma cells. Med J Kagoshima Univ 43:27
13. Mickish GH, Merlino GT, Aiken PM, Gottesman MM, Pastan I (1991) New potent verapamil derivatives that reverse multidrug resistance in human renal carcinoma cells and in transgenic mice expressing the human MDR1 gene. J Urol 146:447
14. Mickisch GH, Roehrich K, Kessig J, Forester S, Tschada RK, Alken PM (1990) Mechanisms and modulation of multidrug resistance in primary human renal cell carcinoma. J Urol 144:755
15. Nygren P, Larsson R (1990) Verapamil and cyclosporin A sensitize human kidney tumor cells to vincristine in absence of membrane P-glycoprotein and without apparent changes in the cytoplasmic free Ca^{2+} concentration. Biosci Rep 10:231
16. Pannuti F, Martoni A, Lenaz GR, Piana E, Nanni P (1978) A possible new approach to the treatment of metastatic breast cancer: massive doses of medroxyprogesterone acetate. Cancer Treat Rep 62:499
17. Paulson DG, Perez CA, Anderson T (1982) Genito-urinary malignancies. In: Vita VT, Hellman S, Rosenberg SA (eds) Cancer. Johnwiley & Sons, Philadelphia, p 732
18. Schneider E, Horton JK, Yang CH, Nakagawa M, Cowan KH (1994) Multidrug resistance-associated protein gene overexpression and reduced drug sensitivity of topoisomerase II in a human breast carcinoma MCF7 cell line selected for etoposide resistance. Cancer Res 54:152
19. Slater LM, Sweer P, Stupecky M, Gupta S (1986) Cyclosporin A reverses vincristine and daunorubicin resistance in acute lymphatic leukemia in vitro. J Clin Invest 77:1405
20. Tsuruo T, Iida H, Tsukagoshi S, Sakurai Y (1981) Overcoming of vincristine resistance in P388 leukemia in vivo and in vitro through enhanced cytotoxicity of vincristine and vinblastine by verapamil. Cancer Res 41:1967