Effect of a Dihydropyridine Analogue, 2-[Benzy1(phenyl)amino]ethyl 1,4-Dihydro-2,6-dimethyl-5-(5,5-dimethyl-2-oxo-1,3,2-dioxaphosphorinan-2-yl)-1-(2-morpholino-ethy1)-4-(3-nitrophenyl)-3-pyridinecarboxylate on Reversing in Vivo Resistance of Tumor Cells to Adriamycin

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ABSTRACT

A newly synthesized dihydropyridine analogue, 2-[benzyl(phenyl)amino]ethyl 1,4-dihydro-2,6-dimethyl-5-(5,5-dimethyl-2-oxo-1,3,2-dioxaphosphorinan-2-yl)-1-(2-morpholinoethyl)-4-(3-nitrophenyl)-3-pyridinecarboxylate (PAK-200), at 5 μM inhibited the efflux of [3H]vincristine from KB-C2 cells and increased the accumulation of [3H]vincristine (VCR), at 5 pM inhibited the efflux of [3H]vincristine (VCR), at 5 pM inhibited the efflux of [3H]vincristine (VCR), and also inhibited the efflux of [3H]azidopine from KB-C2 cells. The level of MDR1 expression of COK-36LN was about 3 times higher than that of KB-3-1 cells, but lower than that of KB-8-5 cells. These results suggest that the interaction of PAK-200 with P-glycoprotein may be partly correlated with the enhancement of the antitumor effect of Adriamycin on xenografted KB-8-5 and COK-36LN cells in nude mice.

INTRODUCTION

Resistance of tumors to multiple, structurally unrelated chemotherapeutic agents is frequently seen clinically and remains a major impediment to successful cancer chemotherapy. Multidrug-resistant cells have been isolated from human carcinoma cell lines and studied to elucidate the molecular basis for the MDR.3 Overexpression of a M, 170,000 transmembrane glycoprotein called P-gp has been observed in various multidrug-resistant cell lines. P-gp is the product of the MDR1 gene and is believed to function as an energy-dependent efflux pump (1, 2).

MDR is reversed by a variety of compounds including calcium channel blockers (3). Most drugs that reverse multidrug resistance block drug efflux from cells and also inhibit photoaffinity labeling of P-gp by a photoaffinity analogue of vinblastine (4). The photoactive dihydropyridine calcium channel blocker, azidopine, has been shown to photolabel P-gp. Vinblastine and nimodipine inhibit this labeling (5). P-gp seems to be an acceptor for some calcium channel blockers such as verapamil, dilatazem, and dihydropyridine analogues that are reported to reverse MDR. The correlation between reversal of drug resistance and inhibition of the [3H]azidopine photo-labeling of P-gp by dihydropyridine analogues suggested a role for P-gp in naturally occurring MDR (6). Recently some dihydropyridine analogues have been shown to reverse MDR in vivo (7, 8).

We have screened dihydropyridine analogues and found some that inhibit photolabeling of P-gp with [3H]azidopine and also reverse drug resistance in multidrug-resistant KB-C2 cells in vitro. In this study, we investigated whether one of these analogues, PAK-200, enhances the effect of ADR on the growth of drug-sensitive KB-3-1, multidrug-resistant KB-8-5, and intrinsically drug-resistant colorectal carcinoma cells in vivo as well as in vitro.

MATERIALS AND METHODS

Chemicals. PAK-200 was synthesized by Nissan Chemical Industries Co., Ltd. (Chiba, Japan). The structures and purities of the analogues were determined using the following procedures. Melting points were determined on a Yanako micro-melting point apparatus.

The [3H]-nuclear magnetic resonance spectrum in CDCl3 solution was recorded on a Jeol PMX60S1 spectrometer, and chemical shifts were given in ppm with trimethylsilane as an internal standard. Mass spectrum was obtained on a Jeol JMS D-300. Column chromatography was carried out on Merck silica gel 60 (70 to 230 mesh American Society for Testing Materials).

To synthesize PAK-200, 0.44 g (11.0 mmol) of NaH (60%) were added to a solution of 3.62 g (5.00 mmol) of 2-[benzyl(phenyl)amino]ethyl 1,4-dihydro-2,6-dimethyl-5-(5,5-dimethyl-2-oxo-1,3,2-dioxaphosphorinan-2-yl)-1-(2-morpholinoethyl)-4-(3-nitrophenyl)-3-pyridinecarboxylate, P-oxide in 18.1 g of dimethylformamide dried over 3A molecular sieves and stirred for 15 min at room temperature. To the reaction mixture, 0.93 g (5.00 mmol) of 4-(2-chloroethyl)morpholine hydrochloride was added and maintained at 80°C for 6 h. The solvent was removed under reduced pressure, and the residue was dissolved with 100 ml of CHCl3, and washed with 100 ml of water. The organic layer was dried over Na2SO4, followed by removal of the solvent under reduced pressure. The residue was purified by silica gel column chromatography (ethyl acetate:ethanol, 5:1) to give 0.91 g (yield, 24%) of PAK-200 as a yellow oil. Nuclear magnetic resonance δ 0.87 (s, 3H), 0.98 (s, 3H), 1.9 to 2.4 (m, 6H), 2.42 (s, 3H), 2.50 (d, J = 2.2 Hz, 3H), 3.3 to 4.7 (m, 16H), 4.99 (d, J = 14 Hz, 1H), and 6.3 to 8.3 (m, 14H); mass spectrum (Electron Impact Ionization), m/e 744 (5, M+), 727 (6%), 595 (5%), 404 (9%), 210 (26%), and 100 (100%).

[3H]VCR (6.75 Ci/mmol) and [3H]azidopine (40 Ci/mmol) were synthesized by Nissan Chemical Industries Co., Ltd. (Chiba, Japan). The structures and purities of the analogues were determined using the following procedures. Melting points were determined on a Yanako micro-melting point apparatus.

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2 To whom requests for reprints should be addressed.

3 The abbreviations used are: MDR, multidrug resistance; P-gp, P-glycoprotein; ADR, Adriamycin; VCR, vincristine; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PAK-200, 2-[benzyl(phenyl)amino]ethyl 1,4-dihydro-2,6-dimethyl-5-(5,5-dimethyl-2-oxo-1,3,2-dioxaphosphorinan-2-yl)-1-(2-morpholinoethyl)-4(3-nitrophenyl)-3-pyridinecarboxylate; s, singlet; d, doublet; m, multiplet; t, triplet; d, dodecyl; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PAK-200, 2-[benzyl(phenyl)amino]ethyl 1,4-dihydro-2,6-dimethyl-5-(5,5-dimethyl-2-oxo-1,3,2-dioxaphosphorinan-2-yl)-1-(2-morpholinoethyl)-4(3-nitrophenyl)-3-pyridinecarboxylate; s, singlet; d, doublet; m, multiplet; SDS, sodium dodecyl sulfate; SSC, standard saline-citrate.

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purchased from Amersham Corp., Arlington, Heights, IL. VCR, ADR, verapamil, and nicardipine were obtained from Sigma Chemical Co., St. Louis, MO.

Cell Culture and Cell Lines. Human epidermal KB carcinoma cells were obtained from Dr. Gottesman (National Cancer Institute, Bethesda, MD). The multidrug-resistant mutants, KB-8-5 and KB-C2, were selected from KB cells with increasing concentrations of colchicine and maintained as described previously (9).

Cell Survival by MTT Assay. MTT colorimetric assay performed in a 96-well plate was used for an in vitro chemosensitivity test (10). The assay is dependent on the reduction of MTT by the mitochondrial dehydrogenase of viable cells to a blue formazan product which can be measured spectrophotometrically. Equivalent numbers of cells (2000 for KB, 3000 for KB-8-5, and 5000 for KB-C2) were inoculated into each well with 0.18 ml of culture medium. After overnight incubation (37°C, 5% CO2), 20 µl of vincristine solution and 0.5 µl of sample solution were added and incubated for 4 days. Then 50 ml of MTT (1.1 mg/ml of phosphate-buffered saline) were added to each well and incubated for a further 4 h. The resulting formazan was dissolved in 100 µl of dimethyl sulfoxide after aspiration of the culture medium. Plates were placed on a plate shaker for 5 min and read immediately at 570 nm.

Determination of Calcium Antagonistic Activities. The calcium-antagonistic activity of PAK-200 and nicardipine was determined by the ability of the compounds to inhibit the contraction of the spirally sheared rabbit thoracic aorta in the presence of calcium.

The arteries were cut helically into strips and fixed vertically between hooks under a tension of 2 g in an organ bath containing Krebs-Henseleit solution (composition in mM: NaCl, 118.4; KCl, 4.7; MgSO4, 1.2; CaCl2, 2.5; KH2PO4, 1.2; NaHCO3, 4.9; glucose, 11.1), which was maintained at 37°C and aerated with a mixture of 95% O2 and 5% CO2.

Before the start of experiments, all the preparations were allowed to equilibrate for 60 min in the bathing solution, during which time the bathing solution was changed every 20 min.

The preparations were contracted by exposure to high potassium solution (composition in mM: KCl, 50; NaCl, 73.1). After the KCl-induced contractions had stabilized, the agents were added cumulatively.

To assess and compare the relaxant activity, papaverine, at a concentration of 10-5 M, was added at the end of experiments, and the result was expressed as a negative logarithm to the dose required for 50% of the maximum relaxation produced by 10-4 M papaverine.

Membrane Vesicle Preparation. Membrane vesicles from KB-C2 cells were prepared as described (11). Protein concentrations were determined by the method of Bradford (12).

Photoaffinity Labeling. Membrane vesicles (100 µg of protein) were incubated with 0.75 µM [3H]azidopine (53 Ci/mmol) for 15 min at room temperature in the presence of the indicated concentrations of PAK-200. After continuous irradiation at 366 nm for 20 min at 25°C, samples were solubilized in a sodium dodecyl sulfate sample buffer as described previously (13).

Sodium Dodecyl Sulfate Gel Electrophoresis. Samples labeled with [3H]azidopine were adjusted to 50 mM Tris-HCl at pH 6.8, 1% SDS, 5% 2-mercaptoethanol, 5% glycerol, and 0.1% bromphenol blue. Electrophoresis in 8% of polyacrylamide gels was carried out according to the method of Laemmli without heating the sample (13). Proteins were stained with 0.25% Coomassie blue in 50% (w/v) trichloroacetic acid.

Small pieces of tissue or tumors were pulverized with a metal tissue pulverizer (Shinko Seiki, Fukuoka, Japan) on a bed of dry ice prior to RNA extraction. Cellular RNA was extracted by homogenization in guanidinium isothiocyanate followed by acid-phenol extraction (14).

Nitrocellulose filter membranes were moistened in 10x SSC (1x SSC = 0.15 M NaCl plus 15 mm sodium citrate, pH 7) before being placed on a slot blotter (Hybri-Slot manifold; Bethesda Research Laboratories, Gaithersburg, MD). The RNA samples were applied under vacuum, and the filter was dried at room temperature before being cross-linked by UV irradiation in a stratalinker (Stratagene, La Jolla, CA).

The filters were prehybridized for 4 h at 42°C in 50% formamide-5x Denhardt's solution (1x Denhardt's solution = 0.02% Ficoll, 0.02% polyvinylpyrrolidone, and 0.02% acetylated bovine serum albumin)-5x SSC-0.05 M sodium phosphate buffer (pH 6.5)-200 µg of denatured salmon sperm DNA per milliliter.

The filters were then hybridized for 16 h at 42°C in a solution containing 50% formamide, 5x SSC, 1x Denhardt's solution, 20 mm sodium phosphate buffer (pH 6.5), 10% dextran sulfate, 100 µg of denatured salmon sperm DNA per milliliter, and 106 cpm of 32P-labeled P-gp complementary DNA probe per milliliter. The genomic probe pMDR1, which was contained within an 800-base pair PvuII fragment of pBR322, was obtained from Dr. Gottesman (National Cancer Institute, Bethesda, MD).

After hybridization, the filters were washed 4 times with 250 ml of 1x SSC-0.1% SDS for 15 min at 23°C and then washed twice with 500 ml of 0.2x SSC-0.1% SDS for 10 min at 50°C. Autoradiographs were exposed for 4 days. Hybridization with a 32P-labeled β-actin probe was performed to compare amounts of RNA loading.

Animals and Tumors. Eight- to 9-wk-old male BALB/c nude mice used for this study (CLEA, Tokyo, Japan) were kept in autoclaved cages, fed on sterile food, and housed with bedding under specific-pathogen-free conditions. The tumor lines used for this study were established directly from human colorectal carcinomas in the patients operated on in the Surgical Clinic of Kagoshima University Hospital. All tumor lines have been maintained by serial transplantations in nude mice. Two tumor lines, COK-36LN and COK-28LN, were used for the study (CLEA, Tokyo, Japan). COK-36LN expresses MDR1 mRNA and COK-28LN expresses the lower level of MDR1 mRNA than COK-36LN. COK-36LN is a mucinous carcinoma, and COK-28LN is a moderately differentiated adenocarcinoma.

In Vivo Examination. Tumor tissue fragments of approximately 4 x 4 x 4 mm in size or 5 x 106/0.1 ml of viable cells of the KB-3-1 and KB-8-5 were inoculated into the subcutaneous tissue in the back of each nude mouse. When the tumors had reached 100 to 300 mm3 the animals were randomly separated into groups of 4, and treatment was initiated. The size of tumors was measured with a sliding caliper every 2 days. Tumor volume (V) was calculated with the formula: V = length x width x height x 0.5, in accordance with the protocol of Battelle Columbus Laboratories. Estimated tumor volumes were shown as the relative mean value (V/15), V5 being the mean volume at initiation and V5 being at any given day (15-17). ADR at 8 mg/kg was administered i.p. on Day 1. PAK-200 was given i.p. at 80 mg/kg twice a day (160 mg/kg/day) for 2 days. The first injection of PAK-200 was given 2 h before ADR administration, and the second was given approximately 8 h later. Control animals were given 0.2 ml of saline solution i.p. for 2 days.

ADR Content in the Tumor Tissues. The amount of ADR in the xenograft tissues was measured with high-performance liquid chromatography. The tumors were excised 2 days after the start of treatment. The tissue was homogenized and extracted with a mixture of butanol and toluene. The extract was evaporated in vacuo, and the residue was

Fig. 1. Chemical structure of PAK-200.
REVERSAL OF DRUG RESISTANCE BY PAK-200

**Fig. 2.** Effect of PAK-200 and other MDR-reversing agents on resistance to VCR in KB-C2 cells. The effect of verapamil (■), cepharanthine (▲), and PAK-200 (▲) at 5 µM on the resistance to VCR in KB-C2 cells (▲) was examined by the MTT assay. The effect of verapamil (■) and PAK-200 (▲) at 5 µM on sensitivity to VCR of KB-3-1 cells (□) was also examined. Points, mean of duplicate determinations.

**Fig. 3.** Effect of PAK-200 on accumulation of VCR in KB-3-1 cells (□) and KB-C2 cells (▲). Points, mean of triplicate determinations.

dissolved in a mixture of 0.1 M phosphate buffer (pH 3.0) and methanol. This solution was applied to a reversed-phase ODS column and eluted with a mixture of 1 N formic acid and methanol. The fluorescence signal was monitored at 470-nm excitation and 585-nm emission.

**RESULTS**

**Reversal of Resistance to Vincristine in KB-C2 Cells by PAK-200.** We synthesized about 400 dihydropyridine analogues and investigated whether they reversed MDR in KB-C2 cells. Several analogues had similar or higher MDR-reversing activity similar to or higher than those of verapamil and cepharanthine, a bisococlaurine alkaloid that reverses multidrug resistance in KB-C2 cells (18). PAK-200 (Fig. 1) was selected as an analogue for this study because it had the lowest calcium channel blocking activity and was one of the most potent MDR-reversing agents. As shown in Fig. 2, the ability of PAK-200 to reverse resistance to VCR in KB-C2 cells was greater than those of cepharanthine and verapamil. At 5 µM, PAK-200, cepharanthine, and verapamil increased the sensitivity of KB-C2 cells to VCR 544, 141, and 11 times, respectively, when 50% growth-inhibitory concentrations of VCR without the reversing agents were compared with that in the presence of the agents.

We then examined the effect of PAK-200 on the accumulation of VCR in KB-3-1 and KB-C2 cells (Fig. 3). The intracellular level of vincristine in KB-C2 cells was 14% of that in KB-3-1 cells. The addition of PAK-200 at 5 µM enhanced the accumulation of vincristine in KB-C2 cells about 7-fold to the level found in KB-3-1 cells without PAK-200. We examined whether the increased accumulation of VCR in KB-C2 cells with PAK-200 was due to the inhibition of drug efflux (Fig. 4). After incubation of the cells for 10 min in the absence of PAK-200, about 60% of the VCR was lost from the KB-C2 cells, whereas almost all VCR was retained in the KB-3-1 cells. This efflux of VCR from KB-C2 cells was completely blocked for 10 min by the addition of PAK-200 to the culture medium. When KB-C2 cells were incubated for 40 min with and without PAK-200, about 10% and 75% of VCR was lost from the cells, respectively. PAK-200 did not significantly disturb the efflux of VCR from KB-3-1 cells.

The photoactive dihydropyridine calcium channel blocker, azidopine, photolabeled P-gp in membrane vesicles from human KB-C2 cells. The labeling was almost completely inhibited by vinblastine and MDR-reversing dihydropyridine analogues (6). We therefore studied the effect of PAK-200 on the photolabeling of P-gp in membranes from KB-C2 cells (Fig. 5). PAK-200 at 100 µM completely inhibited the labeling of P-gp in membrane vesicles from KB-C2 cells. Verapamil partially inhibited the labeling at the same concentration. The inhibitory activity of PAK-200 was considerably higher than that of verapamil.

**Effect of PAK-200 on the Sensitivity to ADR.** Since drug resistance in clinical tumors usually occurs at a low level, and ADR is widely used for the treatment of various tumors, we examined the effect of PAK-200 on the sensitivity to ADR of drug-sensitive KB-3-1 cells, low level multidrug-resistant KB-8-5 cells, and human colorectal carcinoma tumor lines, COK-28LN and COK-36LN.

First we measured the expression of MDRI mRNA in each
cell line (Fig. 6). The level of MDR1 mRNA in COK-36LN was about 3 times higher than that in COK-28LN, but lower than that in KB-8-5 cells. Xenografted KB-8-5 cells expressed similar but slightly lower levels of MDR1 mRNA than cultured KB-8-5 cells. We could not detect any expression of MDR1 mRNA in KB-3-1 cells.

As shown in Table 1, PAK-200 at 1 μM enhanced the sensitivity to ADR of KB-8-5 and COK-36LN but not of KB-3-1 and COK-28LN cells. PAK-200 at 2 μM completely reversed the resistance to ADR of KB-8-5 cells. PAK-200 at 5 μM enhanced the sensitivity to ADR of KB-3-1, KB-8-5, COK-28LN, and COK-36LN cells 2, 5, 2, and 3 times, respectively.

Calcium Antagonism. The calcium antagonistic activity of PAK-200 was measured and compared with those of nicardipine and verapamil (Table 2).

The antagonistic activity of PAK-200 was about 1/1000 and 1/5 of those of nicardipine and verapamil, respectively.

Effect of PAK-200 on Antitumor Activity of ADR in Vivo. PAK-200 had relatively low toxicity. When PAK-200 was administered i.p. to 5 nude mice at a dose of 200 mg/kg, no animals died. When ADR at 10 mg/kg was administrated i.p. on Day 1, all the mice lost more than 15% of their weights after a week. We thus consider that 8 mg/kg of ADR is a near maximum tolerated dose in these studies. We also tested combined therapy on tumor-bearing nude mice with ADR and PAK-200 (Fig. 7).

PAK-200 alone at 80 mg/kg twice a day (160 mg/kg/day) did not significantly suppress the growth of cell lines xenografted into nude mice, whereas ADR alone at a dose of 8 mg/kg showed considerable suppression of the growth of the KB-3-1 tumor, but not of the other tumors. When the two agents were combined, the growth of KB-3-1 and COK-36LN was completely arrested, and the growth of the KB-8-5 tumor was similar to that of the KB-3-1 tumor treated with ADR alone. On the other hand, the growth of COK-28LN was not significantly suppressed by the combination treatment.

Effect of PAK-200 on the Accumulation of ADR in Tumors. The amount of ADR in the tumors was determined in the presence and absence of PAK-200 (Fig. 8). In the presence of PAK-200, the accumulation of ADR in KB-8-5 tumors was 4.6

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Table 1

<table>
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<tr>
<th>PAK-200 (μM)</th>
<th>KB-3-1</th>
<th>KB-8-5</th>
<th>COK-28LN</th>
<th>COK-36LN</th>
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<tr>
<td>0</td>
<td>30 (1.0)*</td>
<td>110 (1.0)</td>
<td>280 (1.0)</td>
<td>200 (1.0)</td>
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<tr>
<td>0.5</td>
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<td>280 (1.0)</td>
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<tr>
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<td>26 (0.2)</td>
<td>200 (0.7)</td>
<td>82 (0.4)</td>
</tr>
<tr>
<td>5</td>
<td>14 (0.5)</td>
<td>22 (0.2)</td>
<td>140 (0.5)</td>
<td>66 (0.3)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, D_{50} of each cell line with or without PAK-200 divided by D_{50} of each line without PAK-200.

* Concentration reducing cell survival by 50%.

Table 2

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Calcium antagonism pID_{50}</th>
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<tr>
<td>Experiment 1</td>
<td></td>
</tr>
<tr>
<td>PAK-200</td>
<td>6.08</td>
</tr>
<tr>
<td>Nicardipine</td>
<td>9.15</td>
</tr>
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| Experiment 2 |  |
| PAK-200 (+)-Verapamil | 6.28 |
| (+)-Verapamil | 6.93 |

pID_{50} is a negative logarithm of the dose required for 50% of the maximum relaxation produced by 10^{-4} M papaverine.
times higher than in the absence of PAK-200 \((P < 0.01)\). Similarly, the ADR content in COK-36LN was 1.5 times higher in the presence of PAK-200 than without PAK-200 \((P < 0.05)\). However, PAK-200 did not significantly increase the accumulation of ADR in KB-3-1 and COK-28LN tumors.

**DISCUSSION**

We have screened 200 newly synthesized dihydropyridine analogues for their ability to reverse MDR in KB-C2 cells. Several analogues that have more potent MDR-reversing activity than cepharanthine in vitro were discovered. PAK-200 was one of the most potent agents and had relatively low calcium channel blocking activity. The effectiveness of PAK-200 at 5 \(\mu M\) in increasing the sensitivity of KB-C2 to VCR was 50 times higher than that of verapamil at the same concentration. The effectiveness of PAK-200 in inhibiting the photolabeling of P-gp with \([3H]\)azidopine was also higher than that of verapamil. On the other hand, the \(Ca^{2+}\) antagonistic activity of PAK-200 was 5 times lower than that of verapamil.

PAK-200 seems to interact with P-gp in KB-C2 cells and inhibits the efflux of drug from the cells. We therefore investigated whether PAK-200 reverses drug resistance in KB-8-5 cells in vivo. We also studied whether the agent sensitizes xenografted colorectal COK-36LN and COK-28LN carcinomas to ADR, since these tumors expressed \(MDR1\) mRNA.

PAK-200 at 80 mg/kg twice a day is a nontoxic dose in mice. We could not inject more PAK-200 as the volume of PAK-200 solution became too large because of the poor solubility of the agent.

PAK-200 reversed resistance to ADR in xenografted KB-8-5 cells and also increased the response of KB-3-1 tumors to ADR. PAK-200 increased the accumulation of ADR in xenografted KB-8-5 cells about 4 times, but did not significantly
increase that in xenografted KB-3-1 cells. Since xenografted KB-8-5 cells in nude mice expressed similar levels of MDR1 mRNA to that in cultured KB-8-5 cells, PAK-200 may interact with P-gp to inhibit the efflux of ADR from xenografted KB-8-5 cells. P-gp seems to be involved in the reversal of drug resistance in xenografted KB-8-5 cells by PAK-200. Since KB-3-1 cells do not express detectable MDR1 mRNA (Fig. 6) and P-gp (19, 20), PAK-200 may interact with molecule(s) in KB-3-1 cells other than P-gp and change the sensitivity of KB-3-1 cells to ADR. This idea is supported by evidence that KB-3-1 cells are partially sensitized by PAK-200 to VCR in vitro (Fig. 2) at concentrations which increase drug accumulation (Fig. 3), but do not inhibit efflux (Fig. 4) as would be expected for P-gp inhibition.

ADR alone could not suppress the growth of colorectal carcinoma xenografts, COK-28LN and COK-36LN. Combined with PAK-200, ADR completely suppressed the growth of COK-36LN, but not of COK-28LN. PAK-200 significantly increased the amount of ADR in COK-36LN but not in COK-28LN tumors. The level of MDR1 expression in COK-36LN was about 3 times higher than that in COK-28LN. These data suggest a role for P-gp in the suppression of the growth of COK-36LN tumors by PAK-200. PAK-200 may be able to sensitize some solid tumors that express P-gp to ADR. Many tumors express MDR1 RNA levels comparable to those seen in COK-36LN and KB-8-5 cells and, thus, would be potential candidates for reversal of drug resistance by PAK-200 (21).

Some newly synthesized dihydropyridine analogues have been shown to reverse drug resistance in P388 mouse leukemia cells in vivo (7, 8). The in vivo effectiveness of agents in reversing MDR has been tested in mouse tumor models, mainly with mouse leukemia P388 and Ehrlich ascites-bearing mice (3). These data have been shown to reverse drug resistance in P388 mouse leukemia xenografts in genetically athymic mice. Anal. Biochem., 72: 248–254, 1976.

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