Two Pyridine Analogues with More Effective Ability to Reverse Multidrug Resistance and with Lower Calcium Channel Blocking Activity Than Their Dihydropyridine Counterparts

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ABSTRACT

Four pyridine analogues and their dihydropyridine counterparts were examined for their ability to reverse drug resistance in a multidrug-resistant human carcinoma cell line, KB-C2. Two pyridine analogues were more able to reverse drug resistance than their dihydropyridine counterparts. The other two pyridine analogues had an effect on drug resistance similar to their dihydropyridine counterparts. The calcium channel-blocking activity of all the pyridine analogues was considerably lower than that of the dihydropyridine analogues.

Of the pyridine analogues, 2-[4-(diphenylmethyl)-1-piperazinyl]ethyl 5-(trans-4,6-dimethyl-1,3,2-dioxaphosphorinan-2-yl)-2,6-dimethyl-4-(3-nitrophenyl)-3-pyridinecarboxylate P-oxide (PAK-104P) was the most effective in reversing multidrug resistance. PAK-104P (1 and 5 μM) completely reversed the drug resistance in KB-8-5 and KB-C2 cells, respectively. The reversing effect of PAK-104P was greater than that of other multidrug resistance-reversing agents, cepharanthine, verapamil, nimodipine, and nicardipine. PAK-104P at 1 μM increased about 10-fold the accumulation of vinblastine in KB-C2 cells, whereas verapamil at the same concentration increased the accumulation about 2-fold.

The inhibition of [3H]azidopine photolabeling of P-glycoprotein by the pyridine and dihydropyridine analogues except 2-[methyl(phenylmethyl)amino]ethyl 4-(2-chlorophenyl)-5-(4-methyl-1,3,2-dioxaphosphorinan-2-yl)-2,6-dimethyl-3-pyridinecarboxylate P-oxide correlated with the reversing of drug resistance by the analogues. Some newly synthesized pyridine analogues seemed to have lower calcium channel-blocking activity and more potent resistance-reversing ability than verapamil and other calcium channel blockers.

INTRODUCTION

Neoplastic cells resistant to multiple drugs including ADR, VCR, VBL, actinomycin D, etoposide, and teniposide often arise after exposure to antineoplastic agents. This problem of acquired multidrug resistance presents a major obstacle in the management of cancer.

Many MDR sublines of mammalian cells have been used to understand the mechanism of resistance during cancer treatment (1–3). Most cell lines with the MDR phenotype show increased expression of MDR1 gene, which encodes a M, 170,000 plasma membrane glycoprotein (P-glycoprotein) (4–9). P-glycoprotein is now thought to be an energy-dependent multidrug efflux pump (10–14). MDR is reversed by a variety of compounds including verapamil (15), quinidine (16), reserpine (17), a synthetic isoprenoid, SDB-ethylenediamine (18), and a biscochlorine alkaloid, cepharanthine (32). The agents that reverse MDR apparently did not seem to have common features. However, it appears that hydrophobicity at physiological pH, cationic charge, and molar refractivity are important physical properties for modulators of MDR (19, 20). They are supposed to inhibit the pump activity of P-glycoprotein (21, 22). When the activity of the pump is inhibited, anticancer agents accumulate in MDR cells and this may be why MDR is reversed.

Recently, dihydropyridine analogues have been shown to reverse MDR (23–25). However, most of such analogues reverse MDR in KB-C2 cells at the 10 μM level and block the calcium channel. We need to find or synthesize new agents that reverse MDR at much lower concentrations and that have lower calcium channel-blocking activity.

Pyridine analogues are known to have lower calcium channel-blocking activity and higher lipid solubility than their dihydropyridine analogue counterparts. Thus we synthesized four pyridine analogues and their dihydropyridine analogue counterparts and investigated their MDR-reversing activity.

MATERIALS AND METHODS

Chemicals. Dihydropyridine and pyridine analogues were newly synthesized by Nissan Chemical Ind. Co., Ltd. (Chiba, Japan). The structures and purities of these analogues were determined using the following procedures. Melting points were determined on a Yanaco micro-melting point apparatus. 1H NMR spectra in CDCl3 solution were recorded on a Joel PMX60S1 spectrometer and chemical shifts were given in ppm with tetramethylsilane as an internal standard. Mass spectra were obtained on a Joel JMS D-300 instrument. Column chromatography was carried out on a Merck Kieselgel 60 (70–230 mesh ASTM).

For PAK-101, 2-[methyl(phenylmethyl)amino]ethyl 3-aminocrotonate (1.24 g) was added to a solution of diethyl-1-acetyl-2(3-trifluoromethyl)phenyl]-ethyl-phosphonate (1.75 g) in toluene (30 ml). The reaction mixture was heated to reflux for 8 h with azeotropic removal of water. The solvent was evaporated in a vacuum and the residue was subjected to column chromatography (ethyl acetate:ethanol, 5:1) to give 1.20 g (41%) of pure PAK-101 as pale yellow oil. 1H NMR (CDCl3) 0.96 (t, J = 7.0 Hz, 3H), 1.20 (t, J = 7.0 Hz, 3H), 2.18 (s, 3H), 2.26 (d, J = 2.2 Hz, 3H), 2.28 (s, 3H), 2.62 (d, J = 6.0 Hz, 2H), 3.47 (s,
For PAK-104P, 2-(methylphenylmethyl)aminoethyl 3-aminocrotonate (10.0 g) was added to a solution of 3-(4-methyl-1,3,2-dioxaphosphorinan-2-yl)-4-(3-nitrophenyl)-3-buten-2-one P-oxide (10.0 g) in toluene (20 ml). The reaction mixture was allowed to reflux for 3 h. After concentration of the reaction mixture in a vacuum, the residue was subjected to column chromatography (ethyl acetate:ethanol, 9:1) to give 5.0 g (93%) of pure PAK-104 as yellow oil: \[ ^1H \text{NMR (CDCl}_3) \delta 0.97-1.36 (m, 6H), 1.74 (t, J = 5.4 Hz, 3H), 2.61 (t, J = 6.0 Hz, 2H), 4.16 (t, J = 6.0 Hz, 2H), 4.19 (s, 1H), 4.80 (d, J = 11.0 Hz, 1H), 4.00-4.95 (m, 3H), 7.10-8.30 (m, 12H); MS(EL), m/e 700 (M+), 683, 641, 167 (100), 68 (93).]

For PAK-104P, 36% nitric acid (10 ml) was added to a solution of PAK-104 (6.8 g) in acetic acid (20 ml). After stirring for 30 min at room temperature, saturated NaHCO₃ aqueous solution (100 ml) was added to the reaction mixture, followed by extraction with ethyl acetate (100 ml in 2 additions). The organic extract was dried over Na₂SO₄ and evaporated in a vacuum. The residue was subjected to column chromatography (ethyl acetate:ethanol, 2:1) to give 6.0 g of pure PAK-104P as yellow oil: \[ ^1H \text{NMR (CDCl}_3) \delta 0.97-1.36 (m, 6H), 1.40-1.80 (m, 2H), 2.00-2.60 (m, 10H), 2.59 (s, 3H), 2.95 (s, 3H), 3.90-4.10 (m, 3H), 3.91 (s, 1H), 4.60-4.80 (m, 1H), 7.10-8.30 (m, 14H); MS(FD), m/e 699 (M+ + 1, 10), 485 (4), 354 (20), 157 (100). \]

**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 (3).

**Cell Survival by MTT Assay.** MTT colorimetric assay performed in a 96-well plate was used for an in vitro chemosensitivity test (26). 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. Equal 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 μl of MTT (1.1 mg/ml PBS) was added to each well and incubated for a further 4 h. The resulting formazan was dissolved with 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 each compound was determined by the ability of the compound to inhibit the contraction of the taenia caecum from a guinea pig in the presence of calcium (25).

A taenia caecum was excised from a male Hartley guinea pig (300-400 g) and suspended in an organ bath containing physiological salt solution (135 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, 15 mM NaHCO₃, and 5.5 mM glucose) aerated with 95% O₂ and 5% CO₂ at 37°C. The taenia caecum was then stretched to an initial tension of 1 g and isometric contraction was measured with a force displacement transducer. After 30 min equilibration, the bathing solution was replaced by a Ca²⁺-free, high K⁺ (100 mM KCl, 40 mM NaCl) solution. The taenia caecum relaxed completely within 30 min. Then CaCl₂ (10 mM) was added. After further equilibration (30 min), the test compound solution (10⁻⁶-10⁻³ M) was added cumulatively. The result was expressed as a minus logarithm of the dose required for 50% of the maximum relaxation produced by 10⁻⁶ M papaverine.

**Calcium Current Measurement in Guinea Pig Ventricular Myocytes.** Single ventricular myocytes were prepared as described by Taniguchi et al. (27). Cells were superfused with modified Tyrode’s solution with the following composition (mM): NaCl 137, KCl 5.4, CaCl₂ 1.8, MgCl₂ 0.5, NaHCO₃ 5, NaH₂PO₄ 0.16, glucose 5.5, and N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid 5 (pH was adjusted to 7.4 by adding NaOH). Glass electrodes were filled with the solution which contained (mM): KCl 140, MgCl₂ 2, CaCl₂ 1, ethyleneglycol bis (β-aminoethyl ether)-N,N’,N’-tetraacetic acid 11, N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid 5, creatine phosphate 5, ATP-Na₂ 2 (pH was adjusted to 7.2 by adding KOH). Whole cell voltage clamp studies were
conducted as described by Marty and Neher (28). Compounds were first dissolved in dimethyl sulfoxide at a concentration of $10^{-3}$ M and then diluted in Tyrode's solution to make a desired final concentration. Experiments were done at 33°C.

Membrane Vesicle Preparation. Membrane vesicles from KB-C2 cells were prepared as described (29) from cells grown in 24- x 24-cm dishes (GIBCO) under standard growth conditions (3). Protein concentrations were determined by the method of Bradford (30).

Photoaffinity Labeling. Membrane vesicles were incubated with 0.75 mM $[^3H]$azidopine for 15 min at room temperature in the presence and absence of various drugs. After continuous irradiation at 366 nm for 20 min at 25°C, samples were solubilized in a sodium dodecyl sulfate sample buffer as described by Debenham et al. (31).

Sodium Dodecyl Sulfate Gel Electrophoresis. Samples labeled with $[^3H]$azidopine were analyzed by electrophoresis using a modification of the system described by Debenham et al. (31) on a 5% polyacrylamide/4.5 M urea gel. pH 7.6, without a stacking gel as described previously (25). Proteins were stained with 0.25% Coomassie blue in 50% (w/v) trichloroacetic acid.

Drug Accumulation and Efflux. For the study of drug accumulation, confluent monolayers of KB and KB-C2 cells in a 24-well plate were incubated with 1 mM $[^3H]$VBL in the absence and presence of indicated concentrations of agents for 1 h at 37°C. After washing with ice-cold PBS three times, the cells were solubilized in 0.2% Triton X-100 in 10 mM phosphate buffer (pH 7.4), harvested, and then counted.

For efflux study, the confluent monolayers of KB and KB-C2 cells were incubated with 1 $\mu$M $[^3H]$VBL in the presence and absence of 10 $\mu$M agents for 1 h at 37°C. After two washings with ice-cold PBS, the cells were further incubated in the medium with and without 10 $\mu$M agents. At the indicated times the cells were harvested and counted as described above.

RESULTS

Reversal of Drug Resistance in KB-C2 Cells by Pyridine and Dihydropyridine Analogues. We synthesized 4 pyridine analogues and their dihydropyridine analogue counterparts and examined their ability to reverse drug resistance in KB-C2 cells. The chemical structures of these newly synthesized compounds are shown in Fig. 1. PAK-101, PAK-102, PAK-103, and PAK-104 are dihydropyridine analogues, and PAK-101P, PAK-102P, PAK-103P, and PAK-104P are their pyridine analogue counterparts.

We examined the cytotoxic effect of the analogues on KB-C2 cells in the presence and absence of 22 nM VCR by the MTT method. PAK-101, PAK-101P, PAK-102, PAK-102P, PAK-104, and PAK-104P at 20 $\mu$M and PAK-103 and PAK-103P at 10 $\mu$M had no cytotoxic effect on KB-C2 cells (Fig. 2). VCR at 22 nM had no cytotoxic effect on KB-C2 cells (data not shown) but increased the sensitivity of KB-C2 to PAK-101, PAK-101P, PAK-102, PAK-102P, PAK-103P, PAK-104, and PAK-104P about 4.1, 4.8, 2.3, 2.1, <1.7, 5.3, 5.0, and 20 times, respectively. VCR at the concentration did not increase the cytotoxic effect of PAK-103 on KB-C2. PAK-104P reversed the resistance to 22 nM VCR in KB-C2 cells at about 1/20 of the concentration required to kill the cells (50% growth inhibition concentration of PAK-104P without VCR/50% growth inhibition concentration of PAK-104P with VCR = 20.0).

Next, the dose-response curves of various anticancer agents with and without the compounds were assayed by the MTT method. Table 1 summarizes the data from the curves. PAK-101 at 5 $\mu$M moderately reversed the resistance to VCR, VBL, ADR, and DAU in KB-C2 cells. PAK-102 at 10 $\mu$M moderately reversed the resistance to VCR and VBL and completely reversed the resistance to ADR and DAU. PAK-102 at 5 $\mu$M had little effect on drug resistance (data not shown). The pyridine analogue counterparts of these two dihydropyridine analogues had effects similar to the dihydropyridine analogues.

Table 1 Effects of dihydropyridine and pyridine analogues on drug resistance of KB-C2 cells

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Chemicals (µM)</th>
<th>Relative resistance to</th>
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<tr>
<td></td>
<td>VCR</td>
<td>VBL</td>
</tr>
<tr>
<td>KB</td>
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<td>KB-C2</td>
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<td>100.0</td>
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<td>7.4</td>
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<td>PAK-103</td>
<td>272.7</td>
<td>27.4</td>
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<td>0.5</td>
</tr>
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<td>16.2</td>
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<tr>
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<td>&lt;1.5</td>
<td>0.7</td>
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Fig. 2. Sensitivity of KB-C2 cells to the dihydropyridine and pyridine analogues in the presence and absence of 22 nM VCR. A, effect of PAK-101 (C), PAK-101P ( ), PAK-102 ( ), and PAK-102P ( ) on cell survival of KB-C2 in the absence (---) or presence (——) of 22 nM VCR. B, effect of PAK-103 (C), PAK-103P ( ), PAK-104 ( ), and PAK-104P ( ) on cell survival of KB-C2 in the absence (---) or presence (——) of 22 nM VCR. Each point represents the mean of 2 experiments.
The effect of PAK-103 on drug resistance was nil at 5 µM (data not shown) and weak at 10 µM. PAK-104 at 5 µM almost completely reversed the resistance to ADR and moderately reversed the resistance to VCR, VBL, and DAU. The pyridine analogues, PAK-103P and PAK-104P, had a more potent effect on drug resistance than their dihydropyridine analogue counterparts. PAK-103P at 10 µM completely reversed the resistance to VBL and DAU and moderately reversed the resistance to VCR and ADR. PAK-104P completely reversed the resistance to all agents tested and was the most potent MDR-reversing agent among the tested compounds.

Calcium Antagonism. The calcium-antagonistic activities of the 4 dihydropyridine analogues and their pyridine analogue counterparts were measured (Table 2). The antagonistic activities of all the pyridine analogues were considerably lower than those of their dihydropyridine analogue counterparts. PAK-103 was the most active agent among them, and PAK-101P was the weakest antagonist. No correlation was observed between the calcium-antagonistic activity of the analogues and the reversing of drug resistance.

Effect of PAK-103, PAK-103P, PAK-104, and PAK-104P on Calcium Current of Guinea Pig Ventricular Cells. We compared the potencies of 4 agents (PAK-103, PAK-103P, PAK-104, and PAK-104P) to block the calcium current of guinea pig ventricular myocytes using a whole-cell voltage clamp technique. In the absence of agents (controls in Fig. 3), inward calcium currents (transient downward deflections) with amplitudes ranging from 0.5 to 1.5 nA were recorded. PAK-103 and PAK-104, at a concentration of 1 µM, completely abolished the calcium current (the right current traces in Fig. 3, A and C) with remaining time-independent outward current. On the contrary, PAK-103P and PAK-104P at the same concentration decreased the calcium current only slightly (the right current traces in Fig. 3, B and D).

These results suggest that the potencies of calcium channel-blocking activity of PAK-103P and PAK-104P is much less or virtually nil, as compared to those of PAK-103 and PAK-104. Similar results were observed for PAK-101P and PAK-102P (data not shown).

Comparison of the Ability of PAK-104P to Reverse MDR with That of Other Reversing Agents. The pyridine analogue, PAK-104P, was the most effective MDR-reversing agent and had an exceedingly low calcium channel-blocking activity in this study. We compared the ability of this agent to reverse MDR in KB-8-5 cells with that of other reversing agents, verapamil, cepharanthine, nimodipine, and nicardipine. Since drug resistance in tumors in patients is usually at a low level, we used a KB-8-5 cell line because of its low resistance to drugs. Fig. 4 shows MTT assays to test the reversing effects of PAK-104P and other agents on resistance to VCR in KB-8-5 cells. KB-8-5 showed about 40 times greater resistance to VCR than KB. PAK-104P at 1 µM completely reversed the resistance in KB-8-5. The sensitivity of KB to VCR was also increased 3 times by 1 µM PAK-104P. None of the other MDR-reversing agents at 1 µM could completely reverse the resistance in KB-8-5. Cepharanthine, verapamil, nimodipine, and nicardipine increased the sensitivity of KB-8-5 to VCR 12, 4.3, 2.3, and 2.7 times, respectively. Cepharanthine and verapamil at 1 µM increased the sensitivity of KB to VCR only 1.5 times. Thus we observed that PAK-104P had the most potent ability to reverse MDR in vitro among MDR-reversing agents tested.

Effect of Agents on Cellular Accumulation of [³H]Vinblastine. To study how PAK-104P reverses MDR, we examined its effect on the accumulation of VBL in KB and KB-C2 cells and compared this with the effect of other MDR-reversing agents (Fig. 5). The intracellular level of VBL in KB-C2 cells was one-tenth of that in KB cells. The addition of PAK-104P at 2.5 µM enhanced the accumulation of VBL in KB-C2 cells 12-fold to the level of that in KB cells without the pyridine. PAK-104 had little effect at 2.5 µM on the accumulation of VBL in KB-C2 cells.
REVERSAL OF DRUG RESISTANCE BY PYRIDINE ANALOGUES

Fig. 5. Effect of agents on the accumulation vinblastine in KB and KB-C2 cells. Effect of agents on accumulation of vinblastine in KB cells (A) and KB-C2 cells (B) at the indicated concentrations; PAK-101 (C), PAK-101P (O), PAK-104 (D), PAK-104P (•), verapamil (A), cepharanthine (A). Points represent the mean of duplicate determinations.

At 2.5 μM, verapamil, cepharanthine, PAK-101, and PAK-101P enhanced the cellular accumulation of VBL in KB-C2 cells by factors of 5.1, 4.0, 2.6, and 2.0, respectively. Accumulation of VBL in KB-C2 cells plateaued at higher concentrations than 2.5 μM for PAK-104P and 10 μM for cepharanthine and verapamil.

The effect of PAK-104P on the accumulation of VBL in KB-C2 cells was greater than the effect of cepharanthine and verapamil at any concentrations tested. The accumulation of VBL in KB-C2 cells increased with increasing concentrations of PAK-101P, PAK-101, and PAK-104 added to the medium until 20 μM, and above 20 μM these 3 agents enhanced the accumulation of VBL more effectively than PAK-104P. In the sensitive KB cells, the accumulation of VBL was enhanced up to 1.4 times by PAK-104P, 1.8 times by PAK-101 and PAK-101P, and 1.3 times by verapamil. Cepharanthine had no enhancing effect.

Effect of PAK-104P on Efflux of VBL. We examined whether increased accumulation of anticancer agents in KB-C2 cells by PAK-104P was due to the inhibition of drug efflux. After incubation of the cells for 3 min in the absence of PAK-104P, about 60% of VBL was lost from KB-C2 cells, whereas about 70% of VBL was retained in KB cells. Addition of 10 μM PAK-104P to the culture medium completely terminated this efflux of VBL from KB-C2 cells at 1 min and thereafter. PAK-104P did not affect the efflux of VBL from KB cells (Fig. 6).

Effect of Agents on Photoaffinity Labeling of P-glycoprotein by [3H]Azidopine. The radioactive photoactive dihydropyridine calcium channel blocker, [3H]azidopine, photolabeled P-glycoprotein in membrane vesicles from KB-C2 cells (25). This photolabeling was almost completely inhibited by dihydropyridine analogues that reversed drug resistance but was not significantly inhibited by analogues that do not reverse resistance (25).

Since the reversing of the drug resistance by dihydropyridine analogues seems to be correlated with the inhibition of the [3H]azidopine photolabeling of P-glycoprotein by dihydropyridine analogues, we studied the effect of newly synthesized dihydropyridine analogues and their pyridine analogue counterparts on the labeling. The effect of verapamil and cepharanthine on the labeling was also investigated and compared with that of newly synthesized agents (Fig. 7).

Two pyridine analogues, PAK-103P and PAK-104P, that had a greater ability to reverse drug resistance than their dihydropyridine analogue counterparts were more potent inhibitors of the photolabeling than their counterpart dihydropyridine analogues, PAK-103 and PAK-104. PAK-104P, the most potent MDR-reversing agent among the tested compounds, had the greatest ability to inhibit photolabeling.

The MDR-reversing ability of the compounds seems to be correlated with their binding activity to P-glycoprotein except PAK-102. PAK-102 was the weakest inhibitor of the photolabeling among the used compounds, but it had greater ability to reverse drug resistance than PAK-103.

DISCUSSION

The focus of our research has been to find potent MDR-reversing agents which have low calcium channel-blocking activity (18, 25, 32). In this study all the newly synthesized pyridine analogues showed more than 1 order of magnitude lower calcium-antagonistic activity than their dihydropyridine analogue counterparts.

Further, the ability of two pyridines, PAK-103P and PAK-
104P, to reverse MDR was greater than that of their dihydro-pyridine analogue counterparts. PAK-104P had the greatest ability to reverse MDR among agents used in this study. The reversing ability of PAK-104P was about 10 times that of verapamil, and the calcium-antagonistic activity of the pyridine analogue was about 50% of that of verapamil.

PAK-104P at $5 \times 10^{-6}$ and $10^{-6}$ M completely reversed resistance to VCR in strongly resistant KB-C2 and weakly resistant KB-8-5 cells, respectively. KB-C2 is 939 times and KB-8-5 is 40 times as resistant to VCR as drug-sensitive KB. Since the tumor cells in patients are expected to have a relatively low degree of resistance, there seems to be a good prospect that we will be able to reverse drug resistance in such tumors at lower doses of PAK-104P.

PAK-104P also enhanced the sensitivity of KB cells to vin-cristine by a factor of 3. The pyridine analogue increased the accumulation of vinblastine in KB cells. Since we could not detect any expression of P-glycoprotein in KB cells with Western blotting analysis, we assume that molecules other than P-glycoprotein are involved in these phenomena.

It is known that a photoaffinity analogue of vinblastine labels P-glycoprotein (33, 34) and the photolabeling was inhibited by most of the agents that reverse MDR (22). Safa et al. (35) reported that a dihydropyridine analogue, azidopine, photolabeled P-glycoprotein and that this labeling was inhibited by vinblastine and actinomycin D. A photoanalogue of other MDR-reversing agents, verapamil (36) and SDB-ethylendiamine* (submitted) was also shown to label P-glycoprotein. These results indicate that hydrophobic anticancer agents that are involved in MDR bind to P-glycoprotein and that the binding site of these agents may be identical with that of MDR-reversing agents, verapamil, SDB-ethylendiamine, and azidopine.

Recently, we suggested a role for P-glycoprotein in the reversal of resistance by dihydropyridine analogues by demonstrating the correlation between the reversal of drug resistance and the inhibition of $[^3H]$azidopine photolabeling of P-glycoprotein by dihydropyridine analogues (25). Now it seems that potent MDR-reversing agents have a high affinity for P-glycoprotein. Two pyridine analogues, PAK-103P and PAK-104P, had a greater ability to inhibit $[^3H]$azidopine photolabeling of P-glycoprotein than their dihydropyridine analogue counterparts. PAK-104P was the most potent inhibitor in this study, suggesting that it has a higher affinity to P-glycoprotein than the other agents. Since $[^3H]$azidopine labeling was inhibited by PAK-104P and since PAK-104P inhibited the efflux of VBL from KB-C2 cells and increased the accumulation of VBL, we conclude that PAK-104P binds to the same site on P-glycoprotein as that where the hydrophobic anticancer agent vinblastine binds. Inhibition of photoaffinity labeling correlated well with MDR-reversing activity of each of the compounds used in this study except PAK-102. MDR-reversing activity of PAK-102 was similar to that of PAK-102P and higher than that of PAK-103, but the PAK-102 was the weakest inhibitor of the labeling among them. PAK-102 may need to be metabolized by a cell before functioning as a blocker of P-glycoprotein.

Zamora et al. (19) and Ford et al. (20) reported that hydrophobicity seems to be one of the common features of MDR-reversing agents. The pyridine analogues used in this study are more hydrophobic than their dihydropyridine counterparts. Increased hydrophobicity of the pyridines may be correlated with their increased MDR-reversing activity and their binding to P-glycoprotein. However, the increased hydrophobicity alone seems not to be sufficient. Other features of two pyridines, PAK-103P and PAK-104P, must also be concerned with their increased MDR-reversing activity, because the reversing activity of PAK-101P and PAK-102P was similar to that of their dihydropyridine analogue counterparts. More precise studies with more analogues are needed to formulate a hypothesis about structure-activity relationships of these new compounds.

This is the first report that has shown the high ability of two pyridine analogues to reverse MDR. We may be able to find even more potent MDR-reversing agents by further screening pyridine analogues.

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Two Pyridine Analogues with More Effective Ability to Reverse Multidrug Resistance and with Lower Calcium Channel Blocking Activity Than Their Dihydropyridine Counterparts

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