Reversal of P-Glycoprotein and Multidrug-Resistance Protein-Mediated Drug Resistance in KB Cells by 5-O-Benzoylated Taxinine K

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ABSTRACT
A newly synthesized taxoid originally from the Japanese yew Taxus cuspidata, 5-O-benzoylated taxinine K (BTK) was examined for its ability to reverse P-glycoprotein (P-gp) and multidrug resistance protein (MRP)-mediated multidrug resistance. BTK reversed the resistance to paclitaxel, doxorubicin (ADM), and vincristine (VCR) of KB-8–5 and KB-C2 cells that overexpress P-gp by directly interacting with P-gp. BTK also moderately reversed the resistance to ADM of KB/MRP cells that overexpress MRP. However, BTK neither inhibited the transporting activity of MRP nor reduced intracellular glutathione levels in KB/MRP cells. BTK shifted the distribution of ADM in KB/MRP cells from punctate cytoplasmic compartments to the nucleoplasm and cytoplasm by inhibiting acidification of cytoplasmic organelles. These two functions of BTK make it able to reverse both P-gp- and MRP-mediated MDR. BTK in combination with ADM should be useful for treating patients with tumors that overexpress both P-gp and MRP.

The mechanism of multidrug resistance (MDR) has been studied intensively in tissue culture cells and human cancers. P-glycoprotein (P-gp) is a 170-kDa transmembrane glycoprotein that is expressed in various MDR cell lines and that functions as an ATP-dependent drug efflux pump (Gottesman and Pastan, 1988; Endicott and Ling, 1989). P-gp-mediated MDR is reversed by a variety of compounds that inhibit drug efflux from the cells (Safa et al., 1987; Akiyama et al., 1988; Ford and Hait, 1990). Multidrug resistance protein (MRP) is a 190-kDa membrane glycoprotein that is overexpressed in many non-P-gp-mediated MDR cell lines (Cole et al., 1992; Roe et al., 1996). MRP is an ATP-dependent transporter of glutathione (GSH)-conjugates, such as leukotriene C4 (LTC4) and S-(2,4-dinitrophenyl)-glutathione and might confer drug resistance on MRP-expressing cells by transporting anticancer agents out of the cells (Leier et al., 1994). Most agents that reverse P-gp-mediated MDR do not reverse MRP-mediated MDR (Cole, 1992). Some agents, however, such as buthionine sulfoximine (Versantvoort et al., 1995), MK571 (Gekeler et al., 1995), ONO-1078 (Nagayama et al., 1998), and PAK-104P (Sumizawa et al., 1997) reverse MRP-mediated MDR. Buthionine sulfoximine, an inhibitor of glutathione synthesis, enhances the toxicity of anticancer agents in MRP-expressing MDR cells by decreasing the levels of intracellular GSH and inhibiting the enhanced efflux of the anticancer agents (Versantvoort et al., 1995). The leukotriene D4 (LTD4) receptor antagonist, MK571, inhibits the photolabeling of MRP by [3H]LTC4, a substrate for MRP, and the transport of [3H]LTC4 into membrane vesicles from MDR cells overexpressing MRP (Gekeler et al., 1995). The LTD4 receptor antagonist ONO-1078 (Nagayama et al., 1998) and the pyridine analog PAK-104P (Sumizawa et al., 1997) competitively inhibit the ATP-dependent [3H]LTC4 transport by membrane vesicles isolated from MDR cells expressing MRP.

5-O-benzoylated taxinine K (BTK) is a new taxoid from the Japanese yew Taxus cuspidata (Sako et al., 1998). In this study, we examined whether synthetic BTK reverses the resistance to paclitaxel (Taxol; Bristol-Myers Squibb Company, New York, NY), doxorubicin (ADM; Bristol-Myers Squibb Company, New York, NY), and vincristine (VCR; Bristol-Myers Squibb Company, New York, NY) in KB-8–5 and KB-C2 cells that overexpress P-gp by directly interacting with P-gp. BTK also moderately reverses the resistance to ADM of KB/MRP cells that overexpress MRP. However, BTK neither inhibited the transporting activity of MRP nor reduced intracellular glutathione levels in KB/MRP cells. BTK shifted the distribution of ADM in KB/MRP cells from punctate cytoplasmic compartments to the nucleoplasm and cytoplasm by inhibiting acidification of cytoplasmic organelles. These two functions of BTK make it able to reverse both P-gp- and MRP-mediated MDR. BTK in combination with ADM should be useful for treating patients with tumors that overexpress both P-gp and MRP.

ABBREVIATIONS: MDR, multidrug resistance (or resistant); P-gp, P-glycoprotein; MRP, multidrug resistance protein; GSH, glutathione; LTC4, leukotriene C4; LTD4, leukotriene D4; PAK-104P, 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; BTK, 5-O-benzoylated taxinine K; ADM, doxorubicin; VCR, vincristine; AO, acridine orange; MEM, minimal essential medium; APMSF, α-aminophenyl methanesulfonyl fluoride hydrochloride; LRP, lung resistance-related protein; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.
New York, NY), doxorubicin (ADM), and vincristine (VCR) of P-gp-mediated MDR and MRP-mediated MDR cells.

Materials and Methods

Chemicals. [14C]ADM (54.0 mCi/mmol) and [3H]azidopine (2200 Ci/mmol) were obtained from Amersham International Ltd. (Buckinghamshire, UK), [3H]paclitaxel (6.5 Ci/mmol) from Moravek Biochemicals Inc., [14,15,19,20-3H]LTC4 (150 Ci/mmol) from DuPont-New England Nuclear (Boston, MA), Acridine orange (AO) from Molecular Probes (Eugene, OR), and PAK-104P from Nissan Chemical Industries (Chiba, Japan). BTK (Fig. 1) was synthesized by one of the authors (M.S.). Other chemicals were obtained from Sigma Chemical (St. Louis, MO).

Cells and Cell Culture. A human epidermoid KB carcinoma cell line, KB–3–1 (Akiyama et al., 1985), was the parental drug-sensitive cell line. KB–3–1 cells and KB/MPR cells (Taguchi et al., 1997), KB–3–1 cells transfected with MRP cDNA, were cultured in minimal essential medium (MEM) (Nissui Seiyaku Co., Tokyo, Japan) containing 10% newborn calf serum. The P-gp mediated MDR mutant KB–8–5 and KB–C2 cells, which have increased levels of MDR1 mRNA, were originally selected from KB–3–1 cells and cultured in medium containing 10 ng/ml and 2 μg/ml colchicine, respectively (Akiyama et al., 1985).

Preparation of Membrane Vesicle and Total Cell Lysates. Membrane vesicles from KB–C2 and KB/MPR cells were prepared as described previously (Cornwell et al., 1986). To prepare the total cell lysates, cells were washed with PBS and scraped into PBS containing 1% Igepal CA-630 (Sigma), 0.5% sodium deoxycholate, 0.1% SDS, 1% aprotinin (Sigma), and 1 mM p-amidinophenyl methanesulfonyl fluoride hydrochloride (APMSF; Wako, Osaka, Japan). The lysates were passed through a 21-gauge needle to shear the DNA, incubated for 30 min on ice, and centrifuged at 15,000 g for 20 min at 4°C. Protein concentrations were determined by the method of Bradford (1976).

Immunoblot Analyses of P-gp, MRP, and Lung Resistance-Related Protein (LRP) in MDR KB Cells. Membrane vesicles (20 μg of protein) for the analysis of P-gp and MRP expression, and total cell lysates (100 μg of protein) for the analysis of LRP were prepared from KB–3–1, KB–8–5, KB–C2, and KB/MPR cells and proteins were separated by SDS-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes. The transferred proteins were reacted with a monoclonal antibody against P-gp (C-219) (Sumizawa et al., 1997), and a polyclonal antibody against MRP (Sumizawa et al., 1997), a polyclonal antibody against MRP (MR3PAB) (Chen et al., 1999), and a polyclonal antibody against LRP (anti-LRP) (Kitazono et al., 1999) and incubated for 1 h at room temperature for with horseradish peroxidase-conjugated goat anti-rabbit IgG. Antibody binding was visualized with the Enhanced Chemiluminescence Western blotting detection system (Amersham).

Drug Accumulation. Accumulation of paclitaxel in KB–3–1 and KB–C2 cells was measured using [3H]paclitaxel, and accumulation of ADM in KB–3–1 and KB/MPR cells was measured using [14C]ADM.

To measure drug accumulation, confluent monolayers of cells in 24-well plates were incubated with 0.1 μM [3H]paclitaxel or 1 μM [14C]ADM in MEM with or without the reversing agents (3 μM BTK or 3 μM verapamil) for 1 h at 37°C after they had been incubated in MEM with or without the reversing agents for 30 min at 37°C. After 3 washes with ice-cold PBS, the cells were solubilized in 10 mM phosphate buffer, pH 7.4, containing 1% Triton X-100 and 0.2% SDS and the incorporated radioactivity was determined.

Photoaffinity Labeling with [3H]Azidopine and SDS Polyacrylamide Gel Electrophoresis. Membrane vesicles (100 μg of protein) were incubated with 1 μM [3H]azidopine for 15 min at room temperature in the presence of the indicated concentrations of BTK or verapamil. After continuous irradiation at 366 nm for 30 min at 25°C, samples were solubilized in an SDS buffer as described previously (Debenham et al., 1982). Electrophoresis in 7.5% polyacrylamide gels was carried out according to the method of Laslett et al. (1970) without heating the samples. Gels were fixed, stained with 0.25% Coomassie blue in 50% trichloroacetic acid and subjected to fluoroxygraphy using ENLIGHTNING (DuPont-New England Nuclear). The dried gels were exposed to Kodak XAR film (Kodak, Rochester, NY) at −80°C.

[3H]LTC4 Uptake by Membrane Vesicles. LTC4 uptake by the vesicles was measured by filtration essentially as described by Ishikawa et al. (1989). The standard incubation medium contained membrane vesicles (50 μg of protein), 1.34 nM [3H]LTC4, 0.25 M sucrose, 10 mM Tris-HCl, pH 7.4, 10 mM MgCl2, 1 mM ATP, 10 mM creatine phosphate, and 100 μg/ml creatine kinase in a final volume of 100 μl. The reaction was carried out at 37°C and stopped with 1 ml of ice-cold 0.25 M sucrose, 100 mM NaCl, and 10 mM Tris-HCl, pH 7.4 (stop solution). The diluted samples were passed through Millipore filters (GVWP, 0.22 μm pore size) under a light vacuum. The filters were washed with 4 ml of ice-cold stop solution and then dried in an oven at 50°C for 10 min. Each filter was placed...
in scintillation fluid and radioactivity was measured in a liquid scintillation counter.

**GSH Assay.** Cells incubated with or without agents were harvested, suspended in 0.5 ml of 5% metaphosphoric acid and disrupted by sonication. After centrifugation at 15,000g for 10 min at 4°C, the GSH levels in the supernatants were measured using a GSH-400 kit (BIOTECH S.A., Bonneuil/Marne, France).

**Labeling of Cells with ADM.** KB-3–1 and KB/MRP cells were incubated for 60 min at 37°C in MEM containing 10% newborn calf serum and 30 μM ADM in the presence or absence of 3 μM BTK or verapamil. The cells were then washed with PBS and examined by confocal microscopy (Leica TCS4D; Leica, Wetzlar, Germany). ADM fluorescence was observed with λex at 488 nm.

**Labeling of Cells with AO.** KB-3–1 and KB/MRP cells were incubated for 15 min at 37°C in MEM containing 10% newborn calf serum and 6 μM AO in the presence or absence of 10 μM BTK, 10 μM tamoxifen, or 10 μM verapamil. The cells were then washed with PBS and examined by confocal microscopy with λem at 530/30 nm (green fluorescence) and λem at 600/long pass nm (red fluorescence).

**Results**

**Expression of P-gp, MRP, and LRP.** Using immunoblotting we investigated the P-gp, MRP, and LRP expression in KB-3–1, KB-8–5, KB-C2, and KB/MRP cells. The 170 kDa P-gp was detected in KB-8–5 and KB-C2 cells but not in KB-3–1 or KB/MRP cells. The 190-kDa MRP was detected in KB/MRP cells but not in KB-3–1, KB-8–5, or KB-C2 cells. The 110 kDa LRP was weakly detected in KB-3–1, but not in KB-3–1, KB-8–5, KB-C2, or KB/MRP cells (Fig. 2).

**Effect of MDR Reversing Agents on Sensitivity of KB Cells to Paclitaxel, ADM, and VCR.** The ability of BTK to reverse drug resistance in cells overexpressing P-gp and MRP was examined and compared with that of verapamil and cepharanthine which reverse P-gp-mediated MDR. The cytotoxic effects of the MDR-reversing agents were examined with the MTT assay. BTK (≤3 μM), verapamil (≤3 μM), and cepharanthine (≤5 μM) had no cytotoxic effects on KB-3–1, KB-8–5, KB-C2, and KB/MRP cells (data not shown). Table 1 summarizes the MTT assay data. The reversing effects of BTK in KB cells to VCR and ADM were concentration dependent. BTK and cepharanthine at 3 μM completely reversed the resistance of KB-8–5 cells and almost completely reversed the resistance of KB-C2 cells to paclitaxel. BTK at 1 μM considerably increased the sensitivity of KB-8–5 cells to ADM and VCR but could not completely reverse the resistance to these agents in the cells. The reversing effects of BTK were concentration dependent. Verapamil almost completely reversed the resistance of KB-8–5 cells and moderately reversed the resistance of KB-C2 cells to paclitaxel. BTK almost completely reversed the resistance of KB-8–5 cells and moderately reversed the resistance of KB-C2 cells to ADM and VCR. Other reversing agents (verapamil and cepharanthine) moderately reversed the resistance of KB-8–5 cells and weakly reversed the resistance of KB-C2 cells to ADM and VCR. These data suggest that the ability of BTK to reverse drug resistance in cells that overexpress P-gp is greater than that of the other reversing agents.

On the other hand, BTK moderately reversed the resistance of KB/MRP cells to ADM and VCR. Verapamil and cepharanthine did not reverse the resistance of KB/MRP cells to ADM, although they moderately reversed resistance to VCR. However, BTK, verapamil, and cepharanthine did not enhance the sensitivity of KB/MRP cells to paclitaxel, which is not transported well by MRP.

**Effect of MDR-Reversing Agents on Cellular Accumulation of Paclitaxel and ADM.** To investigate how BTK reversed the resistance of KB-C2 cells to paclitaxel, we examined its effect on the accumulation of paclitaxel in KB-3–1 and KB-C2 (Fig. 3A). The accumulation of paclitaxel in KB-C2 cells was 40% of that in KB-3–1 cells. BTK at 3 μM enhanced the accumulation of paclitaxel in KB-C2 cells to a level 1.2 fold higher than that in KB-3–1 cells not treated with BTK. Although addition of verapamil at 3 μM also enhanced the accumulation of paclitaxel in KB-C2 cells, its effect was significantly less than that of BTK. We examined whether the increased accumulation of paclitaxel in KB-C2 cells caused by BTK was attributable to inhibition of paclitaxel efflux. We investigated release of paclitaxel as a function of time after it accumulated for 60 min. KB-C2 cells released a higher percentage of accumulated paclitaxel than KB-3–1 cells. At 60 min, 83% of cell-associated paclitaxel was lost from KB-C2 cells, whereas 67% was retained in KB-3–1.

<table>
<thead>
<tr>
<th>TABLE 1</th>
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<tr>
<td>Effects of MDR-reversing agents on the cytotoxicity of paclitaxel, ADM, and VCR</td>
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<tr>
<td>Cell survival was determined by MTT assay. Values are the means ± S.D. of three experiments. Relative resistance (IC50 value for paclitaxel, ADM, or VCR of KB cells with or without the reversing agents was divided by IC50 value for paclitaxel, ADM, or VCR of KB-3–1 without the reversing agents).</td>
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<tr>
<th>KB-3–1</th>
<th>KB-8–5</th>
<th>KB-C2</th>
<th>KB/MRP</th>
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<tr>
<td><strong>Taxol (nM)</strong></td>
<td>6.2 ± 0.5 (1)</td>
<td>78.6 ± 5.5 (12.7)</td>
<td>2733.3 ± 32.8 (441)</td>
</tr>
<tr>
<td>+ BTK (3μM)</td>
<td>7.3 ± 0.4 (1.16)</td>
<td>2.7 ± 0.1 (0.44)</td>
<td>8.4 ± 0.3 (1.29)</td>
</tr>
<tr>
<td>+ Verapamil (3μM)</td>
<td>6.5 ± 0.2 (0.15)</td>
<td>4.3 ± 0.5 (0.70)</td>
<td>12.1 ± 0.2 (2.0)</td>
</tr>
<tr>
<td>+ Cepharanthine (3μM)</td>
<td>26.2 ± 9.0 (1.39)</td>
<td>73.5 ± 5.8 (4.06)</td>
<td>513.0 ± 36.6 (28.5)</td>
</tr>
<tr>
<td>+ Tamoxifen (10μM)</td>
<td>15.1 ± 1.0 (0.85)</td>
<td>N.D.</td>
<td>N.D.</td>
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<tr>
<td><strong>ADM (nM)</strong></td>
<td>16.1 ± 0.8 (0.87)</td>
<td>56.4 ± 1.5 (3.05)</td>
<td>222.3 ± 12.4 (12.0)</td>
</tr>
<tr>
<td>+ BTK (1μM)</td>
<td>11.7 ± 0.8 (0.67)</td>
<td>21.7 ± 0.5 (1.22)</td>
<td>78.5 ± 0.7 (4.39)</td>
</tr>
<tr>
<td>+ Verapamil (3μM)</td>
<td>20.6 ± 0.7 (1.11)</td>
<td>138.3 ± 4.5 (7.72)</td>
<td>290.1 ± 8.6 (16.1)</td>
</tr>
<tr>
<td>+ Cepharanthine (3μM)</td>
<td>24.6 ± 0.9 (1.39)</td>
<td>73.5 ± 5.8 (4.06)</td>
<td>265.5 ± 15.2 (14.7)</td>
</tr>
<tr>
<td>+ Tamoxifen (10μM)</td>
<td>15.1 ± 1.0 (0.85)</td>
<td>N.D.</td>
<td>N.D.</td>
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<tr>
<td><strong>VCR (nM)</strong></td>
<td>12.0 ± 0.9 (1)</td>
<td>659.3 ± 17.6 (54.9)</td>
<td>2033.3 ± 98.8 (169)</td>
</tr>
<tr>
<td>+ BTK (1μM)</td>
<td>8.4 ± 0.4 (0.70)</td>
<td>34.5 ± 1.5 (2.88)</td>
<td>255.6 ± 12.5 (21.3)</td>
</tr>
<tr>
<td>+ Verapamil (3μM)</td>
<td>6.1 ± 0.5 (0.50)</td>
<td>14.2 ± 1.2 (1.17)</td>
<td>99.2 ± 2.4 (8.25)</td>
</tr>
<tr>
<td>+ Cepharanthine (3μM)</td>
<td>10.5 ± 0.7 (0.82)</td>
<td>76.2 ± 1.9 (6.33)</td>
<td>345.8 ± 11.2 (28.8)</td>
</tr>
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</table>

N.D., not determined.
Fig. 3. A, accumulation of paclitaxel in KB-3-1 and KB-C2 cells. The intracellular levels of paclitaxel in the absence or presence of BTK or verapamil were determined as described under Materials and Methods. B, accumulation of ADM in KB-3-1 and KB/MRP cells. The intracellular levels of ADM in the absence or presence of BTK were determined as described under Materials and Methods. Columns represent means of triplicate determinations; bars represent S.D.
cells. When 3 μM BTK was added, 63% of paclitaxel was retained in KB-C2 cells. These data suggest that BTK inhibits the efflux of paclitaxel from KB-C2 cells (data not shown).

We also examined the accumulation of ADM in KB-3–1 and KB/MRP to investigate how BTK reversed the resistance of KB/MRP cells to ADM (Fig. 3B). The accumulation of ADM in KB/MRP cells was 68% of that in KB-3–1 cells. The addition of BTK at 3 μM did not enhance the accumulation of ADM in KB-3–1 or KB/MRP cells.

**Effect of BTK on Photoaffinity Labeling with \[^{3}H\]Azidopine.** We studied the effect of BTK on the photolabeling of P-gp in membrane vesicles from KB-C2 cells. BTK at 100 μM almost completely inhibited the labeling of P-gp and, at the same concentration of verapamil, partially inhibited the labeling. The inhibitory activity of BTK was more than 10 times higher than that of verapamil, because it was greater with BTK at 10 μM than with verapamil at 100 μM (Fig. 4).

**Effect of BTK on \[^{3}H\]LTC₄ uptake by KB/MRP Membrane Vesicles.** The effect of BTK on the ATP-dependent \[^{3}H\]LTC₄ uptake by KB/MRP vesicles was examined and compared with that of verapamil and PAK-104P. ATP-dependent \[^{3}H\]LTC₄ uptake by KB/MRP vesicles was 5.8-fold higher than that by KB-3–1 vesicles. PAK-104P reduced the \[^{3}H\]LTC₄ uptake by KB/MRP vesicles by 96%. However, BTK and verapamil did not significantly reduce \[^{3}H\]LTC₄ uptake by KB/MRP vesicles (Fig. 5).

**The Effect of BTK on GSH levels.** Because GSH is involved in MRP1-mediated drug resistance, we examined the effect of BTK on GSH levels in the cells. The intracellular GSH levels were 108.6 and 110.3 nmol per 10⁷ cells in KB-3–1 and KB/MRP cells, respectively. With 3 μM BTK, they were 110.7 and 112.7 nmol per 10⁷ cells in KB-3–1 and KB/MRP cells, respectively. BTK did not affect the GSH levels in KB-3–1 and KB/MRP cells.

**Fig. 4.** Effect of BTK on photoaffinity labeling of P-gp in membrane vesicles from KB-C2 cells. Membrane vesicles (100 μg of protein) were incubated with 1 μM \[^{3}H\]azidopine (47 Ci/mmol) for 15 min at room temperature in the presence of the indicated concentrations of BTK or verapamil and then processed as described under Materials and Methods.

**Fig. 5.** Effect of the various agents on the ATP-dependent \[^{3}H\]LTC₄ uptake by KB/MRP membrane vesicles. Membrane vesicles (50 μg of protein) were incubated with 1.34 nM \[^{3}H\]LTC₄ and with the indicated concentrations of each agent in the presence or absence of 1 mM ATP as described under Materials and Methods. ATP dependent accumulation was calculated from the difference in the radioactivity incorporated into the vesicles in the presence or absence of ATP. Columns represent means of triplicate determinations; bars represent S.D.
The Effect of BTK on ADM Localization. KB-3–1 and KB/MRP cells were incubated at 37°C with ADM in the absence or presence of BTK or verapamil and examined without fixation by confocal laser microscopy (Fig. 6A). In KB-3–1 cells, ADM was found in the nucleoplasm and cytoplasm in the absence or presence of BTK. In contrast, ADM was observed to be localized primarily in punctate cytoplasmic organelles in KB/MRP cells in the absence of BTK. In the presence of BTK, the distribution of ADM in KB/MRP cells shifted from punctate cytoplasmic compartments to the nucleoplasm and cytoplasm, which is similar to the distribution of ADM in KB-3–1 cells in the absence of BTK. Tamoxifen, which reduces organelle acidification (Altan et al., 1999), also shifted the distribution of ADM from punctate cytoplasmic compartments to the nucleoplasm and cytoplasm in KB/MRP cells (data not shown). However, verapamil did not change the distribution of ADM in KB/MRP cells.

The Effect of BTK on Acidification of Intracellular Organelles. To examine whether the BTK-induced increase of ADM in the nucleus could have been caused by release of ADM from cytoplasmic organelles, the effect of BTK on organelle acidification was examined (Fig. 6B). AO was used to detect acidic organelles (Schindler et al., 1996; Altan et al., 1999). It is a weakly basic fluorescent probe that accumulates in acidic organelles and emits green fluorescence at low concentration and red fluorescence at high concentration. AO produced a red fluorescence in the cytoplasm of KB-3–1 and KB/MRP cells. This finding is similar to that observed in nontransformed cells (Altan et al., 1999). Incubation with 10 μM BTK for 30 min caused a decrease of red AO fluorescence in KB-3–1 and KB/MRP cells. Similar effects have been observed with 10 μM tamoxifen (Schindler et al., 1996). BTK reduced organelle acidification more efficiently than tamoxifen at the same concentration. However, verapamil did not reduce organelle acidification. BTK and tamoxifen also reduced organelle acidification in KB-8–5 cells (data not shown).

Discussion

P-gp-mediated MDR is reversed by a variety of compounds, including calcium channel blockers and their analogs. Most of the agents that reverse P-gp-mediated MDR do not reverse MRP-mediated MDR. Recently, some new agents, the leukotriene D4 receptor antagonists MK571 (Gekeler et al., 1995) and ONO-1078 (Nagayama et al., 1998) and the pyridine analog PAK-104P (Sumizawa et al., 1997), were shown to reverse MRP-mediated MDR. These agents seem to suppress...
the transporting activity of MRP and thus inhibit drug efflux from the cells.

In this study, we examined whether BTK reverses resistance to paclitaxel, ADM and VCR in P-gp-mediated MDR cells (KB-8–5, KB-C2) and MRP-mediated MDR cells (KB/MRP). BTK is a newly synthesized taxoid originally from the Japanese yew T. cuspidata and one of the analogs of taxinine K (Sako et al., 1998). BTK inhibits P-gp function and increases VCR accumulation in MDR human ovarian cancer cells (Sako et al., 1998).

Here, we showed that BTK completely reversed resistance to paclitaxel, ADM, and VCR by inhibiting the function of P-gp and that this activity of BTK is greater than that of verapamil and cephaloridine. In particular, BTK completely reversed the resistance of KB-8–5 and KB-C2 cells to paclitaxel.

On the other hand, BTK, but not verapamil and cephaloridine, moderately reversed the resistance of KB/MRP cells to ADM. BTK did not restore the accumulation of ADM in KB/MRP cells, inhibit the transport of LTC₄ by KB/MRP vesicles, or reduce intracellular GSH levels in KB/MRP cells. These findings indicate that BTK does not inhibit the transporting activity of MRP. However, BTK blocked acidification of organelles in KB/MRP cells, resulting in the release of ADM from the organelles into the cytoplasm and nucleus. ADM localized in acidic organelles such as lysosomes, the trans-Golgi network and endosomes has been observed in drug resistant cells (Willingham et al., 1986). Disruption of the pH of acidic organelles in resistant cells with the tamoxifen, the protonophore monensin or nigericin, the proton pump inhibitor bafilomycin A₁, or the lysosomotropic agent chloroquine causes redistribution of weak base chemotherapeutics from acidic organelles to the nucleus and reverses drug resistance (Schindler et al., 1996; Hurwitz et al., 1997; Altan et al., 1998; Altan et al., 1999). In KB/MRP cells, BTK inhibited the acidiﬁcation of acidic organelles. This may be the reason why KB/MRP cells became more sensitive to ADM when treated with BTK. In this study, tamoxifen also inhibited the acidification of acidic organelles in KB/MRP cells and increased their sensitivity to ADM (Table 1).

Recently Kitazono et al. reported that LRP has an important role in the transport of ADM from the nucleus to the cytoplasm (Kitazono et al., 1999). Therefore, we examined the expression of LRP in KB/MRP and KB-3–1 cells. LRP was not detected in either cell line (Fig. 2). These findings suggest that BTK reversed the resistance of KB/MRP cells to ADM by inhibiting the acidification of acidic organelles.

In summary, BTK has two distinct functions. It inhibits the function of P-gp by directly interacting with the drug binding site of P-gp and also suppresses the acidification of acidic organelles. These two functions of BTK make it able to reverse both P-gp- and MRP-mediated MDR. BTK may be a useful modiﬁer of MDR for treating patients with tumors that overexpress both P-gp and MRP.

References


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