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Research paper

Reversal of multidrug resistance by novel verapamil analogs in cancer cells

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The present study was performed to evaluate the ability of KR-30032 and KR-30035 to overcome multidrug resistance (MDR) by measuring the cytotoxicity and the accumulation rate of rhodamine. Additionally, the adverse cardiac toxicity of KR-30032 and KR-30035 was evaluated by measuring the changes of tension in isolated rat aorta and left ventricular pressure (LVP) in guinea pig heart. KR-30035 potentiated the paclitaxel-induced cytotoxicity to HCT15 [P-glycoprotein (P-gp)-expressed cells] to over 15-fold greater than that of verapamil and KR-30032 was equipotent with verapamil (EC_{50}: 0.07, 5.0 and 3.3 nM at 1.0 μg/ml). KR-30032 and KR-30035 were without effect on cytotoxicity to SK-OV-3 cells (P-gp-non-expressing cells), as well as to tamoxifen-induced cytotoxicity in the above cell types. Maximal rhodamine accumulation rates with KR-30032, KR-30035 and verapamil were 290, 291 and 271% in HCT15 cells; and 451, 970 and 440% in HCT15/CL02 cells, respectively. KR-30032 and KR-30035 were 20- to 25-fold less potent than verapamil in relaxing aorta (EC_{50}: 8.13, 6.40 and 0.32 μM, respectively) and were 12- to 35-fold less potent than verapamil in decreasing LVP in isolated hearts (EC_{50}: 41.8, 14.1 and 1.2 μM, respectively). The results of this study suggest that KR-30032 and KR-30035 are active modulators of MDR with potentially minimal cardiovascular toxicity. [© 1998 Rapid Science Ltd.]

Key words: Cardiac toxicity, KR-30032, KR-30035, multidrug resistance, P-glycoprotein.

Introduction

The emergence of drug-resistant tumor cells remains a major problem in cancer chemotherapy. This resistant phenotype of cancer cell reveals a broad spectrum to structurally and/or functionally unrelated anticancer drugs, hence the term multidrug resistance (MDR). MDR is frequently associated with the overexpression of a membrane protein, P-glycoprotein (P-gp), encoded in human cells by the *mdr1* gene, which is thought to act as an energy-dependent drug efflux pump that results in decreased drug accumulation and diminished cytotoxicity to cancer cells.[1,2] It has been suggested that MDR associated with P-gp overexpression can occur in cancer patients during treatment with a number of drugs such as paclitaxel (Taxol®), anthracyclines (doxorubicin and daunorubicin), vinca alkaloids (vinblastine and vincristine), actinomycin D, mitomycin C and colchicine[3] or even in the absence of drug treatment. The MDR associated with P-gp overexpression could be reversed or modulated by inhibition of P-gp-mediated transport, via increased cellular accumulation of anticancer drugs with various agents such as calcium channel blockers,[4,5] calmodulin inhibitors,[5] antiarrythmics,[6] steroids,[7] antiestrogens[8] and cyclic peptide antibiotics.[9]

Verapamil, a calcium channel blocker, is one of the most extensively characterized modulators of P-gp-mediated MDR and was the first MDR-reversal agent that reached clinical trial.[10–12] However, the usefulness of verapamil was limited since plasma concentrations that were required to reverse MDR resulted in cardiac toxicity such as hypotension, congestive heart failure and heart block.[10,12] This dose-limiting toxicity was also observed in clinical trials with other MDR reversal agents such as cyclosporin A.[13] Accordingly, considerable effort is directed towards the development of compounds that inhibit P-gp, reverse the MDR phenotype and sensitize cancer cells to conventional chemotherapy without undesired toxicological effects. Thus, the present study was performed to evaluate the MDR reversal activities and possible cardiovascular adverse effects of KR-30032 and KR-30035.

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Materials and methods

Drugs and chemicals

Verapamil hydrochloride and (−)norepinephrine hydrochloride (NE) were purchased from Sigma (St Louis, MO). Reagents for the physiological solution used in the isolated aorta and Langendorff experiments were purchased from Junsei (Tokyo, Japan). KR-30032 and KR-30035 were synthesized at Bio-Organic Science Division of Korea Research Institute of Chemical Technology (KRICT, Taejon, Korea). The anticancer drugs (paclitaxel, doxorubicin and tamoxifen), agents for cell culture [gentamycin, amphotericin, 1,2-cyclohexanediaminetetraacetic acid (CDTA) and sodium bicarbonate] and agents for cytotoxicity test [trichloroacetic acid (TCA), sulforhodamine B (SRB), Tris base and rhodamine 123] were purchased from Sigma. RPMI 1640 cell growth medium, trypsin, fetal bovine serum and Hank's balanced salt solution (HBSS) were obtained from Gibco (Grand Island, NY). Verapamil and its analogs were dissolved in dimethyl sulfoxide, and diluted with distilled and deionized water. All drugs and reagents were prepared just prior to use.

Vasorelaxant effects on isolated rat aorta

Thoracic aorta was isolated from male Sprague-Dawley rats, weighing 350–450 g. Each aorta was cut into 2–3 mm wide rings with extreme care to preserve the endothelium. The aortic preparations were suspended between wire hooks in an organ bath containing 20 ml of Krebs' bicarbonate buffer (mM: NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $NaHCO_3$, 25; $MgSO_4$, 1.2; KH_2PO_4, 1.2; and glucose, 11.0) bubbled with a gas mixture (95% O_2, 5% CO_2) and maintained at 37 C. The aortic preparations were allowed to equilibrate for 60 min under 2 g of resting tension. Isometric contraction was measured with a force displacement transducer (FT03; Grass Instruments, Quincy, MA) and displayed on a chart recorder (Multicorder MC 6625; Hugo Sachs Electronic, March, Germany). The aortic preparations were precontracted submaximally with 10^{-7} M NE, washed three times for 45 min, and rechallenged with NE to obtain reproducible and stable response. After obtaining a plateau NE response, KR-30032, KR-30035 and verapamil (10^{-8} to 3×10^{-5} M) were cumulatively added to the tissue bath. Results were expressed as EC_{50} values (molar concentration that induces 50% relaxation of the maximal NE-induced contraction).

Langendorff studies in guinea pig heart

Hearts were rapidly excised from male guinea pigs, weighing 350–500 g, under pentobarbital anesthesia (100 mg/kg, i.v.). The aorta was cannulated and immediately perfused (70 mmHg) in a retrograde fashion with modified Krebs-Henseleit solution (mM: NaCl, 112; KCl, 4.7; $CaCl_2$, 1.25; $NaHCO_3$, 25; $MgSO_4$, 1.2; KH_2PO_4, 1.0; dextrose, 11.5; and pyruvate 2.0) bubbled with a gas mixture (95% O_2, 5% CO_2), and maintained at 37 C. Left ventricular pressure (LVP) was measured by a pressure transducer (P23XL; Grass Instruments) connected to a small rubber balloon inserted into the left ventricle and recorded on the physiograph (model 7; Grass Instruments). After the isolated hearts were allowed to equilibrate for 60 min, KR-30032, KR-30035 and verapamil (10^{-7} to 10^{-4} M) were cumulatively injected to the hearts. Results were expressed as EC_{50} values (molar concentration that decreases LVP by 50%).

Cancer cells

HCT15 and SK-OV-3 cells were originally provided by the National Cancer Institute (NCI, Bethesda, MD) and maintained at the Korea Research Institue of Chemical Technology (KRICT) continuously. HCT15 human adenocarcinoma cells were established from a colorectal cancer after surgical resection before chemotherapeutic treatment. It has been reported that HCT15 cells revealed a high level of P-gp expression and a high activity in rhodamine efflux.[14] On the other hand, human ovarian carcinoma cell line SK-OV-3 has been reported as P-gp-negative cancer cells.[15] The multidrug-resistant HCT15/CL02 cells were established from parental HCT15 cells by stepwise and continuous doxorubicin exposure in KRICT.[16] Stock cell cultures were conducted in Falcon T-25 flasks containing 10 ml of RPMI 1640 medium with glutamine, sodium bicarbonate, gentamycin, amphotericin and 5% fetal bovine serum. Cells were dissociated with 0.25% trypsin and 3 mM CDTA solution, and were maintained at 37 C in 100% humidified atmosphere containing 5% CO_2 in air.

Cytotoxicity assay

As described previously,[16] tumor cells were inoculated over a series of standard 96-well flat-bottom microplates (Falcon) and were then preincubated for 24 h to allow attachment to the microtiter plate. The attached cells were then incubated with serially diluted anti-

cancer drugs in the absence or presence of 0.25, 1.0 or 4.0 μg/ml of KR-30032, KR-30035 and verapamil. After continuous exposure to the compounds for 72 h, the culture medium was removed from each well and the cells fixed with 10% cold TCA at 4 C for 1 h. After wash with tap water, the cells were stained with 0.4% SRB dye and incubated for 30 min at room temperature. The cells were washed again and then solubilized with 10 mM unbuffered Tris base solution (pH 10.5). The absorbance was measured spectrophotometrically at 520 and 690 nm with a microtiter plate reader (E-max; Molecular Devices, Sunnyvale, CA). To eliminate the effects of non-specific absorbance, the absorbance at 690 nm was subtracted from that at 520 nm. The data were transferred and transformed into Micro Excel format (Microsoft, Santa Rosa, CA) and survival fractions were expressed as a percent of control. In these experiments, control means the well contained each corresponding concentration of the compounds without anticancer drugs. All experiments were performed in triplicate.

Rhodamine accumulation and efflux assay

Rhodamine accumulation was performed according to Lee *et al.*[14] with minor modifications. In brief, cells were seeded in two sets of 24-well flat-bottom plates in equivalent volumes with 1.5 ml of growth medium and were incubated in 5% CO_2 at 37 C for 24–72 h. At the semiconfluent logarithmic growth phase of the cells, the culture medium was removed from each well, and 10 μM rhodamine 123 in 1 ml of growth medium was added in the presence or absence of 4.0 μg/ml of KR-30032, KR-30035 and verapamil. After incubation in 5% CO_2 at 37 C for 40 min, the rhodamine-containing medium was removed and the cells washed twice with cold HBSS. For assay of rhodamine efflux, fresh growth medium that did not contain rhodamine and the compounds were added to one set of plates. These cells were incubated for an additional 2 h in 5% CO_2 at 37 C, followed by wash with cold HBSS. Following the accumulation or efflux of rhodamine, cells were lysed by addition of 1.5 ml of distilled water and the plates kept in the dark and cold until quantification of fluorescence. The green fluorescence of rhodamine 123 was measured at 485/20 nm excitation and 530/25 nm emission with a fluorescence microplate reader system (Cytofluor 2300; Millipore, Bedford, MA). Each plate included a blank control which had no cells (BC_{acc} or BC_{eff}; the subscript 'acc' and 'eff' mean accumulation and efflux, respectively), a cell control which had cells without the compounds (CC_{acc} or CC_{eff}) and test groups which had cells with the compounds (T_{acc} or T_{eff}). The percent of rhodamine accumulation and residual rate were calculated as $(T_{acc}-BC_{acc})/(CC_{acc}-BC_{acc})$ and (CC_{eff} or $T_{eff}-BC_{eff})/(CC_{acc}-BC_{acc})$, respectively.

Statistical analysis

Values for accumulation and efflux were expressed as mean ± SD while all other values were expressed as mean ± SEM. Data were analyzed by one-way analysis of variance (ANOVA) followed by Student-Newman Keuls test for multiple comparisons (Sigma Stat®; Jandel, San Rafael, CA). In all the comparisons, the difference was considered to be statistically significant at $p<0.05$.

Results

Vasorelaxant effects on isolated rat aorta

KR-30032, KR-30035 and verapamil (Figure 1) produced concentration-dependent relaxations of the aorta precontracted with NE (10^{-5} M) (Figure 2A). KR-30032 and KR-30035 were 20- to 25-fold less potent as vasorelaxants as compared to verapamil (EC_{50}: 8.13 ± 2.17, 6.40 ± 0.90 and 0.32 ± 0.06 μM, respectively; $p<0.05$ versus verapamil; $n=4$).

Langendorff studies in guinea pig heart

KR-30032, KR-30035 and verapamil induced a concentration-dependent decrease in LVP of isolated hearts (Figure 2B). KR-30032 and KR-30035 were 12- to 35-fold less potent than verapamil at decreasing LVP in isolated hearts (EC_{50}: 41.8 ± 8.1, 14.1 ± 3.5 and 1.2 ± 0.4 μM, respectively; $p<0.05$ versus verapamil; $n=4$).

Cytotoxicity assay

The cytotoxic effects of paclitaxel to HCT15, HCT15/CL02 and SK-OV-3 cells were concentration-dependently increased (Figure 3). The paclitaxel concentrations required to achieve 50% inhibition of growth (EC_{50}) in HCT15, HCT15/CL02 and SK-OV-3 cells were 41.3, 792.8 and 0.03 nM, respectively, demonstrating that HCT15 and HCT15/CL02 cells were about 1300- and 26 000-fold more resistant to paclitaxel than SK-OV-3.

KR-30032, KR-30035 and verapamil concentration-dependently potentiated paclitaxel-induced cytotoxicity to HCT15 cells (Figure 3A). KR-30032, KR-30035 and verapamil (0.25 μ/ml) potentiated the paclitaxel-induced cytotoxicity to HCT15 cells with similar potencies (EC_{50}: 29.5, 20.4 and 24.5 nM, respectively, compared with a control value of 41.3 nM). At 1.0 μg/ml, KR-30035 was about 50-fold more potent and KR-30032 was slightly less potent than verapamil in potentiation of the paclitaxel-induced cytotoxicity (EC_{50}: 0.07, 5.0 and 3.3 nM, respectively). At 4.0 μg/ml, KR-30035 was about 2000-fold more potent and KR-30032 was 2- to 3-fold less potent than verapamil in potentiation of the paclitaxel-induced cytotoxicity (EC_{50}: 0.000018, 0.1 and 0.04 nM, respectively).

KR-30032, KR-30035 and verapamil concentration-dependently enhanced the paclitaxel-induced cytotoxicity to HCT15/CL02 cells (Figure 3B). At 0.25 μg/ml, KR-30032, KR-30035 and verapamil slightly potentiated the paclitaxel-induced cytotoxicity to HCT15/CL02 cells (EC_{50}: 477.7, 488.9 and 690.5 nM, respectively, compared with a control value of 792.8 nM). At 1.0 μg/ml, KR-30035 was about 4-fold more potent and KR-30032 was equipotent with verapamil in potentiation of the paclitaxel-induced cytotoxicity (EC_{50}: 83.0, 301.4 and 315.6 nM, respectively). At 4.0 μg/ml, KR-30035 was about 15-fold more potent and KR-30032 was equipotent with verapamil in potentiation of the paclitaxel-induced cytotoxicity (EC_{50}: 4.2, 64.4 and 56.2 nM, respectively). KR-30032, KR-30035 and verapamil at all concentrations studied (0.25, 1.0 and 4.0 μg/ml) had no effects on the paclitaxel-induced cytotoxicity to SK-OV-3 cells (Figure 3C).

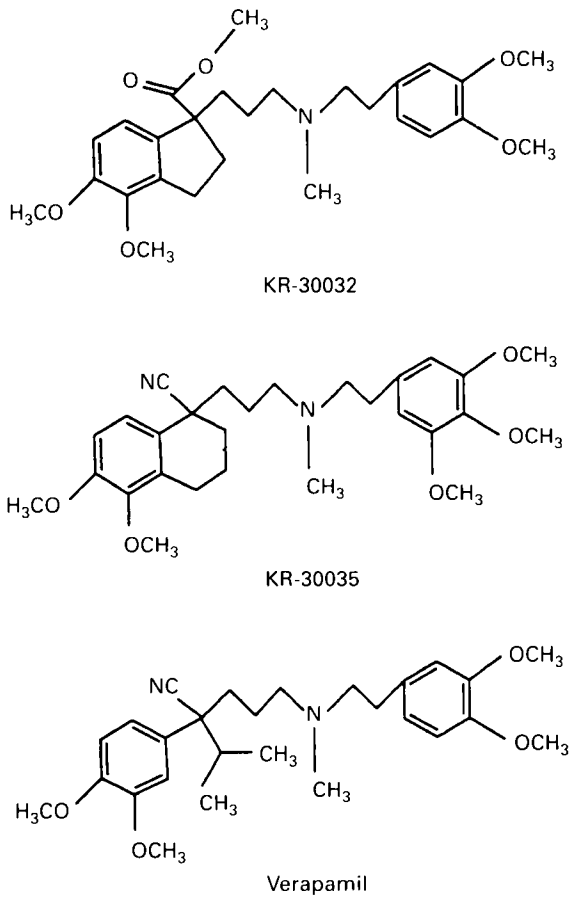

Figure 1. Chemical structures of KR-30032, KR-30035 and verapamil.

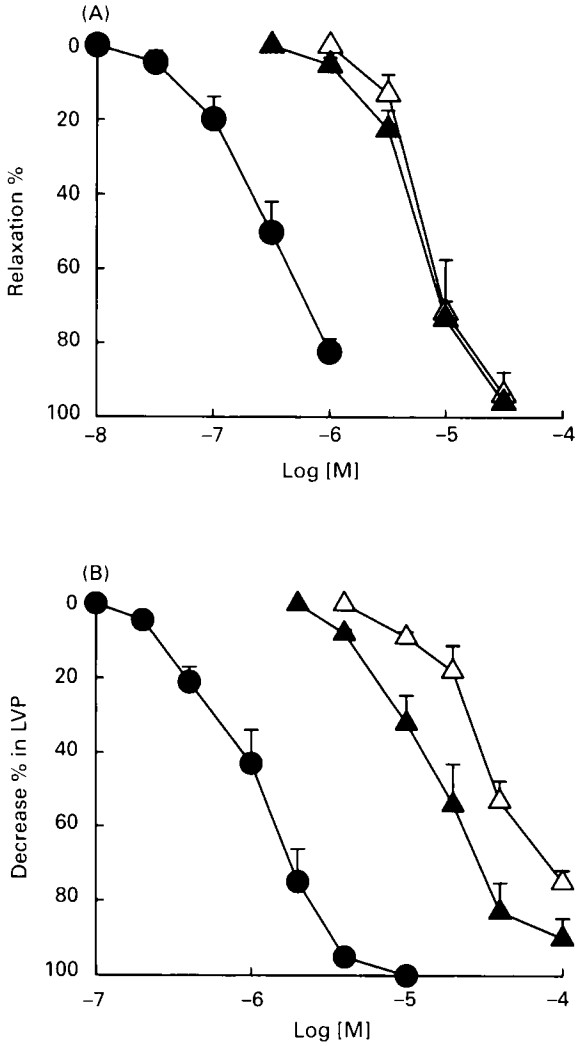

Figure 2. Effects of KR-30032 (open triangles), KR-30035 (solid triangles) and verapamil (solid circles) on isolated rat aorta precontracted with norepinephrine (10^{-7} M) (A) and left ventricular pressure (LVP) in isolated guinea pig heart (B). Each data point represents the mean of at least four experiments and the bar shows the SEM.

The cytotoxic effects of doxorubicin to HCT15, HCT15/CL02 and SK-OV-3 cells were concentration-dependently increased (Figure 4). The EC_{50} values of doxorubicin to HCT15, HCT15/CL02 and SK-OV-3 cells were 526.2, 14469.3 and 55.5 nM, respectively, demonstrating that HCT15 and HCT15/CL02 cells were about 10- and 250-fold more resistant to paclitaxel than SK-OV-3.

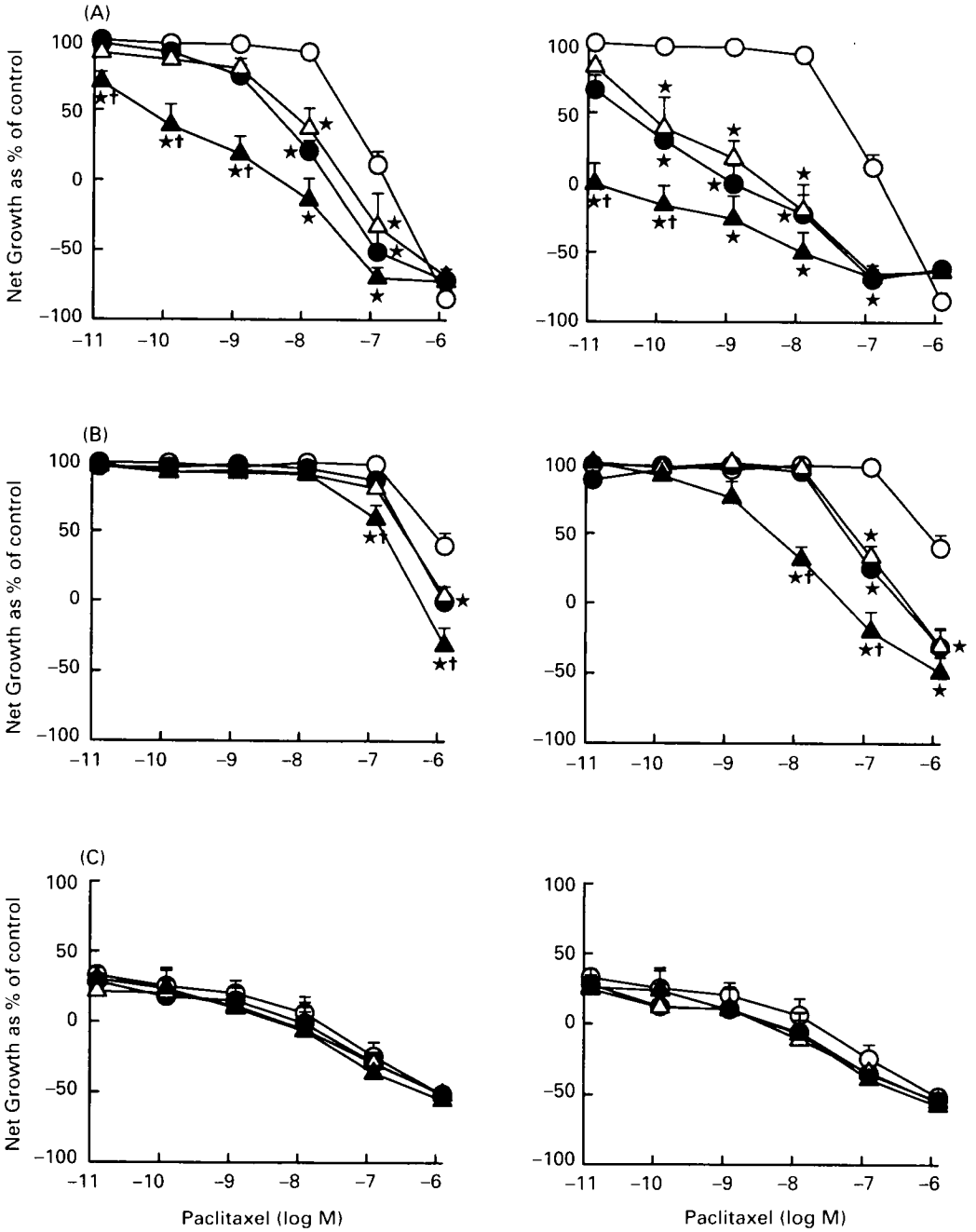

Figure 3. Effects of KR-30032, KR-30035 and verapamil on the cytotoxicities of paclitaxel to HCT15 (A), HCT15/CL02 (B) and SK-OV-3 (C) human cancer cells *in vitro*. The cells were cultured with 10-fold serial dilutions of paclitaxel in the absence (open circles) or presence of KR-30032 (open triangles), KR-30035 (solid triangles) and verapamil (solid circles) at the concentrations of 1.0 (left panel) or 4.0 (right panel) μg/ml. Cell survival fractions were assessed after continuous drug-exposure for 3 days by SRB assay. Each data point represents the mean of at least three experiments and the bar shows the SEM. *$p<0.05$, significantly different from control. †$p<0.05$, significantly different from verapamil.

KR-30032, KR-30035 and verapamil concentration-dependently potentiated the doxorubicin-induced cytotoxicity to HCT15 cells (Figure 4A). Although 0.25 μg/ml KR-30032, KR-30035 and verapamil did not potentiate the doxorubicin-induced cytotoxicity to HCT15 cells (725.2, 576.0 and 362.4 nM, respectively, compared with a control value of 526.2 nM), the compounds potentiated the cytotoxicity at 1.0 and 4.0 μg/ml. KR-30032 and KR-30035 were 2- to 5-fold less potent than verapamil in potentiation of the doxorubicin-induced cytotoxicity (EC_{50}: 214.0, 129.0 and

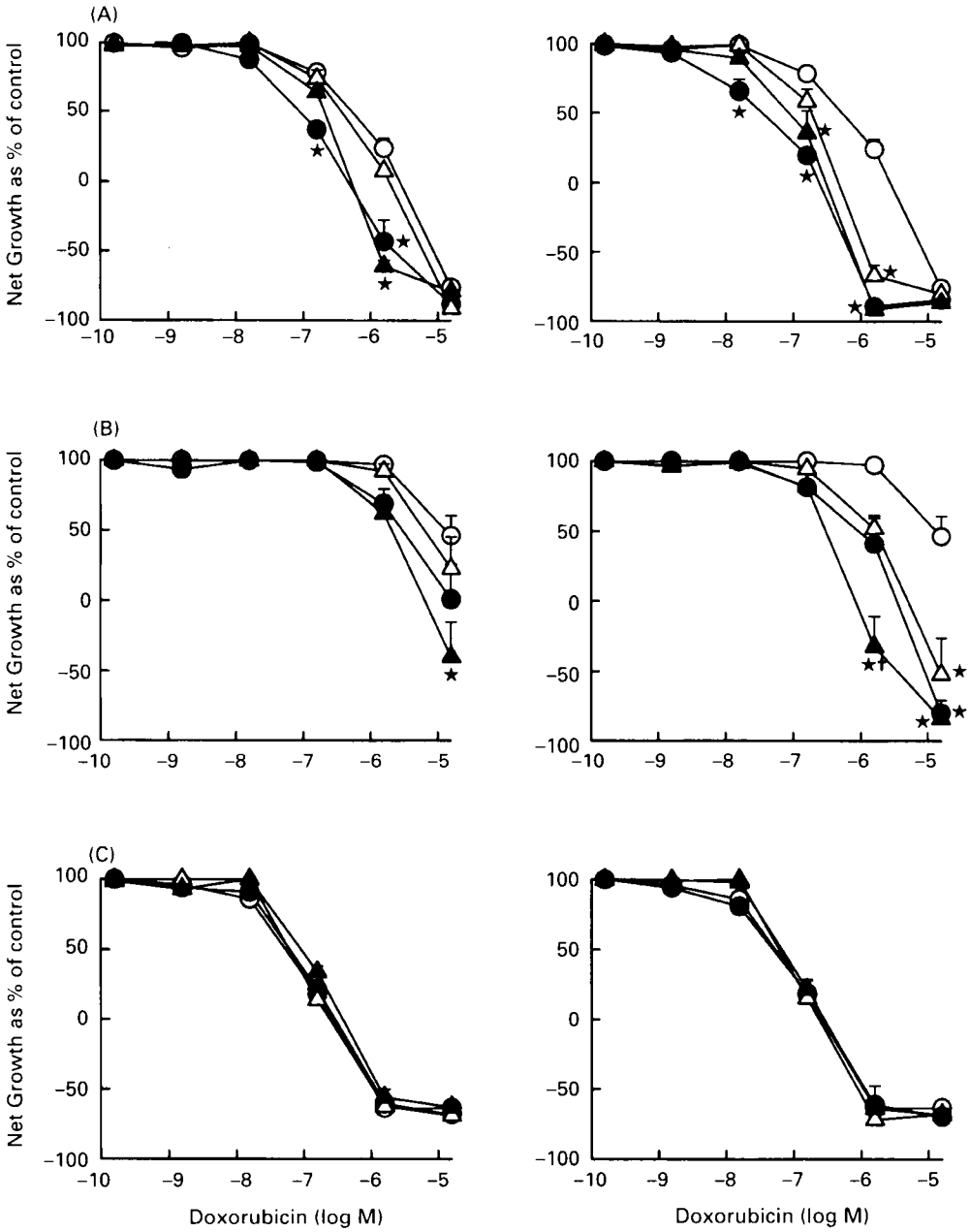

Figure 4. Effects of KR-30032, KR-30035 and verapamil on the cytotoxicities of doxorubicin to HCT15 (A), HCT15/CL02 (B) and SK-OV-3 (C) human cancer cells *in vitro*. The cells were cultured with 10-fold serial dilutions of paclitaxel in the absence (open circles) or presence of KR-30032 (open triangles), KR-30035 (solid triangles) and verapamil (solid circles) at the concentrations of 1.0 (left panel) or 4.0 (right panel) μg/ml. Cell survival fractions were assessed after continuous drug exposure for 3 days by SRB assay. Each data point represents the mean of at least three experiments and the bar shows the SEM. $^*p<0.05$, significantly different from control. $\dagger p<0.05$, significantly different from verapamil.

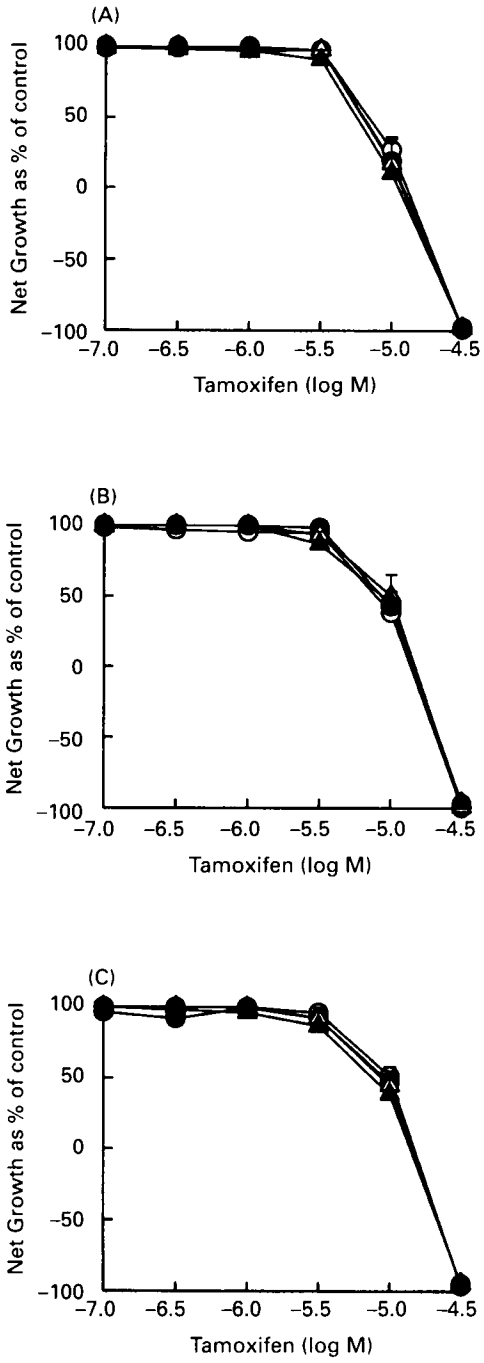

Figure 5. Effects of KR-30032, KR-30035 and verapamil on the cytotoxicities of tamoxifen to HCT15 (A), HCT15/CL02 (B) and SK-OV-3 (C) human cancer cells *in vitro*. The cells were cultured with 3.2-fold serial dilutions of tamoxifen in the absence (open circles) or presence of KR-30032 (open triangles), KR-30035 (solid triangles) and verapamil (solid circles) at the concentration of 4.0 μg/ml. Cell survival fractions were assessed after continuous drug exposure for 3 days by SRB assay. Each data point represents the mean of at least three experiments and the bar shows the SEM.

77.7 nM at 1.0 μg/ml; 102.5, 67.7 and 20.4 nM at 4.0 μg/ml, respectively).

KR-30032, KR-30035 and verapamil concentration-dependently enhanced the doxorubicin-induced cytotoxicity to HCT15/CL02 cells (Figure 4B). At all concentrations studied, KR-30035 was about 1.5-fold more potent and KR-30032 was 2- to 3-fold less potent than verapamil (EC_{50}: 8157.3, 11661.3 and 12821.7 nM at 0.25 μg/ml; 1274.8, 6925.5 and 2256.6 nM at 1.0 μg/ml; and 334.6, 987.4 and 661.3 nM at 4.0 μg/ml, respectively). KR-30032, KR-30035 and verapamil at all concentrations studied (0.25, 1.0 and 4.0 μg/ml) had no effects on the doxorubicin-induced cytotoxicity to SK-OV-3 cells (Figure 4C).

The cytotoxic effects of tamoxifen to HCT15, HCT15/CL02 and SK-OV-3 cells were concentration-dependently increased (Figure 5). The potencies of resistance of HCT15 and HCT15/CL02 cells to tamoxifen were similar with that observed to SK-OV-3 (EC_{50}: 5.91, 6.15 and 6.63 nM, respectively). KR-30032, KR-30035 and verapamil had no effects on tamoxifen-induced cytotoxicity in all the cell types.

Rhodamine accumulation and efflux assay

In HCT15 cells, KR-30032, KR-30035 and verapamil increased the rhodamine accumulation to similar magnitudes (290 ± 45, 291 ± 50 and $271 \pm 32\%$, respectively) (Figure 6). After additional incubation with fresh medium for 2 h, residual rhodamine was also significantly increased by KR-30032, KR-30035 and verapamil (19 ± 0.2, 38 ± 6.6 and $18 \pm 1.2\%$, respectively; $p < 0.05$ compared with control of $12 \pm 1.0\%$). The potentiation due to KR-30035 was significantly greater than that due to verapamil ($p < 0.05$). In HCT15/CL02 cells, KR-30032, KR-30035 and verapamil significantly increased the accumulation of rhodamine (451 ± 110, 970 ± 135 and $440 \pm 82\%$, respectively) (Figure 6). The potentiation due to KR-30035 was significantly more than that due to verapamil ($p < 0.05$). After additional incubation with fresh medium for 2 h, residual rhodamine was not significantly increased by KR-30032, KR-30035 and verapamil (5 ± 0.3, 11 ± 1.3 and $6 \pm 2.4\%$, respectively, compared with a control of $10 \pm 4.7\%$). In SK-OV-3 cells, KR-30032, KR-30035 and verapamil had no effects on rhodamine accumulation (89 ± 2.8, 89 ± 8.1 and $95.1 \pm 12.7\%$, respectively) and on residual rhodamine after 2 h (40 ± 6.7, 36 ± 8.6 and $37 \pm 4.7\%$, respectively, compared with control of $39 \pm 8.7\%$).

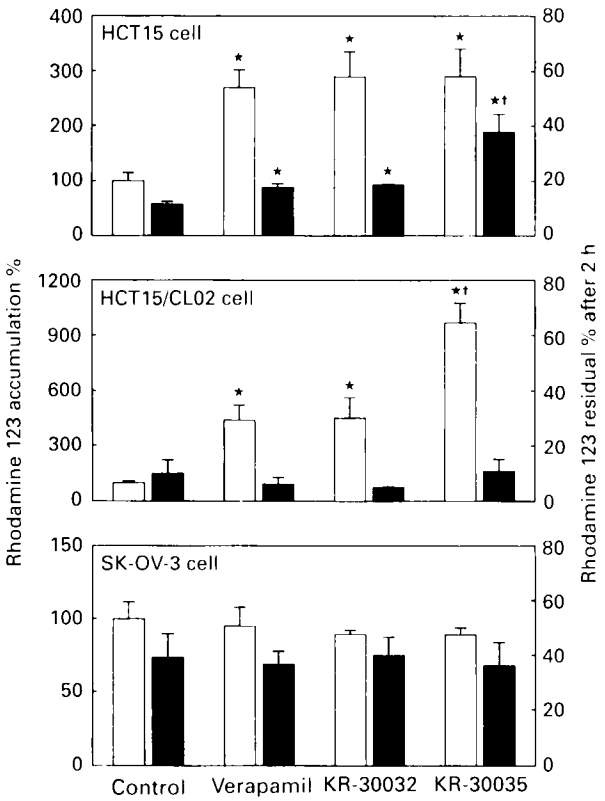

Figure 6. Effects of KR-30032, KR-30035 and verapamil on the accumulation (open bar; corresponding to left axis) and residual rate of rhodamine (closed bar; corresponding to right axis) in SK-OV-3, HCT15 and HCT15/CL02 human cancer cells. The cells were incubated with 10 mM rhodamine 123 in the absence or presence of verapamil, KR-30032 and KR-30035 at the concentration of 4.0 μg/ml for 40 min. Then, one set of cell culture plates was stopped rhodamine accumulation and extracellular rhodamine washed out by adding cold HBSS, and the other set of plates was incubated with fresh medium for an additional 2 h. At the end of both procedures, the intracellular rhodamines were measured by a fluoresecnce microplate reader at 485/20 nm excitation and 530/25 nm emission. Data are presented as the mean + SD of at least three experiments. $^{*}p < 0.05$, significantly different from control. $^{\dagger}p < 0.05$, significantly different from verapamil.

Discussion

KR-30032 and KR-30035 enhanced the paclitaxel-induced cytotoxicity to HCT15 and HCT15/CL02 cells that reveal a high level of P-gp expression. Similar results were obtained on doxorubicin-induced cytotoxicity to HCT15 and HCT15/CL02 cells. However, KR-30032 and KR-30035 were without effect on paclitaxel- and doxorubicin-induced cytotoxicity to SK-OV-3 cells that do not express P-gp.[15] These compounds also did not influence the cytotoxicity of paclitaxel to other P-gp-negative cells such as human non-small cell lung cancer cell line A549 and human skin cancer cell line SK-MEL-2 (unpublished data). Furthermore, these compounds had no effect on cytotoxicity of tamoxifen that was not transported by P-gp. These are in line with the recently published reports suggesting that verapamil enhances the cytotoxicity to P-gp-expressing cells, but not to P-gp-non-expressing cells.[16,17] These results suggest that KR-30032 and KR-30035 enhance the cytotoxicity of cancer drugs via modulation of PGP.

KR-30032 at doses tested was equipotent with verapamil in enhancing the paclitaxel-induced cytotoxicity to HCT15 and HCT15/CL02 cells; and 2- to 5-fold less potent than verapamil in enhancing doxorubicin-induced cytotoxicity to HCT15 and HCT15/CL02 cells. KR-30035 at doses tested was over 15- to 50-fold more potent than verapamil in enhancing the paclitaxel-induced cytotoxicity to HCT15 and HCT15/CL02 cells; and equipotent with verapamil in enhancing doxorubicin-induced cytotoxicity to HCT15 and HCT15/CL02 cells. KR-30032 and KR-30035 as well as verapamil were more effective to paclitaxel- than doxorubicin-induced cytotoxicity, which give one possibility that paclitaxel might be more sensitive to membrane P-gp than that of doxorubicin resulting from the different mode of action of paclitaxel and doxorubicin.

Recent studies have established a good correlation between rhodamine efflux and *mdr1* expression.[14] On the basis of this report, to investigate differential MDR-reversal activities of KR-30032, KR-30035 and verapamil, the accumulation and residual rates of rhodamine were measured. KR-30032 significantly increased the accumulation of rhodamine with a similar magnitude to verapamil in HCT15 (290 and 271%, respectively) and HCT15/CL02 cells (451 and 440%, respectively). KR-30035 was equipotent in HCT15 (291%) and significantly more potent than verapamil in HCT15/CL02 cells (970%, $p < 0.05$). However, KR-30032 and KR-30035 had no effects on rhodamine accumulation in SK-OV-3 cells as verapamil. These results suggest that KR-30032 is equipotent and KR-30035 is more potent than verapamil on the accumulation of rhodamine, and these compounds might increase the accumulation of rhodamine by modulating P-gp. After additional incubation with fresh medium for 2 h, in HCT15 cells, the residual rate of rhodamine was significantly increased by KR-30032, KR-30035 and verapamil; particularly, KR-30035 was significantly more potent than verapamil. In HCT15/CL02 cells, however, the compounds did not influence the residual rate of rhodamine. These differential effects of the compounds on residual rate of rhodamine

between HCT15 and HCT15/CL02 cells indicate that the compounds more potently inhibit the efflux of rhodamine in HCT15 cells than in HCT15/CL02 cells, which could explain the results that the compounds were more active to HCT15 cells than HCT15/CL02 cells on paclitaxel-induced cytotoxicity.

On the other hand, the cardiovascular effects of KR-30032 and KR-30035 were relatively lower than verapamil; the vasorelaxant effects of KR-30032 and KR-30035 on isolated aorta were 25- and 20-fold smaller than verapamil, respectively; and the decreasing effects of LVP in isolated heart were 35- and 12-fold smaller than verapamil, respectively, suggesting that the possibility to induce cardiac toxicity would be much lower than verapamil.

In summary, our results suggest that KR-30032 and KR-30035 enhance the cytotoxicity of cancer drugs via modulation of P-gp. Furthermore, KR-30035 was at least 15-fold more potent than verapamil on the enhancing effects of paclitaxel-induced cytotoxicity as also supported by the data of rhodamine accumulation. Importantly, KR-30035 were 20- and 12-fold less potent than verapamil as a vasorelaxant and in decreasing LVP, suggesting little cardiovascular adverse effects at concentrations that effectively inhibit cell growth. These results suggest that KR-30035 is a potent modulator of MDR with reduced cardiovascular activity and could be potentially useful for the treatment of cancer in combination with anticancer agents.

Acknowledgments

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