Effects of Quinidine and Related Compounds on Cytotoxicity and Cellular Accumulation of Vincristine and Adriamycin in Drug-resistant Tumor Cells

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ABSTRACT

Quinidine, which has antiarrhythmic activity, greatly enhanced the cytotoxicity of vincristine (VCR) in tumor cells and especially in VCR-resistant sublines of P388 leukemia (P388/VCR) and human myelogenous leukemia. A nontoxic concentration of quinidine increased VCR cytotoxicity in these resistant tumor cells about 50 to 80 times, and the drug in combination with VCR could completely reverse VCR resistance of these cell lines. Quinidine also enhanced the cytotoxicity of Adriamycin, especially in the Adriamycin-resistant subline of P388 leukemia; this enhancement (8-fold) was less than that of VCR toxicity in the VCR-resistant tumor line. When administered daily for 10 days with VCR, quinidine at doses of 50 to 125 mg/kg significantly enhanced the chemotherapeutic effect of VCR in P388/VCR-bearing mice. Some other antiarrhythmic agents also showed similar effects in vitro, but these effects were considerably lower than that of quinidine.

Quinidine increased the cellular levels of VCR and daunomycin in VCR-resistant sublines of mouse and human tumors and the ADM-resistant mouse tumor line in vitro, respectively. Quinidine also enhanced the cellular accumulation of VCR in P388/VCR cells in vivo. Thus, the therapeutic effect observed in P388/VCR-bearing mice might be due to the enhanced accumulation of VCR in P388/VCR cells by quinidine. The increase of cellular accumulation of VCR was partly explained by inhibition of efflux of VCR and daunomycin from the resistant tumor cells. The mechanism of this phenomenon is discussed in relation to previous findings on calcium channel blockers.

INTRODUCTION

In experimental systems, it is known that anthracycline-resistant tumor cells are also resistant to other DNA intercalating drugs and to mitotic spindle poisons, including Vinca alkaloids, inhibitors of protein synthesis, and other structurally unrelated compounds (3, 5-7, 13, 16, 21, 33). The molecular mechanism of this pleiotropic phenotype of drug resistance remains far from clear; however, these pleiotropic drug-resistant tumor cells possess an enhanced drug efflux function common to various antitumor agents described above (7, 12, 18, 20, 21, 29). Recent findings on calcium channel blockers.

MATERIALS AND METHODS

Drugs. VCR and ADM for clinical use were obtained from Shionogi and Co., Osaka, Japan, and Kyowa Hakko Kogyo Co., Tokyo, Japan, respectively. [3H]VCR sulfate (5.8 Ci/mmol) and [3H]DAU (4.34 Ci/mmol) were purchased from the Radiochemical Center, Amersham, England. Quinidine sulfate, propranolol hydrochloride, 5,5-diphenylhydantoin sodium salt, lidocaine, and ajmalin were purchased from Nakarai Chemical Co., Kyoto, Japan. Procainamide was a product of Sigma Chemical Co., St. Louis, MO.

Animals and Tumor Cells. Adult female BALB/c × DBA/2Cr F1 (hereafter called CD2F1) mice weighing 20 to 23 g were obtained from Charles River Japan, Inc., Tokyo, Japan. P388, P388/VCR, and P388/ADM cells lines were supplied by the National Cancer Institute, NIH, Bethesda, MD (26). The human myelogenous leukemia K562 cell line (17) was provided by Dr. Ezaki, and the K562/VCR cell line was established in this laboratory (29).

Cell Culture and Drug Treatment. Tumor cells were maintained in suspension in plastic dishes (Corning Glass Works, Corning, NY) in Roswell Park Memorial Institute Medium 1640 supplemented with 10% fetal bovine serum (Flow Laboratories, Stanmore, New South Wales, Australia) and kanamycin (100 µg/ml) (growth medium) (26, 29). Cultures were incubated at 37°C in a humidified atmosphere of 5% CO2 in air. For experiments on the effects of drugs, tumor cells (P388, 2 x 10^6; P388/VCR, 3 x 10^6; P388/ADM, 2 x 10^6; and K562 and K562/VCR, 4 x 10^6) were cultured at 37°C for 5 hr in Falcon No. 2054 culture tubes containing 2 ml of growth medium in a humidified atmosphere of 5% CO2 in air. Then, the cells were treated with graded concentrations of VCR (0.1 to 100 nM) or ADM (0.01 to 10 µM) in the absence or presence of antiarrhythmic drugs (1.0 to 10 µM, depending on the drug), reincubated for 72 hr in the presence of drugs, and counted in a Model ZBI Coulter...
Counter (29). Three tubes were used for each drug concentration. In the control experiment, tumor cells grew exponentially during the incubation period; their final cell numbers are given in each chart and table. Drugs were dissolved in dimethyl sulfoxide at a final concentration of 100 mM and diluted with phosphate-buffered saline (0.2 M sodium phosphate:0.15 M NaCl, pH 7.4). The final concentration of dimethyl sulfoxide in the culture was less than 0.1% (v/v), which had no detectable effect on cell growth.

The IC50 values in the presence or absence of drugs were determined by plotting the logarithm of the drug concentration versus the growth rate (percentage of control) of the treated cells (26, 29).

**Evaluation of Antitumor Activity.** A sample of 0.1 ml of diluted ascites fluid containing 10⁶ P388/VCR cells was transplanted i.p. into CD2F1 mice. VCR was dissolved in 0.9% NaCl solution. Quinidine was suspended in a small volume of 2% (w/v) methyl cellulose (No. 25; Nakarai Chemicals, Kyoto, Japan) and diluted with 0.9% NaCl solution. The final concentration of methyl cellulose was less than 0.1%. This vehicle had no effect on the antitumor activity of VCR. The 2 drugs were mixed, and the mixture was administered at a dose of 0.02 ml/g body weight i.p. daily for 10 days starting from the day after tumor inoculation (26, 29).

The doses of quinidine and VCR were 50 to 125 mg/kg and 100 to 200 μg/kg, respectively. Groups of 10 mice were used in the experiments. As described previously (26, 29).

\[
\text{Antitumor activity (\%)} = \frac{\text{Mean survival time of treated mice}}{\text{Mean survival time of control mice}} \times 100.
\]

**Cellular Uptake of [3H]VCR and [3H]DAU in the Presence of Quinidine.** Cellular uptake of the drugs was measured in growth medium (30). The effects of quinidine were compared at the same nontoxic concentration; tumor cells (2 x 10⁶) Falcon No. 2054 culture tubes containing 1 ml of growth medium with 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer and incubated at 37°C in the absence or presence of quinidine. The effects of quinidine were determined by measuring the amounts of intracellular VCR and DAU at various times as described previously (29, 30).

Cellular uptake of VCR in the presence of quinidine was also determined in animal experiments. In this case, experimental conditions were similar to those of therapeutic experiments. Groups of mice (3 mice/group) were inoculated i.p. with 10⁶ P388 or P388/VCR cells. One week later, when both cell lines (P388 and P388/VCR) were in good condition and had reached approximately 1.2 to 1.6 x 10⁵ cells/mouse, mice were given i.p. injections of [3H]VCR (10 nM; specific activity, 5.8 Ci/mmol) and [3H]DAU (10 nM; specific activity, 4.34 Ci/mmol), respectively, and the amounts of intracellular VCR and DAU were measured. As described previously (29, 30).

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**Efflux of [3H]VCR or [3H]DAU and Effect of Quinidine.** VCR- and ADM-resistant tumor cells carry out efflux of VCR and DAU to the outside of the cell and usually maintain very low intracellular levels of these drugs (7, 12, 18, 20, 21, 29). To obtain sufficiently high intracellular concentrations of drugs, we initially loaded P388/VCR, K562/VCR, and P388/ADM cells with 33 nM [3H]VCR and 33 nM [3H]DAU in glucose-free growth medium supplemented with 10% dialyzed fetal bovine serum (Grand Island Biological Co.) in the presence of 10 μM sodium azide for 30 min as described previously (12, 20, 30). Then, each cell suspension was centrifuged (80 x g, 10 min, 5°C), and the precipitated cells (2 x 10⁵) were resuspended (>98% of the cells excluded trypan blue) in 1 ml of growth medium containing 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer and incubated at 37°C in the absence or presence of quinidine. At the indicated times, the intracellular [3H]VCR and [3H]DAU levels were determined as described previously (29, 30).

**RESULTS**

**Potentiation of VCR and ADM Cytotoxicity by Quinidine.** The various tumor cells used in these experiments demonstrated equivalent cytotoxic responses to quinidine. At a concentration of 10 μM, it inhibited the growth of mouse cell lines only slightly (<5%) and had no effect on growth of human-derived cell lines, while at a concentration of 30 μM, it caused about 10% growth inhibition.

The sensitivities of P388 and P388/VCR cells to VCR and the effect of quinidine on these sensitivities are illustrated in Chart 1. P388/VCR cells were resistant to VCR, and the index of resistance was approximately 16, as determined by comparison of the IC50 values for the 2 cell lines (Chart 1). Quinidine enhanced the cytotoxicity of VCR in P388 cells; at 10 μM, it increased the cytotoxicity 2-fold as compared with the IC50 value. It also greatly increased the cytotoxicity of VCR against P388/VCR cells, the increase being 82-fold at 10 μM quinidine. As quinidine shifted the IC50 value of VCR for P388/VCR cells to smaller values than that (1.78 nm) of P388 cells, it completely overcame the VCR resistance of these cells.

The effects of quinidine on the sensitivities of K562 and K562/VCR cells to VCR and of P388 and P388/ADM cells to ADM were also examined in the same manner, as illustrated in Chart 1. The IC50 values of VCR and ADM in absence and presence of quinidine are summarized in Table 1. Quinidine enhanced VCR cytotoxicity against human K562 cells and especially VCR-resistant lines (Table 1). It caused a 2-fold increase in VCR cytotoxicity in K562 cells as determined from IC50 values. It greatly enhanced the cytotoxicity of VCR against K562/VCR cells, causing 50-fold enhancement at 10 μM. Thus, it could completely overcome the VCR resistance of these cells. Quinidine also enhanced the cytotoxicity of ADM, especially on P388/ADM cells, causing an 8-fold increase in ADM cytotoxicity at 10 μM; however, it had a negligible effect in P388 cells. Quinidine only partially reversed ADM resistance in P388/ADM cells, because the IC50 value did not reach a similar range to that for P388 cells.

Chart 1. Effects of quinidine on the growth-inhibitory actions of VCR on P388 and P388/VCR cells. Tumor cells were treated with graded VCR concentrations with or without quinidine 5 hr after seeding the cells at 2 x 10⁶ (P388) and 3 x 10⁶ (P388/VCR) per ml of medium, and cell numbers were counted after 72 hr of continuous exposure to drugs as described in "Materials and Methods." In the absence of drugs, the cells grew exponentially, and the final cell numbers of P388 and P388/VCR were (2.75 ± 0.03) (S.D.) x 10⁵ and (2.35 ± 0.04) x 10⁵ per 2 ml of medium, respectively. When P388 cells were incubated with VCR at the indicated concentrations in the absence (O) or presence of quinidine at 1 (A), 3 (B), or 10 (C) μM, the IC50 of VCR were 28.8 ± 1.47, 22.2 ± 3.2, 1.49 ± 0.28, and 0.35 ± 0.01 nm, respectively. When P388/VCR cells were treated with VCR at the indicated concentrations in the absence (O) or presence of quinidine at 1 (A), 3 (B), or 10 (C) μM, the IC50 of VCR were 22.8 ± 1.47, 22.2 ± 3.2, 1.49 ± 0.28, and 0.35 ± 0.01 nm, respectively. Points, mean for 3 determinations. All IC50 in the presence of quinidine, except that for 1 μM on P388/VCR, were significantly different from the corresponding IC50 without quinidine (p < 0.05 by Student's t test). Quinidine alone at 1 to 10 μM was nontoxic.

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Table 1
Enhancement of cytotoxicities of VCR and ADM by quinidine in drug-sensitive and -resistant tumor cells

<table>
<thead>
<tr>
<th>Quinidine (μg)</th>
<th>K562/VCR</th>
<th>K562/ADM</th>
<th>P388/VCR</th>
<th>P388/ADM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>10.2 ± 0.5*</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>VCR (200 μg/kg) + Quinidine (125 mg/kg)</td>
<td>12.3 ± 1.2</td>
<td>119</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VCR (200 μg/kg) + Quinidine (100 mg/kg)</td>
<td>14.4 ± 1.6*</td>
<td>140</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VCR (200 μg/kg) + Quinidine (75 mg/kg)</td>
<td>13.9 ± 1.0</td>
<td>135</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VCR (200 μg/kg) + Quinidine (50 mg/kg)</td>
<td>13.6 ± 0.7</td>
<td>132</td>
<td></td>
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</tr>
<tr>
<td>VCR (100 μg/kg) + Quinidine (125 mg/kg)</td>
<td>11.0 ± 1.2</td>
<td>107</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VCR (100 μg/kg) + Quinidine (100 mg/kg)</td>
<td>13.5 ± 1.0*</td>
<td>131</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VCR (100 μg/kg) + Quinidine (75 mg/kg)</td>
<td>13.0 ± 0.8*</td>
<td>126</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VCR (100 μg/kg) + Quinidine (50 mg/kg)</td>
<td>13.1 ± 0.9*</td>
<td>127</td>
<td></td>
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</tr>
</tbody>
</table>

* Mean ± S.D. of the values obtained from 3 mice.

Combined Effect of VCR and Quinidine on P388/VCR-bearing Mice. VCR administered daily for 10 days starting from Day 1 increased the life span of P388 leukemia-bearing mice. The antitumor activity of treated versus control mice was 173 and 223% at VCR dosages of 100 and 200 μg/kg, respectively. VCR given according to the above schedule had little effect on P388/VCR-bearing mice, and at 200 μg/kg, it caused slight increase in the survival time (Table 2). Quinidine alone also did not have a significant therapeutic effect on P388/VCR-bearing mice. However, quinidine given 10 times with VCR significantly increased the life span of P388/VCR-bearing mice (Table 2). The result was not impressive, but an increase in life span of 2 to 3 days was observed in mice treated with quinidine and VCR.

Effects of Quinidine on Cellular Accumulation of [3H]VCR and [3H]DAU in Vitro and in Vivo. Cellular uptake of VCR was examined in the presence of 10 nm [3H]VCR or [3H]DAU. More than 98% of the P388 and P388/VCR cells excluded trypsin blue after treatment with 10 nm antitumor drug and 10 μM quinidine for 2 hr. Uptake of [3H]VCR into cultured P388/VCR cells almost reached a plateau after 30 min under the condition of constant drug exposure (Chart 2). Approximately 0.1 pmol of VCR was found at 1 hr in 10⁶ P388/VCR cells. Quinidine added to the culture at 3 or 10 μM greatly increased the amount of cellular VCR in P388/VCR cells. Treatment with 10 μM quinidine for 1 to 2 hr resulted in about 4- to 5-fold accumulation of VCR in P388/VCR cells (Chart 2A). The effects of quinidine in enhancing the cytotoxicity of VCR in P388/VCR cells and overcoming the resistance in P388/VCR cells to VCR in vivo and in vitro could be explained by this phenomenon.

Similar enhancement of the cellular uptake of VCR by quinidine was also observed in an animal experiment. In this experiment, the doses of quinidine and VCR were 100 and 0.2 mg/kg, respectively, to mimic the therapeutic experiment. After 30 min of [3H]VCR administration, an average of 1.12 pmol of VCR was found in P388/VCR cells, while 3.27 pmol of VCR was found in P388 cells (Table 3). The intracellular VCR level is clearly reduced in resistant cells as has been widely observed in in vitro experiments (6, 21, 29, 33). These values are higher than those observed in in vitro experiments (Chart 2A), because the VCR concentration in the peritoneal cavity in mice (VCR was given at 0.2 mg/kg) might be higher than that of the cellular experiment (10 nm). When quinidine was given to the P388/VCR-bearing mice at 100 mg/kg with VCR at 0.2 mg/kg, 2.94 pmol of [3H]-VCR was found in 10⁶ P388/VCR cells. Thus, approximately 2.6-fold accumulation of [3H]VCR occurred in P388/VCR cells. This enhancement was almost similar to that observed in in vitro experiments (Chart 2). These results indicate that similar reactions could occur in both in vitro and in vivo experiments by quinidine. Potentiation of the VCR effect in vivo by quinidine could also be explained by the quinidine-induced accumulation of VCR in resistant tumor cells.

Quinidine enhanced cellular uptake of [3H]VCR similarly in K562/VCR cells (Chart 2B). The cellular level of VCR in K562/
VCR cells was less than that in P388/VCR cells, and in fact, VCR resistance of K562/VCR cells was stronger than that of P388/VCR cells (Chart 1; Table 1). However, more than 10-fold accumulation of VCR occurred in 1 to 2 hr during treatment of K562/VCR cells with 10 μM quinidine.

Quinidine enhanced the uptake of [3H]DAU by P388/ADM cells (Chart 2C). In this experiment, we used [3H]DAU instead of [3H]VCR, because the latter compound is not available commercially, and P388/ADM cells show almost equal resistance to DAU and ADM. Uptake of [3H]DAU into P388/ADM cells almost reached a plateau after 10 min of incubation, and only a very slight increase occurred thereafter. Quinidine at 3 or 10 μM greatly enhanced the cellular accumulation of DAU in resistant cells. These concentrations of quinidine caused about 5- to 6-fold accumulation of VCR. The effect of 3 μM quinidine was greater in P388/ADM cells than in other resistant tumor cells.

Quinidine slightly enhanced cellular uptake of VCR and DAU in sensitive P388 and K562 cells. One million P388 cells accumulated 0.38 and 10.0 pmol of [3H]VCR and [3H]DAU, respectively, and one million K562 cells accumulated 0.65 pmol of [3H]-VCR at 1 hr after the incubation under the conditions described in Chart 2 (data not shown). These values were considerably higher than those observed in resistant P388 and K562 cells. Quinidine at 10 μM enhanced VCR and DAU accumulation 1.5- and 1.2-fold in P388 cells, respectively (data not shown). The drug also enhanced VCR accumulation 1.3-fold in K562 cells. This enhancement, however, was considerably smaller than that observed for resistant tumor cells (Chart 2).

**Effect of Quinidine on Efflux of VCR and DAU in Resistant Tumor Cells.** [3H]VCR and [3H]DAU accumulated in resistant tumor cells in the presence of sodium azide in glucose-free growth medium (30) and were rapidly reduced on incubation of the cells in fresh medium (Chart 3). On incubation for 1 hr, about 80 to 90% of the intracellular VCR was lost from P388/VCR and K562/VCR cells, and almost all the intracellular DAU was lost from P388/ADM cells. Addition of 3 or 10 μM quinidine to the culture effectively decreased this efflux of VCR or DAU from the cells. After incubation with quinidine for 1 hr, approximately 50 to 70% of the initial amount of VCR still remained in the cells. Moreover, 50 to 65% of the initial intracellular DAU still remained in P388/ADM cells after reincubation with quinidine for 10 min, while in the absence of quinidine, more than 90% of the drug was transported from the cells during this incubation period.

**Effects of Quinidine-related Compounds on VCR Cytotoxicity.** In studies on the mechanism of the effect of quinidine, we examined the effects of other drugs with an antiarrhythmic action. At noncytotoxic and maximum effective concentrations, antiarrhythmic drugs in general enhanced the cytotoxicities of VCR and ADM, especially in VCR- and ADM-resistant tumor cells, but their effects were significantly lower than those of quinidine (Table 4). Of the compounds tested, lidocaine, propanolol, and ajmalin possess a rather strong effect, especially in P388/VCR cells, causing a 4- to 17-fold enhancement. Similar enhancement was also observed in K562/VCR cells (data not shown). In P388/ADM cells, propanolol and ajmalin enhanced the cytotoxicity of ADM severalfold.

**DISCUSSION.**

We have reported that calcium channel blockers and calmodulin inhibitors efficiently inhibited drug efflux from resistant tumor cells (24, 25, 29, 30), thereby endowing these cells with the same sensitivity to antitumor agents as sensitive cells (24-27, 28). Moreover, 50 to 65% of the initial intracellular DAU still remained in P388/ADM cells after reincubation with quinidine for 10 min, while in the absence of quinidine, more than 90% of the drug was transported from the cells during this incubation period.

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29–32). These results suggest that cellular calcium and calmodulin may be related to the drug efflux mechanism in the plasma membrane of the resistant tumor cells. In this paper, we found that quinidine, an antiarrhythmic drug, possesses the ability to enhance the cytotoxicity of VCR and ADM in resistant tumor cells, although its effects in vitro are considerably lower than those of calcium channel blockers and calmodulin inhibitors (26, 29, 31). Potentiation of VCR and ADM effects by quinidine in vivo was also lower than that observed by calcium channel blockers (26, 29); however, the effect of quinidine in vivo was rather stronger than that observed for calmodulin inhibitors (32), because calmodulin inhibitors usually were toxic in animal experiments.

Calcium channel blockers inhibit the slow calcium channel in the plasma membrane of smooth muscle. Quinidine has no calcium channel blocking action. Quinidine and other antiarrhythmics perturb the organization of membrane lipids and affect nonspecifically various membrane-bound enzymes which are believed to be influenced by the physical state of surrounding lipids (22). Quinidine depresses the lip-phase transition temperature and broadens the transition (22). This interaction of quinidine with phospholipid is suggested to contribute the mechanisms by which quinidine exerts its physiological and pharmacological effects reported previously (1, 8, 10, 11, 15, 22). Calcium channel blockers are also known to interact with the plasma membrane (2, 23, 34). As a mechanism of overcoming drug resistance by these agents, we must consider the possibility that the interaction of calcium channel blockers and quinidine with the membrane of tumor cells might lead to an inhibition of drug efflux from resistant tumor cells.

Another feature of quinidine is that the drug depresses myocardial contractility (9, 10). The real mechanism of this action is not known; however, the drug has been reported to decrease micromodal and mitochondrial calcium uptake in cardiac muscle (10). A decrease in calcium uptake in heart subcellular particles can be conceived to reduce the intracellular calcium stores. Thus, both calcium channel blocker and quinidine can induce similar results, i.e., a decrease in the intracellular level of free calcium, although the processes are different from each other (10).

The present results are interesting in cancer chemotherapy, and the mechanisms that underlie these phenomena are worth being elucidated. Interaction of calcium channel blockers and quinidine with membrane and the modulation of the calcium environment which was induced in the membrane might be important in the inhibition of drug efflux of resistant tumor cells.

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