Regression of Established Tumors Expressing P-glycoprotein by Combinations of Adriamycin, Cyclosporin Derivatives, and MRK-16 Antibodies

Toru Watanabe, Mikihiro Naito, Noriko Kokubu, Takashi Tsuruo*

Background: Overexpression of P-glycoprotein, a transmembrane protein capable of transporting a broad spectrum of anticancer drugs out of cells, likely contributes to tumor drug resistance. Strategies for overcoming this resistance include the use of specific compounds, such as cyclosporin derivatives, that modulate P-glycoprotein function and antibodies that bind to the protein, thereby altering its activity.

Purpose: We examined the antitumor activity of combination treatment with the anti-P-glycoprotein monoclonal antibody MRK-16, a cyclosporin derivative (either cyclosporin A [CsA] or PSC 833), and the anticancer drug Adriamycin (ADM) against human colorectal carcinoma cells in vitro and established xenografts of these cells in vivo.

Methods: The human colorectal carcinoma cell line HCT-15 and its ADM-resistant subline HCT-15/ADM2-2 were used in this study. Cellular staining with a tetrazolium dye was used to assess the antitumor (i.e., antiproliferative) effects of treatment in vitro. Caliper measurement of tumor volumes was used to assess the antitumor effects of treatment in vivo. Cell surface binding of MRK-16 was measured by means of an immunofluorescence assay. Differences in the patterns of tumor cell growth in vitro and tumor growth rates in vivo were evaluated by means of repeated measure analysis of variance. Synergy in the combined effects of treatment was evaluated by means of the fractional product method.

Results: HCT-15 cells were found to express P-glycoprotein intrinsically; HCT-15/ADM2-2 cells expressed approximately five times more P-glycoprotein than the parental cells. HCT-15/ADM2-2 were also found to be about eight times more resistant to ADM in vitro than the parental cells. Incubation of both cell types in vitro with either MRK-16 and ADM or one of the cyclosporin derivatives and ADM inhibited cell growth minimally; however, ternary treatment with MRK-16, one of the cyclosporin derivatives, and ADM dramatically reduced the growth of both cell types. An analysis of treatment effects indicated that synergistic effects were obtained with ternary treatment. When athymic mice bearing established tumors (either HCT-15 or HCT-15/ADM2-2) were treated similarly with various combinations of the tested agents, the most pronounced antitumor effects were observed with ternary treatment. In some mice bearing HCT-15/ADM2-2 xenografts, ternary treatment led to complete tumor regression. Finally, CsA and PSC 833 were both shown to enhance MRK-16 binding to HCT-15 cells and HCT-15/ADM2-2 cells in vitro. Conclusion: Combination treatment with a cyclosporin derivative and an anti-P-glycoprotein antibody can be effective in circumventing P-glycoprotein-mediated drug resistance.

[A J Natl Cancer Inst 1997;89:512-8]

Notes

Supported by Public Health Service grants CA17613 and CA46589 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

We thank Donna Virgil for preparing and Ilse Hoffmann for editing the manuscript and Rachid Hamid and Jeff Rigoty of the Research Animal Facility and Beverly Gambrell of the Histopathology Facility for providing expert technical assistance.

Manuscript received September 10, 1996; revised December 17, 1996; accepted January 24, 1997.

*Affiliations of authors: T. Watanabe, Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, Tokyo, Japan, and Sandoz Tsukuba Research Institute, Sandoz Pharmaceuticals, Ltd., Ibaraki, Japan; T. Tsuruo, Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, and Institute of Molecular and Cellular Biosciences, The University of Tokyo; M. Naito, Institute of Molecular and Cellular Biosciences; N. Kokubu, Sandoz Tsukuba Research Institute.

Correspondence to: Takashi Tsuruo, Ph.D., Institute of Molecular and Cellular Biosciences, The University of Tokyo, Yayoi, Bunkyo-ku, Tokyo 113, Japan.

See “Notes” following “References.”
resistant to those drugs. One strategy for overcoming multidrug resistance is to modulate P-glycoprotein function by means of inhibitors (4). To achieve this, a number of compounds that are capable of modulating P-glycoprotein activity have been identified (4). These compounds include calcium channel blockers, calmodulin inhibitors, cyclosporin A (CsA), FK506, and [3'-keto-Bmt]^[Val2]-cyclosporin (PSC 833) (4-9), which are all believed to be competitive and/or non-competitive inhibitors of the P-glycoprotein-mediated transport of antitumor drugs.

Although considerable effort has been devoted to finding successful combinations of these modulators and multidrug-resistance-related anticancer drugs, the inherent and potential toxic effects of the modulators limit their chemotherapeutic advantages. When two modulators, such as CsA and verapamil, are combined in vitro, a lower concentration of each modulator is required to reverse multidrug resistance than the concentrations needed when the agents are used singly (10). Modulator combinations might be useful, therefore, to avoid the dose-limiting toxicity of the individual modulators. Another strategy that has been investigated is the specific targeting of P-glycoprotein-positive tumor tissue by use of anti-P-glycoprotein monoclonal antibodies (11,12). Antibodies administered alone or in combination with cytotoxic agents have been studied (13-18). We previously reported that two antibodies, MRK-16 and MRK-17, which recognize the external domain of human P-glycoprotein (19), induced tumor regression in multidrug-resistant xenografts grown in athymic mice (13). This tumor regression was induced by complement-dependent cytotoxicity and antibody-dependent, cell-mediated cytotoxicity. In addition, treatment with MRK-16 enhanced the antitumor activity of Adriamycin (ADM), without the potentiation of ADM toxicity, in an athymic mouse model of human colorectal carcinoma (14).

MRK-16 has been shown to enhance synergistically the inhibitory activities of CsA and PSC 833 in vitro in ADM-resistant sublines of leukemia and drug-resistant carcinoma cell lines, but its effect on the activities of FK506 and verapamil was found to be additive (20-22). Although the mechanism(s) of the synergistic interaction between MRK-16 and the cyclosporin derivatives has not been fully elucidated, the enhanced intracellular accumulation of CsA observed with MRK-16 treatment is likely to be relevant.

The findings described above led us to examine the antitumor activity of combination therapy with MRK-16, cyclosporin derivatives, and ADM in athymic mice bearing established human colorectal carcinomas.

Materials and Methods

Materials

CsA and PSC 833 were obtained from Sandoz Pharmaceuticals, Ltd. (Basel, Switzerland). ADM (as doxorubicin) and vincristine (VCR) were purchased from Kyowa Hakko Kogyo Co., Ltd. (Tokyo, Japan) and Shinogi Co., Ltd. (Osaka, Japan), respectively. Paclitaxel (Taxol) was purchased from Sigma Chemical Co. (St. Louis, MO). Galenical forms of etoposide (as Lastet) and mitoxantrone (as Novantron) were purchased from Nihon Kayaku Co., Ltd. (Tokyo) and Lederle Japan, Ltd. (Tokyo), respectively. The murine monoclonal antibody MRK-16 was prepared as described previously (19). Drink solutions of cyclosporin derivatives were also prepared as described previously (23,24). Solutions of ADM and MRK-16 were prepared in physiologic saline.

Animals and Tumors

Female 6-week-old BALB/c nu/nu mice weighing 19-23 g each were purchased from Clea Japan, Inc. (Tokyo, Japan). They were maintained under specific pathogen-free conditions at 25 °C in an atmosphere of 95% air, 5% CO2, 26-28 °C temperature, and 50% humidity. Lighting was operated automatically on a 12-hour light/dark cycle. All animal studies were done under the guidelines of the Sandoz Tsukuba Research Institute, Sandoz Pharmaceuticals, Ltd., Tsukubashi, Ibaraki, Japan. Human colorectal carcinoma HCT-15 (CCl 225) cells were obtained from the American Type Culture Collection (Rockville, MD) through Dainippon Pharmaceutical Co. (Osaka, Japan). The ADM-resistant subline of HCT-15, HCT-15/ADM2-2, was established by continuous exposure of the parental cells to ADM at a concentration of 0.2 μg/mL. HCT-15 cells were maintained in RPMI-1640 medium (Nissui, Tokyo, Japan) supplemented with 10% heat-inactivated fetal bovine serum, 2 mM glutamine, and 100 μg/mL kanamycin in an atmosphere containing 5% CO2 at 37 °C. HCT-15/ADM2-2 cells were maintained in the same growth medium supplemented additionally with 0.2 μg/mL ADM. The human myelogenous leukemia cell line K562 was provided by Kohji Ezaki (Cancer Chemotherapy Center, Tokyo, Japan), and its subline resistant to ADM (K562/ADM) was established as described previously (25).

Immunoblotting to Detect P-glycoprotein

P-glycoprotein in the plasma membrane fraction of cellular extracts was detected by means of immunoblotting, with the monoclonal antibody JSB-1 as the probe, according to a method described previously (26).

In Vitro Growth Inhibition Assay

The growth inhibition assay used in this study has been described previously (27). Here, the method was used with minor modifications. In brief, HCT-15 and HCT-15/ADM2-2 cells were seeded in 96-well tissue culture plates at a density of 5 × 104 cells/well. After incubating the cells for 24 hours, drug treatments were initiated. The cells were exposed to the drugs for 72 hours, and then relative cell growth was assessed by means of staining with 2,3-bis(2-methoxy-4-nitro-5-sulphonyl)-5-[(phenylamino)carbonyl]-2H-tetrazolium hydroxide (Sigma Chemical Co.). After a 4-hour incubation with the tetrazolium dye, the absorbance at 540 nm was measured by use of a microplate reader (Molecular Devices Corp., Menlo Park, CA).

In Vivo Solid Tumor Studies

Antitumor activity in a xenograft model was evaluated as described previously (24). A suspension of cells (107 cells in 0.1 mL Hanks' balanced salt solution) was injected into the right subaxillary region of individual athymic mice (day 0). The control and treatment groups contained 10 and five mice, respectively. Drug treatment was initiated after tumors were established (100-500 mm3, as estimated by caliper measurement), and it consisted of administration of a cyclosporin derivative, MRK-16, and ADM. The dose and route were as follows: 8 mg/kg body weight intravenously for ADM; 30 μg/mouse intravenously for MRK-16; 25 mg/kg body weight perorally for PSC 833; and 50 mg/kg body weight perorally for CsA. The cyclosporin derivatives and MRK-16 were given perorally 4 hours and intravenously 2 hours, respectively, before treatment with ADM. Some groups of mice with HCT-15/ADM2-2 tumors were treated with multiple doses of MRK-16 (50 μg/mouse intravenously on days 7, 10, 14, and 17). Tumor volume (V) was calculated by use of the following equation:

\[ V = a \times b^2 \times \frac{1}{2} \times \frac{V_f}{V_0}, \]

where a and b are the longest and shortest diameters of the tumor mass (in millimeters), respectively. Calculated tumor volumes were expressed in percentages as relative tumor volumes (RVs) by use of the following equation:

\[ RV = \frac{V_f}{V_0} \times 100, \]

where Vf is the tumor volume at day f and V0 is the initial volume immediately before the first drug treatment. The criterion for antitumor activity was tumor growth inhibition. The T/C (treated/control) value was calculated as a percentage by use of the following formula:

\[ T/C = \frac{mean \, RV \, of \, the \, treated \, animals}{mean \, RV \, of \, the \, control \, animals} \times 100. \]

Antibody Binding Assay

Cell surface binding of MRK-16 was determined as described previously (28), with minor modifications. HCT-15 cells were seeded in triplicate wells of 96-well tissue culture plates (5 × 104 cells/well) and cultured overnight. The culture medium was replaced with 2% inactivated fetal bovine serum, 2 mM glutamine, and cultured overnight at 37 °C in a humidified atmosphere of 5% CO2 at 37 °C. After treatment with MRK-16, the cultures were washed and incubated with biotinylated JSB-1 antibody for 1 hour. The cells were washed with 0.1% bovine serum albumin in phosphate buffered saline, and then non-specific binding was blocked with blocking buffer (1% serum/1% bovine serum albumin/0.1% NaN3). The cell surface binding of MRK-16 was determined by using streptavidin-conjugated horseradish peroxidase (Medical & Biological Laboratories Co., Nagoya, Japan). The bound antibody was detected using the horseradish peroxidase method.
moved and replaced by phosphate-buffered saline supplemented with 10% bovine serum albumin and 10 mM NaN₃ (binding buffer) with and without 6 μg/mL CsA or PSC 833. The adherent cells were incubated for 2 hours at 37 °C. Either MRK-16 or a nonspecific mouse immunoglobulin G (IgG)-2a was added to a final concentration of 2.5 μg/mL, followed by further incubation for 1 hour at 37 °C. The wells of the plates were gently washed twice with ice-cold binding buffer. The cells were then incubated with diluted fluorescein isothiocyanate-labeled anti-mouse IgG for 1 hour at 4 °C. After four washes with ice-cold phosphate-buffered saline, the cells were subsequently harvested after trypsinization and suspended in 5% sodium dodecyl sulfate (in Hanks’ balanced salt solution). Fluorescence intensity (emission wavelength = 530 nm; excitation wavelength = 485 nm) was determined by means of a fluorescence plate reader (CytoFluor 2350, Millipore Corp., Bedford, MA). The specific binding of MRK-16 was estimated by subtracting the fluorescence intensity of samples incubated with the nonspecific IgG from the fluorescence of samples incubated with MRK-16.

Statistical Analysis

Statistical analysis involving the comparison of entire inhibition curves from the in vitro growth inhibition studies and from the in vivo solid-tumor studies was performed by use of repeated measure analysis of variance (ANOVA) [Bonferroni/Dunn (29)] with StatView software (version 4.02 for Macintosh, Abacus Concepts, Inc., Berkeley, CA). Presented P values are two-sided. Synergy of MRK-16 and the cyclosporin derivatives was analyzed by means of the fractional product method (30,31). For the in vitro growth inhibition study, if the observed inhibition is less than the calculated inhibition, a synergistic effect is suggested; if the observed inhibition is similar to the calculated inhibition, an additive effect is suggested. For the solid tumor study, if the observed T/C value is less than the calculated T/C value, synergy is suggested; if the observed and calculated T/C values are the same, an additive effect is suggested. In the solid-tumor study, significant differences between treated groups of mice were determined by use of the Mann–Whitney U test. In analyzing results from the antibody-binding assay, P values were determined by means of the Student’s t test for the comparison of fluorescence values obtained with and without cyclosporin derivatives.

Results

P-glycoprotein Expression and Drug Sensitivity Exhibited by HCT-15 and HCT-15/ADM2-2 Cells

Immunoblot analysis, using the anti-P-glycoprotein monoclonal antibody JSB-1 as the probe, demonstrated that the human colorectal tumor cell line HCT-15 intrinsically expressed P-glycoprotein (Fig. 1, A). Densitometry revealed that the ADM-resistant subline HCT-15/ADM2-2 expressed five times more P-glycoprotein than HCT-15. In an in vitro growth inhibition assay, the drug concentrations that produced 50% growth inhibition of HCT-15 and HCT-15/ADM2-2 cells, respectively, were 252 and 1960 ng/mL for ADM, 192 and 1040 ng/mL for VCR, 326 and 1570 ng/mL for paclitaxel, 1740 and 5480 ng/mL for etoposide, and 87 and 272 ng/mL for mitoxantrone. Thus, the HCT-15/ADM2-2 cell line was eight times more resistant to ADM than the HCT-15 cell line in vitro, and the resistant cells showed cross-resistance to VCR, paclitaxel, etoposide, and mitoxantrone.

Effect of MRK-16 on Sensitization by Cyclosporin Derivatives to ADM and VCR In Vitro

We investigated the effect of the MRK-16 monoclonal antibody on the antiproliferative activities of combinations of ADM or VCR and cyclosporin derivatives against HCT-15 cells and HCT-15/ADM2-2 cells in vitro (Fig. 1, B). Tumor cell growth rates were determined after a 72-hour incubation with the drugs by means of an assay that employed staining with 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl]-2H-tetrazolium hydroxide. When ADM or VCR was combined with either PSC 833 at 0.05 μg/mL or CsA at 0.3 μg/mL, cell growth was inhibited by up to 50%. Although MRK-16, by itself and with the anticancer agents, minimally reduced the growth rate, MRK-16 in combination with a cyclosporin derivative significantly enhanced the growth-inhibitory activities of ADM and VCR. The combined effects were synergistic, as determined by an analysis that utilized the fractional product method.

Antitumor Activity in a Xenograft Model of Combination Treatment with ADM, Cyclosporin Derivatives, and MRK-16

The effects of the ternary combination therapy were explored in vivo by use of established xenografts in athymic mice (Fig. 2; Table 1). A pilot study indicated that treatment with either PSC 833 at 25 mg/kg body weight or CsA at 50 mg/kg body weight reduced the maximum tolerated single intravenous dose of ADM from 12 to 8 mg/kg body weight. Therefore, in a series of treatments, we fixed the dose of ADM at 8 mg/kg.

The HCT-15 xenografts achieved a volume of more than 100 mm³ on day 11. At that time, the mice were treated with ADM (intravenously) and/or MRK-16 (30 μg/mouse, intravenously) or with ADM and a cyclosporin derivative (PSC 833 at 25 mg/kg body weight, perorally, or CsA at 50 mg/kg body weight, perorally) with or without MRK-16 (Fig. 2, upper panels). Single agent treatment with ADM slightly inhibited the growth of the HCT-15 xenografts by approximately 20% as measured 14 days after the treatment (Table 1). While either MRK-16 or the cyclosporin derivatives slightly enhanced the antitumor activity of ADM, the triple combinations containing a cyclosporin derivative, MRK-16, and ADM resulted in more pronounced growth inhibition of the xenografts (T/C ≤ 40%).

Additional experiments were designed to examine the effects of the ternary treatments on tumors formed by HCT-15/ADM2-2 cells, which express higher amounts of P-glycoprotein than HCT-15 cells (Fig. 2, lower panels). The average volume of HCT-15/ADM2-2 tumors became greater than 100 mm³ on day 7 after tumor cell injection. At this time, ADM and the cyclosporin derivatives were given. Two different amounts of MRK-16 were used in these experiments. Some mice received a single dose of 30 μg/mouse intravenously on day 7 (30 μg × 1); others received 50 μg MRK-16 per day intravenously on days 7, 10, 14, and 17 (50 μg × 4). The ternary combinations significantly inhibited tumor growth, while MRK-16 combined with ADM in the absence of the cyclosporin derivatives resulted in moderate inhibition. Interestingly, the HCT-15/ADM2-2 xenografts were more sensitive to MRK-16 treatment than the HCT-15 xenografts (Table 1). The combinations with MRK-16 at 30 μg × 1, ADM, and a cyclosporin derivative resulted in complete regression of established HCT-15/ADM2-2 xenografts in 20% (one of five) of the treated mice (63-day survival and tumor free). Multiple doses of MRK-16 increased the xenograft regression rate. MRK-16 at 50 μg × 4 in combination with CsA and ADM resulted in a cure rate of 60% (three of five mice); MRK-16 at 50 μg × 4 in combination with PSC 833 and ADM resulted in a cure rate of 40% (two of five mice). Thus, multiple and single doses of MRK-16 enhanced the antitumor activity of com-
combined treatment with cyclosporin and ADM.

Effect of CsA and PSC 833 on Binding of MRK-16 to the Cell Surface

To examine a possibility that CsA and PSC 833 affect the association of MRK-16 with P-glycoprotein, we measured the binding of MRK-16 to HCT-15 cells and HCT-15/ADM2-2 cells in the presence of cyclosporin derivatives (Fig. 3). Both PSC 833 and CsA at 6 μg/mL increased the binding of MRK-16 to P-glycoprotein on the cell surface. The activity of the cyclosporin derivatives in enhancing MRK-16 binding to P-glycoprotein was more prominent with HCT-15/ADM2-2 cells than with parental HCT-15 cells.

Discussion

A number of studies (1-3) have demonstrated thus far that P-glycoprotein expression is an important indicator of therapeutic outcome. Therefore, efforts to overcome P-glycoprotein-mediated drug resistance have been directed at inhibiting P-glycoprotein function. These efforts have included the use of chemosensitizing agent combinations and inhibitory antibodies that recognize the external domain of P-glycoprotein. In this study, we used PSC 833, CsA, and MRK-16 as modulators of P-glycoprotein function. PSC 833 in combination with multidrug-resistance-related anticancer drugs has resulted in the elimination of murine tumors in mice (24). In addition, treatment with PSC 833 and ADM has resulted in the inhibition of growth of established HCT-15 xenografts; however, this treatment was not curative. MRK-16 in combination with ADM was also unable to induce complete HCT-15 tumor regression (14). These findings suggest that both PSC 833 and MRK-16 are potent agents, but they are still insufficient to induce the complete regression of established tumors. In this study, we combined MRK-16 with the cyclosporin derivatives PSC 833 and CsA in an attempt to establish a more effective

Fig. 1. P-glycoprotein expression by cells of the human colorectal carcinoma cell line HCT-15 and its Adriamycin (ADM)-resistant subline HCT-15/ADM2-2 and sensitivity of the cells to combination treatment with the anti-P-glycoprotein monoclonal antibody MRK-16, cyclosporin derivatives, and anticancer drugs in vitro. A) Samples of membrane fractions from HCT-15 cells and HCT-15/ADM2-2 cells were subjected to immunoblot analysis with the anti-P-glycoprotein monoclonal antibody JSB-1. Membrane fractions from ADM-resistant sublines of human myelogenous leukemia K562 cells and parental K562 cells were used as positive and negative controls, respectively. Lane 1) ADM-resistant K562 cells, 5-μg sample; lane 2) parental K562 cells, 20-μg sample; lane 3) HCT-15 cells, 20-μg sample; and lane 4) HCT-15/ADM2-2 cells, 20-μg sample. The arrow indicates the position of P-glycoprotein. See “Materials and Methods” section for details. B) HCT-15 cells (upper panels) and HCT-15/ADM2-2 cells (lower panels) were incubated for 72 hours in the presence of MRK-16 with or without the addition of an anticancer drug (ADM or vincristine [VCR]) and a cyclosporin derivative (cyclosporin A [CsA] or PSC 833). Cell growth was measured by means of a tetrazolium dye assay, as described in the “Materials and Methods” section. Growth rates are expressed as percentages of the control rate (i.e., the growth rate without drugs and MRK-16) and are plotted in relation to the MRK-16 concentration. The individual culture conditions were as follows: no drug (○); the anticancer drug without the cyclosporin derivative (■); the anticancer drug with 0.05 μg/mL PSC 833 (▲); and the anticancer drug with 0.3 μg/mL CsA (▲). The cell subline and anticancer drug concentration are indicated in each panel. Symbols and bars denote the means and standard deviations from triplicate determinations. The P values (two-sided) shown in the panels were determined by use of repeated measure analysis of variance (Bonferroni/Dunn) for the comparison of entire curves. * = a synergistic effect between MRK-16 and the cyclosporin derivatives as determined by the fractional product method. See the “Materials and Methods” section for details.
method for circumventing P-glycoprotein-mediated drug resistance.

Our results indicate that combination therapy with MRK-16 and cyclosporin derivatives is quite effective in blocking multidrug resistance in a xenograft model. In addition, it is noteworthy that the complete regression of some established tumors was evident in mice receiving MRK-16, a cyclosporin derivative, and ADM (Fig. 2). Interestingly, a single treatment with MRK-16 in combination with ADM and cyclosporin treatment induced the disappearance of some HCT-15/ADM2-2 xenografts but not of HCT-15 xenografts (Fig. 2). The densitometric analysis of immunoblots revealed a P-glycoprotein expression level in HCT-15/ADM2-2 cells that was approximately five times higher than that in HCT-15 cells. These findings suggest that the combination treatment is more effective against tumors with higher expression of P-glycoprotein. In addition, the activity of MRK-16 can be associated with its specific binding to P-glycoprotein, since an irrelevant antibody that does not bind to the surface of the tumor cells did not affect treatment outcome, as previously reported (14). It is possible that an antibody directed against a different cell surface antigen might be able to induce antigen-dependent immune responses similar to those observed with MRK-16 and MRK-17 (13); however, it is likely that the antitumor activity of anti-P-glycoprotein antibodies in combination therapies involves more than just these responses.

The drug-resistance reversal activities of CsA and PSC 833 in vitro were synergistically enhanced by MRK-16 treatment (20-22). The synergistic interaction between MRK-16 and CsA can be attributed to the enhanced intracellular accumulation of CsA in the presence of the anti-

![Fig. 2](image-url)
Table 1. Antitumor activity of combination treatments with cyclosporin derivatives (cyclosporin A [CsA] or PSC 833), the anti-P-glycoprotein monoclonal antibody MRK-16, and Adriamycin (ADM) against established HCT-15 and HCT-15/ADM2-2 tumors in athymic mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean relative tumor volume (± SE in mm³)</th>
<th>Maximum body weight loss in percent and day measured (following first treatment)</th>
<th>No. of animals cured/total No. of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>990 ± 95</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>MRK-16</td>
<td>30 µg x 4§</td>
<td>693 ± 136</td>
<td>100</td>
</tr>
<tr>
<td>ADM</td>
<td>8 mg/kg</td>
<td>833 ± 153</td>
<td>84</td>
</tr>
<tr>
<td>+MRK-16</td>
<td>30 µg x 1§</td>
<td>587 ± 85</td>
<td>59</td>
</tr>
<tr>
<td>+PSC 833</td>
<td>25 mg/kg§</td>
<td>545 ± 125</td>
<td>61.1, day 20</td>
</tr>
<tr>
<td>+MRK-16</td>
<td>30 µg x 4§</td>
<td>270 ± 263.3</td>
<td>92.7, day 17</td>
</tr>
<tr>
<td>+CsA</td>
<td>50 mg/kg§</td>
<td>624 ± 109</td>
<td>8.2, day 26</td>
</tr>
<tr>
<td>+MRK-16</td>
<td>30 µg x 1§</td>
<td>294 ± 95.4</td>
<td>3.2, day 20</td>
</tr>
</tbody>
</table>

**HCT-15 xenografts**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean relative tumor volume (± SE in mm³)</th>
<th>Maximum body weight loss in percent and day measured (following first treatment)</th>
<th>No. of animals cured/total No. of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1176 ± 268</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>MRK-16</td>
<td>30 µg x 1§</td>
<td>568 ± 161</td>
<td>48</td>
</tr>
<tr>
<td>MRK-16</td>
<td>50 µg x 4§</td>
<td>790 ± 261</td>
<td>67</td>
</tr>
<tr>
<td>ADM</td>
<td>8 mg/kg</td>
<td>941 ± 364</td>
<td>80</td>
</tr>
<tr>
<td>+MRK-16</td>
<td>30 µg x 1§</td>
<td>535 ± 112</td>
<td>46</td>
</tr>
<tr>
<td>+MRK-16</td>
<td>50 µg x 4§</td>
<td>639 ± 136</td>
<td>54</td>
</tr>
<tr>
<td>+PSC 833</td>
<td>25 mg/kg§</td>
<td>666 ± 155</td>
<td>15, day 10</td>
</tr>
<tr>
<td>+MRK-16</td>
<td>30 µg x 1§</td>
<td>141 ± 73.4</td>
<td>13, day 10, 15, day 10</td>
</tr>
<tr>
<td>+MRK-16</td>
<td>50 µg x 1§</td>
<td>23 ± 14.5</td>
<td>2/5</td>
</tr>
<tr>
<td>+CsA</td>
<td>50 mg/kg§</td>
<td>602 ± 102</td>
<td>2.4, day 10</td>
</tr>
<tr>
<td>+MRK-16</td>
<td>30 µg x 1§</td>
<td>162 ± 103.4</td>
<td>8.5, day 10, 15, day 10</td>
</tr>
<tr>
<td>+MRK-16</td>
<td>50 µg x 4§</td>
<td>108 ± 67.3</td>
<td>3/5</td>
</tr>
</tbody>
</table>

**HCT-15/ADM2-2 xenografts**

Body (20). However, a previous study (22) has demonstrated that the intracellular accumulation of PSC 833 is not increased by treatment with MRK-16. Furthermore, it has been shown that PSC 833, unlike CsA, is not transported by P-glycoprotein (32). Therefore, even if MRK-16 inhibits P-glycoprotein function, the accumulation of PSC 833 in P-glycoprotein-positive cells might change minimally.

The question arises as to how MRK-16 synergistically enhances the reversal activity of PSC 833. We hypothesize that PSC 833 modulates molecular interactions between the antibody and P-glycoprotein. We have demonstrated that PSC 833 increases MRK-16 binding to P-glycoprotein (Fig. 3). This effect could contribute to the synergistic enhancement of the multidrug-resistance-reversal activity caused by the MRK-16 plus PSC 833 combination. We have detected a similar effect on MRK-16 binding mediated by CsA.

In conclusion, MRK-16 enhances the antitumor activity of combination treatment with cyclosporin and ADM in an established P-glycoprotein-positive xenograft model. The ternary treatment combination is more effective against tumors with higher expression of P-glycoprotein. In clinical trials of MRK-16 for the circumvention of drug-resistance, combining the antibody with cyclosporin derivatives and anticancer drugs could present distinct advantages in the reversal of that resistance.

References


Notes

Supported by a special grant for Advanced Research on Cancer from the Ministry of Education, Science and Culture, Japan.

Manuscript received September 9, 1996; revised December 26, 1996; accepted January 1, 1997.