Reversal of Vinca Alkaloid Resistance by Anti-P-Glycoprotein Monoclonal Antibody HYB-241 in a Human Tumor Xenograft

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ABSTRACT

A panel of monoclonal antibodies (MAbs) to P-glycoprotein was developed by immunization of mice with multidrug-resistant human neuroepithelioma and neuroblastoma cells. All the anti-P-glycoprotein MAbs reacted with the extracellular portion of P-glycoprotein. The MAbs were examined for their ability to enhance accumulation of actinomycin D, vincristine, vinblastine, and doxorubicin in the human mdr1 transfectant cell line, BRO/pFRmdrl.6. HYB-241, an IgG anti-P-glycoprotein MAb, was the most effective modulator, increasing actinomycin D levels in the transfectant line by 6-fold, vincristine by 2-fold, and vinblastine levels by 3-fold. None of the MAbs were capable of modifying the accumulation of doxorubicin. HYB-241 lowered the 50% inhibitory concentration values of actinomycin D by 11-fold, vincristine by 6-fold, and vinblastine by 2-fold. No effect on the 50% inhibitory concentration values of doxorubicin or gramicidin were seen. 111In-labeled HYB-241 localized in human tumor xenografts of BRO/pFRmdrl.6 in nude mice (25% injected dose/g at 120 h). Mice with established drug-resistant xenografts were treated with antibody 24 h prior to the injection of Vinca alkaloid at concentrations known to be non-growth inhibitory. The addition of HYB-241 at 25 mg/kg per injection prior to drug resulted in a significant inhibition of growth of this drug-resistant tumor.

INTRODUCTION

P-glycoprotein is a transmembrane glycoprotein responsible for the ATP-dependent efflux of a broad spectrum of structurally and functionally distinct drugs from multidrug-resistant cells. A variety of small molecules capable of modulating P-glycoprotein have been described in the literature. These include calcium channel blockers (1), calmodulin inhibitors (2), antiarrhythmics (3, 4), antimalarials (5), and other lysoosmotropic agents (6), steroids (7), antitoxins (8), and cyclic peptide antibiotics (9). All of these compounds are effective modulators in vitro. They lower the IC50 values of a variety of drugs included in the MDR family, and they increase intracellular drug concentrations in resistant cells. The mechanism responsible for this reversal of resistance is believed to be competition between the modulator and drug for binding to the ATP-dependent efflux pump, P-glycoprotein (10, 11). The clinical utility of any modulator, however, depends not only on its ability to reverse drug resistance at low concentrations but also on whether it has a low toxicity in vivo. The cardiac toxicity seen during the clinical evaluation of verapamil as a chemosensitizing agent pointed out the need for less toxic modulators (12, 13). Verapamil has been shown to be cytotoxic to cells independent of its ability to increase cancer drug accumulation in cells (14). Recent reports (15, 16) have indicated additional sites of action, since verapamil can modulate resistance in cells lacking P-glycoprotein. Two other antiarrhythmic drugs, quinidine and amiodarone, have also entered clinical trials as chemosensitizing agents (17). Both drugs have produced a number of adverse clinical side effects (18).

Our approach has been to identify MAbs which interact specifically with P-glycoprotein, in an attempt to produce nontoxic P-glycoprotein modulators. The long serum half-life and extended residence time of MAbs on tumors may offer an advantage over small-molecule modulators. In recent clinical trials (13, 19) continuous infusion of verapamil was required in order to maintain optimal serum concentrations, since verapamil has a high systemic clearance and a half-life of 3–7 h (20).

In this paper we describe the production and characterization of a panel of MAbs which recognize P-glycoprotein. The MAbs were evaluated for their ability to modulate P-glycoprotein activity in vitro. HYB-241, the most potent modulator of P-glycoprotein in vitro, was studied further in human tumor xenografts in nude mice.

MATERIALS AND METHODS

Cells. The multidrug-resistant human neuroepithelioma and neuroblastoma cell lines MC-IXC/VCR and SH-SY5Y/VCR (21), as well as the drug-sensitive lines from which they were derived, MC-IXC and SH-SY5Y, were gifts from Dr. June Biedler (Sloan Kettering Cancer Center, New York). The multidrug-resistant human leukemia cell line CEM/VLB180 (22) and its parent drug-sensitive line CCRF-CEM were gifts from Dr. William Beck (St. Jude Children’s Hospital, Memphis, TN). The human melanoma cell line BRO and the mdr I transfectant BRO/pFRmdrl.6 clone 1.1 cell line (23) were supplied by Piet Borst (The Netherlands Cancer Institute). The BRO/pFRmdrl.6 cell line was obtained through transfection of a human mdr I complementary DNA into the BRO cell under the control of a cytomegalovirus promoter and hepatitis B virus polyadenylation signals. All human lines were maintained in RPMI-1640 (Gibco, Grand Island, NY) supplemented with 2 mm l-glutamine and 10% fetal calf serum (growth media) in a humidified atmosphere of 5% CO2/95% air at 37°C. The mouse myeloma cell line P3-X63-Ag8.653 (24), a non-Ig-producing myeloma, was maintained in autoclavable minimal essential medium (Irvine Scientific, Irvine, CA) with 8% horse serum and 2% fetal calf serum. The hybridomas were obtained from Eli Lilly (Indianapolis, Ind.). Actinomycin D, doxorubicin, etoposide, and gramicidin were purchased from Sigma (St. Louis, MO). All radiolabeled drugs were purchased from Amersham (Arlington Heights, IL). 111InCl3 was purchased from Mallinckrodt (St. Louis, MO).

Antibody Production. Six-week-old female BALB/c mice from Charles River Biotechnology Services (Wilmington, MA) were immunized with 5 x 106 MC-IXC/VCR cells i.p. which had been scraped from the surface of tissue culture flasks. Three weeks later mice received a second i.p. injection of 5 x 106 MC-IXC/VCR cells. Four days prior to fusion, the mice received a final i.v. boost of 5 x 106 cells. This same schedule was followed for mice immunized with SH-SY5Y/VCR cells. Spleenocytes from the immunized mice were fused with the nonsecreting mouse myeloma cell line P3-X63-Ag8.653 by treatment with polyethylene glycol according to the method of Gerhard et al. (25) as modified from the method of Kohler and Milstein (26).
Antibody Screening. The initial screening of hybridoma cultures was done by indirect ELISA using a horseradish peroxidase conjugate of goat anti-mouse immunoglobulin (Cappel, Malverne, PA). Tumor cell monolayers of the drug-sensitive and drug-resistant cells were prepared by plating 10,000 cells/well in 96-well microtiter plates (Becton Dickinson, Lincoln Park, NJ) and incubating the plates at 37°C in a CO2 incubator overnight. The following day cells were fixed with 0.01% glutaraldehyde for 45 min at room temperature. After the fixation was removed and the plates were washed three times with PBS, the plates were blocked with 10% bovine serum albumin for at least 45 min. Hybridoma supernatants (50 μl) were added and allowed to incubate for 1 h at 37°C. Plates were washed with PBS and incubated with 50 μl of peroxidase-conjugated goat anti-mouse immunoglobulin diluted 1:1000 in PBS with 10% horse serum. Following five washes with PBS, positive clones were identified by the addition of 100 μl of a solution containing 1 mg/ml O-phenylenediamine, 0.1% hydrogen peroxide, 50 mM citrate, and 100 mM sodium phosphate buffer, pH 5.0. The reaction was quenched by the addition of 50 μl 3 N sulfuric acid, and the plates were read at 490 nm. Clones which produced a 5-fold or greater ELISA signal on at least one drug-resistant line in comparison to its drug-sensitive parental line were expanded. Hybridoma cells were injected into BALB/c mice for ascites production (27). Antibodies were purified from the ascites fluid by protein A chromatography (28).

Isotype. The subclasses of each of the MAbs were determined in an ELISA as described above, where subclass-specific secondary antibodies were utilized (Fisher, Springfield, NJ).

Immunofluorescence. Approximately 1 × 10⁴ cells were incubated with 100 μl of MAb at 10 μg/ml in microtiter plates for 1 h at room temperature. Cells were washed three times with PBS and resuspended in 75 μl of fluorescein isothiocyanate-labeled goat anti-mouse immunoglobulin (Boehringer Mannheim, Indianapolis, IN) diluted 1:75 in growth media and incubated for 1 h. The washed cells were resuspended in 1.0 ml PBS, and the cell suspension was analyzed at a rate of 300–600 events/s on a fluorescent activated cell sorter 400 flow cytometer (Becton Dickinson, Mountain View, CA).

Antigenic Modulation. MAbs were added to exponentially growing CEM/VLB105 cells to a final concentration of 100 μg/ml. The cells plus MAb were maintained in tissue culture flasks at 37°C in a CO2 incubator. At 1, 2, 4, 6, and 24 h following the addition of MAb to the cells, an aliquot of cells (1 × 10⁴ cells) was removed and stained with either HYB-241 or the anti-human T-cell MAb T101 (29) as described above ("Immunofluorescence"). Loss of antigen from the cell surface was monitored by flow cytometry.

Drug Accumulation. BRO/pFRmdrl.6 cells in log phase growth were harvested from tissue culture flasks using mild trypsinization, counted, and replaced with growth media with or without 100 μg/ml of antibody and incubated for 30 min at 37°C. Radiolabeled drugs were added to wells in triplicate at the following concentrations: [3H]Hycanthomin D (3 Ci/mmol) at 0.3 μCi/ml (1 μ); [3H]vincristine sulfate (2 Ci/mmol) at 0.5 μCi/ml (1 μ); [3H]vinblastine sulfate (11.7 Ci/mmol) at 0.5 μCi/ml (0.44 μM); or [3H]doxorubicin (55 mCi/ml) at 0.25 μCi/ml (4.7 μM). After incubation for varying amounts of time at 37°C in the dark, cells were washed with cold PBS and released from the wells by mild trypsinization. Cells were transferred to scintillation vials, and 5 ml of CytoScint (ICN, Irvine, CA) were added to each vial. Radioactivity was measured by liquid scintillation spectrometry.

Growth Inhibition Assay. Between 3 × 10⁴ and 5 × 10⁴ exponentially growing BRO/pFRmdrl.6 cells in 200 μl were plated per well of a 96-well microtiter plate. One day later the growth medium was removed and replaced with growth media with or without 100 μg/ml of antibody and incubated for 30 min at 37°C. Radiolabeled drugs were added to wells in triplicate at the following concentrations: [3H]Hycanthomin D (3 Ci/mmol) at 0.3 μCi/ml (1 μ); [3H]vincristine sulfate (2 Ci/mmol) at 0.5 μCi/ml (1 μ); [3H]vinblastine sulfate (11.7 Ci/mmol) at 0.5 μCi/ml (0.44 μM); or [3H]doxorubicin (55 mCi/ml) at 0.25 μCi/ml (4.7 μM). After incubation for varying amounts of time at 37°C in the dark, cells were washed with cold PBS and released from the wells by mild trypsinization. Cells were transferred to scintillation vials, and 5 ml of CytoScint (ICN, Irvine, CA) were added to each vial. Radioactivity was measured by liquid scintillation spectrometry.

Biodistribution in Mice with Human Tumor Xenografts. BRO/pFRmdrl.6 cells (5 × 10⁶ cells/animal) were injected s.c. into the flanks of 6-8-week-old female nude mice (obtained from Harlan Sprague Dawley, Indianapolis, IN). At day 21, animals with 0.2–0.3-g tumors were selected for experiments. Diethylstilbestrol diacetate-conjugated antibodies (1 μg) containing 10 μCi 111In were injected into the tail vein (0.1 ml in PBS), and groups were separated (5 animals/group) for sacrifice at various time points. The procedures for sacrificing, specimen collection, counting, and data reduction have been described previously (30).

Growth Inhibition of MDRI Transfected Tumors in Nude Mice. BRO/pFRmdrl.6 cells (5 × 10⁶ cells/animal) were implanted by s.c. inoculation into the flanks of 6–8-week-old female nude mice. When tumors reached 0.1–0.2 g, treatment was begun. The control group received i.v. injections of sterile PBS (500 μl) once each week for 3–4 weeks. The experimental group received i.v. injections of HYB-241 (25 mg/kg in 500 μl PBS) followed 24 h later by either vinblastine (5 mg/kg) or vincristine (2 mg/kg). The treatment with antibody followed 24 h later by drug was repeated once each week for the duration of the experiment (3–4 weeks). A group which received antibody only (25 mg/kg i.v.) as well as a group receiving drug only every 7 days were included in each experiment. Tumor size as well as weight of the animals were measured 2–3 times a week.

RESULTS AND DISCUSSION

A wide variety of small-molecule modulators of P-glycoprotein have been described in the literature (1–9). These reagents are highly effective in vitro in reducing the IC50 values of a variety of drugs in resistant cells as well as increasing the intracellular concentrations of chemotherapeutic drugs. Limited success has been achieved in vivo in reversing drug resistance due to the intrinsic toxicity of many of these small-molecule modulators. Although the results were encouraging in the Arizona trial where verapamil was added to a multidrug regimen in P-glycoprotein-positive multiple myeloma and produced responses in 2 of 5 patients (13) and in a later trial in lymphoma (19) which produced responses in 13 of 18 patients, optimal concentrations of verapamil required for the reversal of resistance in vivo could not be administered because of its potent effects on the cardiovascular system. MAbs may represent more specific and less toxic modulators of P-glycoprotein. A variety of MAbs to P-glycoprotein have been described in the literature. These include C219 (31), JSB-1 (32), MRK16, and MRK17 (33). Only MRK16 and MRK17 MAbs bind to cell surface epitopes of P-glycoprotein and therefore have potential therapeutic value. MRK16 has been shown to modulate P-glycoprotein activity and reverse resistance to vincristine and daunorubicin in vitro (34). We have produced a panel of MAbs to P-glycoprotein and studied their ability to modulate the activity of this ATP-dependent efflux pump in vitro and in vivo.

Mice were immunized with either the human multidrug-resistant neuroblastoma cell line MC-IXC/VCR, which is 6000-
fold resistant to vincristine, or the human multidrug-resistant neuroepithelioma line SH-SY5Y/VCR, which is 2500-fold resistant to vincristine. Two fusions produced 1139 hybridomas. Clonates were assayed by ELISA against drug-resistant and parental drug-sensitive cells. Ten clones consistently demonstrated greater reactivity with at least one drug-resistant cell line in comparison to the drug-sensitive control cells. Table 1 shows the binding ratios of each of these MAbs on two sets of drug-resistant and parental drug-sensitive cell lines, MC-IXC/VCR and MC-IXC and SH-SY5Y/VCR and SH-SY5Y. Eight MAbs reacted with the mdrl transfectant and not the parental line BRO, which indicates that these antibodies are directed against the *mdrl* gene product P-glycoprotein. MAbs HYB065 and HYB195 are not directed against P-glycoprotein, since they failed to bind to the transfected cell line. These MAbs may recognize novel drug-resistant related molecules. All of the anti-P-glycoprotein MAbs are IgG1, with the exception of HYB-162, which is an IgG2b, and HYB-243, which is an IgM.

The ELISA data generated on the glutaraldehyde-fixed cells suggested that the panel of MAbs may be recognizing a cell surface component of P-glycoprotein. Fig. 1 shows the typical shift in mean intensity of fluorescence produced by three of the anti-P-glycoprotein MAbs, HYB-241, HYB-612, and HYB-237, when the MAbs were reacted first with the drug-sensitive line CCRF-CEM and then with the drug-resistant line CEM/VLB100 and then examined by flow cytometry. All of the MAbs bound to intact CEM/VLB100 cells, suggesting that they all recognize the extracellular loop portions of P-glycoprotein. No binding to CCRF-CEM cells was seen in this assay.

Previously published experiments (35) indicated that both HYB-241 and HYB-612 were capable of increasing intracellular concentrations of actinomycin D and vincristine in MC-IXC/VCR cells. We were interested in determining whether HYB-241, HYB-612, or any of the other anti-P-glycoprotein MAbs would have similar effects on a cell line with lower levels of resistance, such as the BRO/pFRmdrl.6 cell line, and whether the MAbs would modulate the activity of other drugs in the MDR family. The accumulation of actinomycin D over time in the presence of varying amounts of HYB-241 is shown in Fig. 2. In the presence of 1.25 μg/ml actinomycin D, BRO/pFRmdrl.6 cells reached steady-state levels of drug by 2 h and remained at this level at 6 h. The addition of 0.1 μg/ml HYB-241 had no effect on drug accumulation, but the addition of 1 μg/ml or more of HYB-241 significantly increased levels of drug which accumulated in the cells. The greatest increase in actinomycin D levels (7-fold) was achieved at 50 μg/ml MAb at 6 h.

Fig. 3 illustrates the effect of each of the MAbs as well as 20 μm verapamil on the accumulation of vincristine, vinblastine, and actinomycin D in BRO/pFRmdrl.6 cells. HYB-241 at 50 μg/ml was the most effective MAb at increasing intracellular levels of actinomycin D (7-fold), vinblastine (3-fold), and vincristine (2-fold). Levels of actinomycin D and vinblastine which accumulated in the transfectant cells in the presence of HYB-241 were comparable to levels of drug which accumulated in the drug-sensitive BRO cell line (data not shown). In contrast, levels of vincristine which were obtained in the transfectant line in the presence of HYB-241 were 2-fold less than the levels of vincristine which accumulated in the drug-sensitive BRO line. HYB-241 was as effective as 20 μm verapamil in increasing the accumulation of vinblastine in the transfectant, while verapamil was more effective in increasing levels of actinomycin D and vincristine in these cells. The other anti-P-glycoprotein MAbs modulated the accumulation of actinomycin D and the *Vinc* alkaloids to lesser degrees. None of the MAbs affected the accumulation of doxorubicin (data not shown). The similarity in drug modulation seen with the MAb panel was not surprising, since competition studies suggested that all the MAbs bound to the same or overlapping epitopes (data not shown). The differences in degree of modulation are probably related to differences in MAb affinities. The two drug-resistance-related MAbs HYB-065 and HYB-195, which failed to bind to the MDRI transfectant, had no effect on accumulation of any of the drugs examined.

One mechanism which could account for the ability of a MAb to modulate drug accumulation in drug-resistant cells would be through antibody-mediated internalization of antigen (36).
REVERSAL OF VINCA ALKALOID RESISTANCE BY MONOCLONAL ANTIBODY

Fig. 2. Effect of HYB-241 concentration on the accumulation of [3H]actinomycin D in BRO/pFRmdrl.6 cells. Cells (5 x 10^6) were incubated with [3H]actinomycin D at 0.3 nCi/ml (•) for 1, 2, 3, and 6 h in the presence of MAb HYB-241 at 100 µg/ml (•), 50 µg/ml (O), 10 µg/ml (A), 1 µg/ml (Φ), 0.1 µg/ml (□), or PBS (Δ).

Down-regulation of P-glycoprotein on the cell surface could lead to the increased accumulation of drugs, especially those with lower affinities for P-glycoprotein-binding sites. In order to test this hypothesis, CEM/VLB100 cells were incubated with saturating amounts of HYB-241, and at various time intervals the cells were assayed for the presence of P-glycoprotein on their cell surface. Over a 24-h time period no loss of P-glycoprotein was observed from the surface of the cells (Fig. 4). The MAB T101 (29), known to immunologically modulate its antigen, CD5, was included as a positive control. This result suggests that antigenic modulation is not responsible for the reversal in resistance seen with HYB-241.

Based on the drug accumulation results which indicated that HYB-241 may be the most potent MAb modulator of our panel, we extended our studies of this MAb to examine its ability to lower IC50 values. The IC50 values for each of the drugs were determined for both the drug-sensitive and drug-resistant transfectant lines. The transfectant line was resistant to vincristine (410-fold), vinblastine (63-fold), actinomycin D (12-fold), etoposide (5-fold), granamicin (24-fold), and doxorubicin (3-fold).

In agreement with drug accumulation experiments, the addition of HYB-241 resulted in the greatest decrease in IC50 with actinomycin D. HYB-241 at 100 µg/ml reduced the IC50 of actinomycin D 11-fold from 4.0 to 0.36 nM in mdr1-transfected cells. The actinomycin D IC50 for the mdr1-transfected cell line was 0.36 nM.

Fig. 3. Effect of anti-P-glycoprotein MAbs on the accumulation of actinomycin D, vincristine, and vinblastine in BRO/pFRmdrl.6 cells. Cells (5 x 10^6) were preincubated with MAb at 50 µg/ml for 30 min followed by the addition of [3H]actinomycin D at 0.3 µCi/ml, [3H]vincristine at 0.5 µCi/ml, or [3H]vinblastine at 0.5 µCi/ml. Cells were washed and counted for radioactivity after 4 h. The effect of verapamil (20 µM) on BRO/pFRmdrl.6 cells and the effect of each of the drugs on BRO cells were studied. Values represent the means of three determinations.

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Based on the drug accumulation results which indicated that HYB-241 may be the most potent MAb modulator of our panel,
in the presence of MAb was not significantly different from the IC50 of the drug-sensitive BRO line (Fig. 5). HYB-241 at 100 μg/ml did not alter the IC50 of the drug-sensitive BRO line. In order to determine if the reduction in IC50 of actinomycin D was a specific effect of HYB-241, an IgG, anti-Gardnerella antibody, GDJ352, was added in the same experiment. No reduction in the IC50 of the drug was observed in the presence of 100 μg/ml of the irrelevant MAb (data not shown).

The ability of the MAb to localize to drug-resistant BRO/pFRmdrl.6 tumors in nude mice by anti-P-glycoprotein MAb HYB-241 in combination with vinblastine. A, 25 mg/kg of HYB-241 i.v. followed 24 h later by 5 mg/kg vinblastine i.v. once weekly; Δ, 5 mg/kg vinblastine i.v. once weekly; O, 25 mg/kg HYB-241 i.v. once weekly; PBS control i.v. once weekly. Points, means of five determinations; bars, SE.

Table 2 Reversal of drug resistance in BRO/MDR1 cells by HYB-241

<table>
<thead>
<tr>
<th>Drug</th>
<th>BRO cells IC50 (μM)</th>
<th>BRO/MDR1 cells IC50 (μM)</th>
<th>DMF</th>
<th>DMF</th>
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<tbody>
<tr>
<td>Actinomycin D</td>
<td>0.34 (0.31-0.37)</td>
<td>4.0 (3.0-5.0)</td>
<td>1.5</td>
<td>1.4</td>
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<tr>
<td>+HYB-241</td>
<td>0.23 (0.19-0.27)</td>
<td>0.36 (0.13-0.60)</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>+GDJ-352</td>
<td>ND</td>
<td>2.8 (2.2-3.4)</td>
<td></td>
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<tr>
<td>Vincristine</td>
<td>0.61 (0.50-0.71)</td>
<td>250 (157-343)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+HYB-241</td>
<td>0.58 (0.41-0.75)</td>
<td>42.0 (33-52)</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>+GDJ-352</td>
<td>ND</td>
<td>192 (96-286)</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>Vinblastine</td>
<td>0.49 (0.32-0.66)</td>
<td>31 (25-36)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+HYB-241</td>
<td>0.60 (0.32-0.88)</td>
<td>15 (11-21)</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>+GDJ-352</td>
<td>ND</td>
<td>19 (11-26)</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>86 (71-102)</td>
<td>290 (262-319)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+HYB-241</td>
<td>72 (60-85)</td>
<td>269 (233-305)</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>+GDJ-352</td>
<td>ND</td>
<td>233 (190-276)</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>Etoposide</td>
<td>0.76 (0.47-1.11)</td>
<td>3.4 (2.0-4.9)</td>
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</tr>
<tr>
<td>+HYB-241</td>
<td>1.11 (0.27-1.94)</td>
<td>7.6 (7.0-8.5)</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>+GDJ-352</td>
<td>ND</td>
<td>3.9 (2.2-5.6)</td>
<td>0.9</td>
<td></td>
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<tr>
<td>Gramicidin</td>
<td>2.2 (1.8-2.6)</td>
<td>53 (32-73)</td>
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</tr>
<tr>
<td>+HYB-241</td>
<td>3.1 (1.8-4.4)</td>
<td>39 (22-57)</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>+GDJ-352</td>
<td>ND</td>
<td>53 (26-79)</td>
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* IC50 values represent the drug concentration at which the growth of the cells is 50% of that seen in the control cell wells.
* DMF, dose modifying factor (IC50 of drug divided by the IC50 of drug in the presence of antibody).
* 95% confidence intervals.

The anti-P-glycoprotein monoclonal antibody HYB-241 was added to 100 μg/ml to the cells 30 min prior to the addition of drug. The negative control antibody GDJ352, an anti-gardnarella negative control antibody, was added at 100 μg/ml to the cells prior to the addition of drug.

Increased IC50 value for etoposide by 2.2-fold. The mechanism for this increase in resistance in the presence of an anti-P-glycoprotein antibody is currently being studied.

The ability of the MAb to localize to drug-resistant BRO/pFRmdrl.6 tumors in vivo was studied using 111In-labeled HYB-241. The radiolabeled antibody was injected into tumor-bearing nude mice, and at various time points thereafter mice were sacrificed, and the amount of radioactivity in each of the organs and in the tumor was determined. Fig. 6A shows that by 24 h after i.v. injection of 111In-labeled HYB-241, 16% of the injected dose per gram was bound to the tumor. The amount of MAb continued to accumulate at the tumor site, and at 120 h post-injection 25% of the injected dose per gram was bound to the tumor. 111In-labeled MOPC 21 (37), an irrelevant antibody of the same isotype, bound less than 5% of the injected dose per gram at 120 h postinjection (Fig. 6B). The 111In-labeled HYB-241 did not localize to the drug-sensitive BRO tumors (data not shown).

Based on the results of the biodistribution study which indicated that HYB-241 localized at the tumor 24 h postinjection and remained at the tumor at high concentrations at 120 h, a protocol was developed to study the effect of a 24-h predose of HYB-241 followed by Vinca alkaloid administration on the growth of an established drug-resistant tumor. Doses of Vinca alkaloid were chosen which were ineffective at inhibiting growth of the drug-resistant BRO/pFRmdrl.6 tumor but which were...
effective in inhibiting growth of the drug-sensitive BRO tumor. Predosing animals with 500 µg of HYB-241 (25 mg/kg) i.v. 24 h prior to Vinca alkaloid weekly for 3 weeks inhibited the growth of the drug-resistant tumor. As shown in Fig. 7 vinblastine treatment at 5 mg/kg i.v. once per week did not significantly inhibit the growth of this tumor. The injection of 500 µg of HYB-241 24 h prior to the same dose (5 mg/kg) of vinblastine significantly decreased the growth of the tumor. HYB-241 alone had no effect on the growth of the tumors. Similar inhibition in growth of the tumors was obtained when HYB-241 was preadministered to mice prior to injection of weekly doses of vincristine which alone had no effect (data not shown). In contrast, HYB-241 was unable to potentiate the activity of doxorubicin in vivo.

In summary, these results indicate that HYB-241 may be an effective chemosensitizer of vincristine, vinblastine, and actinomycin D in the treatment of human tumors. Antibodies may offer some unique advantages in the clinic as modulators of drug resistance due to their long residence time at the tumor site and long serum half-life. MAbs may also produce fewer side effects than the small-molecule modulators currently being examined in the clinic. Humanized MAbs substantially reduce drug resistance due to their long residence time at the tumor site and the accumulation of toxic compounds in normal tissues and the accumulation of toxic compounds in normal tissues may not occur with antibody modulators because P-glycoprotein may not be physiologically accessible to antibody in the normal tissues where it is expressed (40). Studies are currently under way to study the effect of HYB-241 on Vinca alkaloid accumulation in normal tissues.

The mechanism by which HYB-241 modulates the pump is purely speculative at this time. The MAb does not modulate the antigen immunologically, and therefore it appears that the ability of the MAb to reduce IC50 values and increase drug accumulation is not due to the loss of P-glycoprotein from the surface of the cells. Alternatively, the binding of MAb to the extracellular portion of P-glycoprotein may induce a conformational change in the molecule which has an impact on the ability of the molecule to transport some drugs (actinomycin D, vincristine, vinblastine) but not others (doxorubicin). This selectivity in the antibody’s ability to modulate drug activity may provide further insight into the structure/function relationship of P-glycoproteins.

REFERENCES


Reversal of *Vinca* Alkaloid Resistance by Anti-P-Glycoprotein Monoclonal Antibody HYB-241 in a Human Tumor Xenograft

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