MAC-321, a novel taxane with greater efficacy than paclitaxel and docetaxel *in vitro* and *in vivo*

Deepak Sampath, Carolyn M. Discafani, Frank Loganzo, Carl Beyer, Hao Liu, Xingzhi Tan, Sylvia Musto, Tami Annable, Patricia Gallagher, Carol Rios, and Lee M. Greenberger

Wyeth Research, Department of Oncology, Pearl River, NY

Abstract

The taxanes, paclitaxel (PTX) and docetaxel (DTX), belong to a novel class of anticancer drugs that stabilize microtubules and lead to tumor cell death. While both agents are widely used for the treatment of lung, breast, and ovarian cancer, many tumor types are refractory or develop resistance to these drugs. We describe here a novel analogue of DTX, designated MAC-321 [*M*icrotubule/*A*poptosis/*C*ytotoxic: 5β, 20-epoxy-1, 2α-, 4-, 7β-, 10β-, 13α-hexahydroxytax-11-en-9-one 4 acetate 2 benzoate 7-propionate 13-ester with (2*R*,3*S*)-*N*-tertbutoxycarbonyl-3-(2-furyl)isoserine], that overcomes P-glycoprotein-mediated resistance to PTX and DTX in preclinical model systems. Similar to PTX or DTX, MAC-321 enhanced the rate of tubulin polymerization *in vitro* and caused the bundling of microtubules in cells. MAC-321 inhibited proliferation of a panel of 14 tumor cell lines with minimal variation in potency (IC$_{50}$ = 2.2 ± 1.4 nM; range = 0.6–5.3 nM). Unlike PTX or DTX, the IC$_{50}$ of MAC-321 did not vary in cells that expressed low to moderate levels of P-glycoprotein. Even under extraordinary conditions in KB-V1 cells, which highly overexpress P-glycoprotein, resistance to MAC-321 was 80-fold compared with that of PTX (1400-fold) and DTX (670-fold). In addition, equivalent or less resistance to MAC-321 compared with PTX or DTX was observed in four cell lines that contain distinct point mutations within the taxane-binding site of β-tubulin. Most importantly, MAC-321 displayed superior *in vivo* efficacy because: (*a*) MAC-321 either partially or completely inhibited tumor growth in three tumor models that overexpressed P-glycoprotein and were resistant to PTX; and (*b*) unlike PTX or DTX, MAC-321 was highly effective when given orally. MAC-321 was also highly effective when given as single i.v. dose. Our findings suggest that MAC-321, which is currently under clinical evaluation, may have broad therapeutic value. (Mol Cancer Ther. 2003;2:873–884)

Introduction

On the basis of current estimates, more than 10 million cases of cancer are documented worldwide resulting in greater than 6 million deaths annually (1). In most patients with solid tumors, some of the most effective anticancer therapies are palliative rather than curative and prolong life only on the order of months rather than years (2). As a result, there remains a significant unmet medical need to develop agents that improve quality of life and prolong survival.

Agents that bind to tubulin and inhibit microtubule function are widely used in the treatment of cancer (2). Such drugs inhibit several processes during cell division, including chromatid separation, leading to inhibition of growth and ultimately cell death. Although the exact mechanism of action is not completely understood, all anti-microtubule agents alter the dynamic equilibrium of microtubules such that they either perturb the net addition of tubulin dimers to one end (polymerization), or the net removal of tubulin dimers from the opposite end (depolymerization) (2).

Paclitaxel (PTX), originally derived from the inner bark of the pacific yew tree, *Taxus brevifolia*, and docetaxel (DTX), derived semisynthetically by esterification of a side chain to 10-deacetyl baccatin III (10-DAB), stabilize microtubules and at stoichiometric concentrations enhance microtubule polymerization (3–8). On the basis of photoaffinity labeling and crystallographic analyses, both PTX and DTX inhibit the function of tubulin by binding to a similar, highly defined region within β-tubulin (9). However, recent studies indicate that the antineoplastic activity of taxanes may originate, in part, from induction of genes encoding transcription factors with tumor suppressor effects as well as enzymes governing proliferation, apoptosis, inflammation, and other anti-proliferative factors (10–12).

The currently approved taxanes have numerous limitations. First, certain tumor types are either completely refractory to these agents (*i.e.*, colon carcinomas) or develop resistance during multiple cycles of therapy (*i.e.*, breast, ovarian, or lung carcinomas) (1, 13). Second, all anti-microtubule drugs induce serious side effects, most notably bone marrow suppression and/or peripheral neuropathy. Third, both PTX and DTX, which are prepared in a lipophilic vehicle, induce hypersensitivity reactions that require patients to be pre-medicated with corticosteroids.

In tissue culture, resistance to PTX or DTX can be attributed to (*a*) overexpression of drug efflux pumps such as P-glycoprotein, (*b*) acquired mutations at the drug binding site of tubulin, (*c*) differential expression of tubulin isoforms, (*d*) alteration in apoptotic mechanisms, (*e*) activation of growth factor pathways, or (*f*) other mechanisms (14, 15). The contribution of each of these mechanisms to clinical resistance remains uncertain, although correlations have been made with P-glycoprotein expression levels in some tumor types.

We describe here a novel DTX analogue designated MAC-321 [*M*icrotubule/*A*poptosis/*C*ytotoxic: 5β, 20-epoxy-1, 2α-, 4-, 7β-, 10β-, 13α-hexahydroxytax-11-en-9-one 4 acetate 2 benzoate 7-propionate 13-ester with (2*R*,3*S*)-*N*-tertbutoxycarbonyl-3-(2-furyl)isoserine]. Similar to DTX and PTX, MAC-321 stimulated microtubule assembly in a cell free system and induced the bundling of microtubules in cells. However, unlike PTX or DTX, MAC-321 is highly effective at inhibiting the growth of tumor cells that are resistant to PTX or DTX when given p.o. or i.v. *in vivo*. The basis for this effect compared with PTX or DTX is related, at least in part, to the reduced interaction of MAC-321 with P-glycoprotein.

Materials and Methods

Chemicals

PTX, vincristine, vinblastine, Adriamycin/doxorubicin, polyoxyl 35 castor oil (Cremophor EL), and polysorbate 80 (Tween 80) were purchased from Sigma Inc. (St. Louis, MO). DTX was obtained from the pharmacy (Taxotere; Aventis, Bridgewater, NJ and LKT Laboratories, St. Paul, MN). Mitoxantrone was manufactured by Wyeth (Pearl River, NY). Epothilone A (EpoA) and B (EpoB) were obtained from Calbiochem (San Diego, CA). Dolastatin-10 was generously provided by the National Cancer Institute (Bethesda, MD). MAC-321, also known as TL-139, was obtained from Taxolog, Inc. (Fairfield, NJ).

Cell Lines

All cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, MD) except as indicated. The 1A9 ovarian parental and resistant cell lines were generously provided by Drs. Tito Fojo and Marianne Poruchynsky (National Cancer Institute) and have been characterized previously (16, 17). The A549 lung adenocarcinoma parental cell lines and its counterpart selected for resistance to EpoB (A549.EpoB40) were kindly obtained from Dr. Susan Band Horwitz (Albert Einstein College of Medicine, Bronx, NY) and have been described previously (18). All other cell lines, including KB-3-1, KB-8-5, KB-V1 (19), A375SM (20), HL60 and HL60/ADR (21), S1 and S1-M1-3.2 (22), and MX-1W have been described and maintained as previously reported.

In Vitro Tubulin Polymerization Assays

For *in vitro* tubulin polymerization assays, lyophilized bovine microtubule-associated protein (MAP)-free tubulin and PEM buffer [80 mM Na-PIPES (pH 6.9), 1 mM MgCl$_2$, 1 mM EGTA] were purchased from Cytoskeleton (Denver, CO). MAP-free tubulin (1.5 mg/ml), prepared as previously described (23), was incubated with test compounds at the following concentrations: 0.1, 0.3, 0.9, 2.7, 8.1, and 24.3 μM in PEM-0.3% DMSO. Absorbance at 340 nm was measured every minute for 60 min at 24°C using a SpectraMax Plus spectrophotometer (Molecular Devices, Sunnyvale, CA).

Immunofluorescence Microscopy

The effect of test agents on tubulin morphology in cells was visualized by immunofluorescence microscopy. KB-3-1 epidermoid carcinoma cells were plated at 5000 cells/chamber on poly-D-lysine-coated eight-chamber microscope slides (Becton Dickinson Labware, Bedford, MA) and cultured overnight. Compounds, diluted in media, were added to each chamber to achieve the desired final concentrations. Details on the detection of tubulin using the anti-α-tubulin antibody (clone DM 1A, Sigma) followed by FITC-conjugated F(ab′)$_2$ fragment of goat anti-mouse IgG (Jackson Immunoresearch, West Grove, PA), as well as the detection of DNA using propidium iodide, have been previously described (23).

Immunoblot Analysis

Total membrane proteins (50 μg) were resolved by 7.5% SDS-PAGE and transferred to polyvinylidene difluoride (PVDF) membranes. Identical samples run in parallel gels were stained with Coomassie blue to confirm equal protein loading. Immunoblots were incubated with a 1:500 dilution of multidrug-resistant transporter (MDR1) primary antibody (clone Ab-1, Oncogene Science, Uniondale, NY) overnight at 4°C in 5% nonfat dried milk/Tris-buffered saline, followed by a 1:2000 dilution of secondary antibody goat anti-rabbit IgG-HRP (Bio-Rad Inc., Hercules, CA) for 1 h at room temperature. Protein signals were detected with enhanced chemiluminescence reagents (Amersham, Arlington Heights, IL) followed by exposure to Hyperfilm-ECL (Amersham).

Quantitative Real-Time Reverse Transcription-PCR

Expression of MDR1 mRNA transcripts in tumor cells lines was determined by real-time reverse transcription (RT)-PCR using the iCycler thermal cycler and analysis software (Bio-Rad). Oligonucleotides for MDR1 [(forward primer: 5′-CTGCTTGATGGCAAAGAAATAAAG-3′), (reverse primer: 5′-GGCTGTTGTCTCCATAGGCAAT-3′), and probe (5′-6-FAM-CAGTGGCTCCGAGCACACCTGG-BHQ1-Q)] were designed with Primer Express software version 1.0 (PE Applied Biosystems, Foster City, CA) using the published GenBank sequence of human MDR1 (accession number M14758). Oligonucleotide sequences for human β-actin [(forward primer, 5′-TGCGTGACATTAAGGAGAAG), reverse primer (5′-GCTCGTAGCTCTTCTCCA), probe (5′-Texas Red-CACGGCTGCTTCCAGCTCCTC-BHQ1-Q)] (accession number NM001101) were provided by Bio-Rad. Relative fluorescence values of PCR product were calculated on a logarithmic scale using a standard curve consisting of 0.1–1000 ng of template cDNA during sample analysis. For MDR1 analysis, 0.1–0.5 μg of cDNA was analyzed in duplicate using a final concentration of 0.3 μM MDR1-specific primer pairs and 0.2 μM probe. MDR1 cDNA levels were normalized by processing the same cell samples in a parallel reaction for β-actin mRNA levels using 0.2 μM of primer pairs and probe. Relative expression values were calculated as defined by Pfaffl (24) and data are reported as the mean relative expression of MDR1 normalized to β-actin.

Drug Accumulation Studies

The intracellular accumulation of MAC-321 in KB-3-1,

KB-8-5, and KB-V1 cells was performed essentially as described previously (22). Briefly, cells were plated in 24-well dishes in serum-containing DMEM media at 1.5×10^5 cells per well. After culturing overnight, cells were incubated in the absence of serum with 500 nM $[^{14}C]$-MAC-321 (104 mCi/mmol, Wyeth) or $[^{14}C]$-PTX (74 mCi/mmol, Moravek Biochemicals, Brea, CA) for 2 h at 37°C. Radiolabeled cells were harvested, washed with cold PBS, and lysed in 0.2 ml of 2 N NaOH for 1 h at 37°C. Lysates (100 μl) were transferred to scintillation vials, vortexed with 100 μl of 2 N HCl, and counted on a Beckman liquid scintillation counter. Activity was normalized for protein content by performing a dye-binding assay (Bio-Rad) and data were reported as mean picomoles drug per milligram total protein from triplicate wells.

Cell Proliferation Assays

Cytotoxicity was assessed by growing cells in the presence of agents for 72 h. Cell survival was measured by the sulforhodamine B (SRB) protein stain method or the ATP-binding assay using the Cell-Titer Glo Luminescent Reporter (GLR) System (Promega, Inc., Madison, WI). The SRB assay was done as previously described (23). For the GLR system, cells were plated robotically at approximately 50% confluency in a 384-well plate and allowed to attach for 12 h at 37°C/5% CO$_2$. After diluting test agents using BioMek 2000 robotic system (Beckman Instruments, Fullerton, CA), agents were added to each well and incubated for 72 h. ATP binding and stabilization of the luminescent signal were performed according to manufacturer's protocol (Promega) following tumor cell lysis. Absorbance was read on a Victor V multilabel plate reader (Perkin-Elmer, Gaithersburg, MD) at a wavelength of A_{595} and data collected using Wallac 1420 Workstation software.

The drug concentration that reduced the viability of cells by 50% (IC$_{50}$) was determined by plotting duplicate data points over a concentration range and manually calculating values or obtained using regression analysis (Data Analysis Toolbox, MDL Information Systems, San Leandro, CA). Cytotoxicity profiles were similar with the SRB or GLR methods.

In Vivo Tumor Xenografts

Athymic nu/nu female mice were implanted s.c. with either of the following cell lines: 1.5×10^6 Lox, 5×10^6 KB-3-1, 8×10^6 A375SM, 2.5×10^6 KB-8-5, 6×10^6 DLD-1, 7×10^6 HCT-15, or 5 MX-1W tumor fragments (passaged from animal to animal). When tumors attained an average mass between 80 and 200 mg (day 0), animals were randomized into treatment groups each containing either 5 or 10 animals. Mice were treated i.v. with the following: (*a*) one or more doses of MAC-321 prepared in a vehicle containing 10% (w/v) safflower and 10% (w/v) soybean oil with 1.25% (w/v) egg phosphatides; (*b*) 60 mg/kg PTX in 6% ethanol/6% polyoxyl 35 castor oil in saline; or (*c*) 25 mg/kg DTX prepared in 1.4% ethanol/3.5% polysorbate 80 in saline. In other experiments, mice were treated p.o. with one or more doses of MAC-321 prepared in 5% ethanol/5% polyoxyl 35 castor oil in normal saline or vehicle alone. Tumor mass [(length

$\times$ width^2)/2] was determined approximately once a week for up to 42 days, depending on the experiment. A drug dose is considered toxic if there is greater than 20% lethality or if animals have lost ≥20% of their initial body weight.

To prepare PTX, the powder was first solubilized in 100% ethanol, followed by mixing with polyoxyl 35 castor oil to yield a 25 mg/ml stock (50% ethanol:50% polyoxyl 35 castor oil) that was diluted in saline immediately before administration. A dose of 60 mg/kg PTX when administered on days 1, 5, and 9 was determined to be approximately 90% of the maximum tolerated dose (based on the maximal acceptable weight loss of 20% compared with control-treated animals). To prepare DTX, 20 mg of powder were dissolved in 100% polysorbate 80 and then further diluted in 13% ethanol to yield a 10 mg/ml stock. In addition, MAC-321 was initially solubilized in 100% ethanol followed by mixing with vehicles used for i.v. or p.o. administration. Injection or gavage volumes for all test agents did not exceed 0.5 ml.

Results

Chemical Structure of MAC-321

The structures of PTX, DTX, and MAC-321 are compared in Fig. 1. MAC-321 is a DTX analogue with a furan ring substitution in the 3′ carbon position and a propionate substitution in the seven-carbon position of DTX (Fig. 1, R_1 and R_3, respectively).

MAC-321 Induces Microtubule Polymerization and Stabilization

To confirm that MAC-321 functioned like a taxane molecule, the effect on microtubule polymerization was studied *in vitro* utilizing MAP-free bovine brain tubulin in the absence of GTP. Under standard test conditions, as little as 0.9 μM of MAC-321 or DTX enhanced tubulin polymerization with an observed 5-min lag time compared with control treatment (Fig. 2). The maximum polymerization of microtubules was achieved with 24.3 μM MAC-321 or DTX, with no apparent delay (Fig. 2). PTX also induced polymerization of microtubules, although only partial induction of polymerization was observed with the maximum concentration of drug tested (24.3 μM) (data not shown). As a negative control, 1 μM vincristine inhibited polymerization of tubulin (23). Thus, MAC-321, like DTX, is a taxane with potent tubulin polymerization activity.

Fluorescent Staining of Microtubules in KB-3-1 Cells

Another hallmark of taxanes is their ability to induce tubulin bundling in tumor cells. Therefore, PTX, DTX, and MAC-321 were examined for their ability to disrupt cell division and induce the bundling of microtubules in cells (Fig. 3). PTX, DTX, and MAC-321 were used at concentrations that were 1-, 3-, or 10-fold higher than the concentration needed to inhibit the growth of KB-3-1 human epidermoid carcinoma cells (Table 1). In control-treated cells, extensive microtubule networks in the cytoplasm (Fig. 3A) and defined spindle poles surrounding
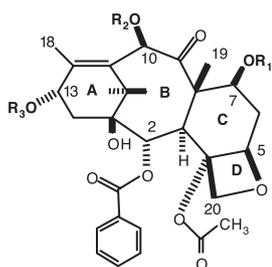
Compound	R₁ =	R₂ =	R₃ =
Paclitaxel	H	CH₃C-(=O)	
Docetaxel	H	H	
MAC-321	CH₃CH₂C-(=O)	H	

Figure 1. Chemical structures of PTX, DTX, and MAC-321. Modifications of the baccatin III core structure (denoted as *A*, *B*, *C*, and *D*) are indicated as R_1, R_2, and R_3.

the metaphase plate in dividing cells were observed (Fig. 3A, *inset*). At low concentrations of MAC-321 (1.25 nM) or PTX (4 nM), normal metaphase plates with characteristic spindle poles were rarely observed and cells were usually rounded (Fig. 3, C and D). Rather, multiple punctate bright fluorescent spots of tubulin were observed in close proximity to nuclear material in many cells. At the highest concentration tested, both 12.5 nM MAC-321 and 40 nM PTX induced the formation of microtubule bundles in the cytoplasm of numerous cells (Fig. 3, E and F and E, *inset*). DTX caused similar effects (data not shown). To insure these effects were specific, cells were treated with 8 nM vinblastine (Fig. 3B). In this case, no bundling of tubulin was observed (Fig. 3B, *inset*).

MAC-321 Is a Potent Inhibitor of Tumor Cell Growth

The growth inhibitory effects of MAC-321, PTX, and DTX were compared among 14 diverse cell lines derived from colon, breast, lung, ovarian, pancreatic, melanoma, and epidermoid tumors. MAC-321 inhibited the growth of all tumor cell lines tested in tissue culture regardless of tumor origin with a IC_{50} value of 2.2 ± 1.4 nM (ranging from 0.6 to 5.3 nM) (Table 1). In addition, MAC-321 was equipotent compared to DTX or vinblastine and 2.5-fold more potent than PTX in those cell lines that had no detectable levels of P-glycoprotein (Table 1).

MAC-321 Overcomes PTX Drug Resistance due to Overexpression of Drug Efflux Pumps

Because MAC-321 is an anti-microtubule drug, we reasoned that it would be most useful in those patients where traditional anti-microtubule therapies had failed. Therefore, the activity of MAC-321 was compared with other taxanes, with special emphasis on PTX-/DTX-resistant

models where the basis of resistance was known to be associated with the overexpression of drug efflux pumps, including MDR1 (P-glycoprotein, ABCB1), or tubulin mutations (16–18). As a benchmark, we used KB-8-5 and KB-V1 cells, which were derived from KB-3-1 cells by selection in colchicine and vincristine, respectively, and known to overexpress P-glycoprotein (19). Increased levels of MDR1 mRNA and protein were confirmed in cell lines selected for P-glycoprotein overexpression (KB-8-5 and KB-V1) and compared with expression levels found in non-selected cell lines (Fig. 4). Relative to KB-8-5 cells, the human colon cell lines, DLD-1 and HCT-15, had approximately equal and higher levels of P-glycoprotein expression, respectively (Fig. 4). While the levels of MDR1 mRNA were markedly higher in the MX-1W and NCI-H1299 cell lines (Fig. 4A), the P-glycoprotein levels were only slightly elevated compared with KB-3-1 cells (Fig. 4B). Interestingly, MAC-321 retained potency in cell lines where resistance to PTX was associated with overexpression of the MDR1 and was reflected in higher IC_{50} values for PTX, DTX, and vinblastine (Table 1). The latter observation was most pronounced in the MDR-positive DLD-1 and HCT-15 cells. For example in HCT-15 cells, the IC_{50} for PTX and DTX was approximately 439 and 55 nM, respectively, *versus* 3.3 nM for MAC-321.

A detailed cytotoxicity profile for the KB-3-1 cell line compared with KB-8-5 and KB-V1 cells to a variety of anti-microtubule agents, including PTX and DTX, has been reported by our laboratory recently (23). Because IC_{50} values were determined by identical methods (SRB protein stain) in the latter and present studies, only the relative resistance of MAC-321, PTX, and DTX is discussed here. In these cell lines, resistance to PTX and DTX was moderate (18- to 19-fold) in the KB-8-5 cells and very high (1400- to 670-fold) in the KB-V1 cells (23). In contrast, no resistance (1.3-fold) to MAC-321 was found in the KB-8-5 cell line (IC_{50} of KB and KB-8-5 cells was 1.3 ± 0.5 and 1.7 ± 1.5 nM, respectively). However, resistance to MAC-321 can be mediated by P-glycoprotein in extreme circumstances because 81-fold resistance to MAC-321 was found in a KB-V1 cell line ($IC_{50} = 100.6 \pm 85.6$ nM) (23). This is likely due to P-glycoprotein, because sensitivity to PTX and MAC-321 was almost completely reversible with
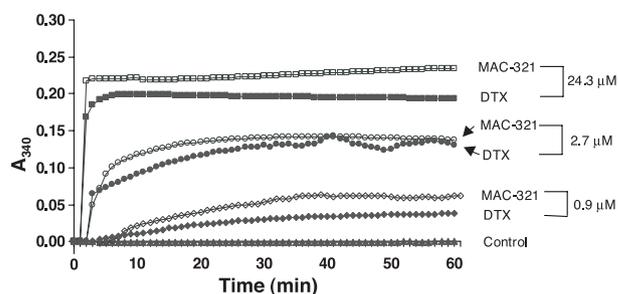

Figure 2. *In vitro* tubulin polymerization assay. Tubulin polymerization assays were conducted at 24°C using 1.5 mg/ml of purified bovine brain MAP-free tubulin in the presence of vehicle control (▲) or the indicated concentrations of DTX (*closed symbols*) or MAC-321 (*open symbols*) as shown. Turbidity was measured by absorbance (340 nm) for up to 60 min.

Figure 3. Immunofluorescence staining of α-tubulin in KB-3-1 cells treated with PTX and MAC-321. Cells were treated for 20 h with vehicle (**A**), 8 nM vinblastine (**B**), 1.25 nM MAC-321 (**C**), 12.5 nM MAC-321 (**E**), 4 nM PTX (**D**), or 40 nM PTX (**F**). After fixation, cells were incubated with an anti-α-tubulin antibody followed by a FITC-conjugated secondary antibody and stained with propidium iodide to visualize DNA. Images were overlaid electronically after cells were examined by fluorescent microscopy for tubulin and propidium iodide staining. *Insets* in panels **A**, **B**, **C**, and **E** were electronically enlarged to demonstrate tubulin morphology. *Arrowheads* in panels **B**, **C**, **E**, and **F** indicate altered microtubule morphology.
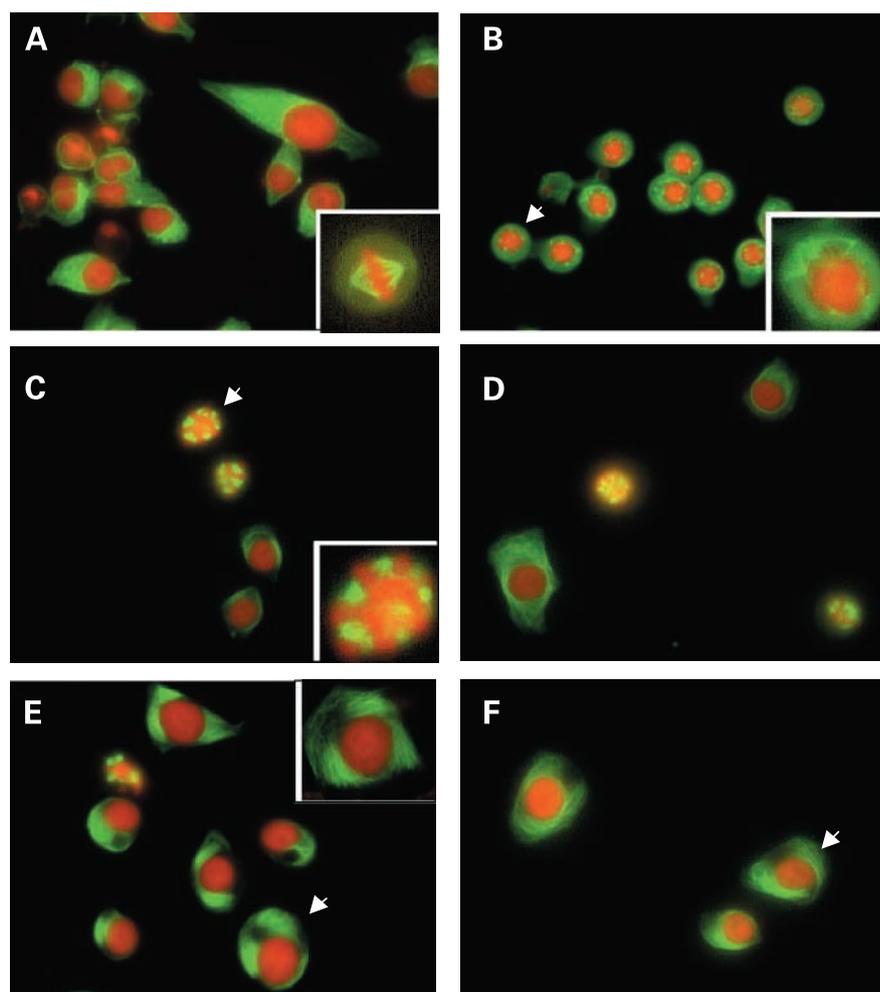
a P-glycoprotein-specific inhibitor, CL-329,753 (Table 2) that has been previously described (25). Consistent with this observation, only KB-V1 cells had low drug accumulation of radiolabeled MAC-321 compared with KB-3-1 cells, while both KB-8-5 and KB-V1 cells had low drug accumulation of radiolabeled PTX (Fig. 5).

Resistance to MAC-321 in cells selected for MRP1 (21) or MXR (22) overexpression was also investigated. No resistance to MAC-321 was found in cells selected for MRP1 overexpression (IC$_{50}$ in HL-60 and HL-60/ADR cells was 0.79 ± 0.01 and 0.89 ± 0.01 nM, respectively) or in MXR (IC$_{50}$ in S1 and S1-M1-3.2 cells was 33.9 ± 7.5 and 20.9 ± 3.3 nM, respectively). In contrast, these cell lines were highly resistant to both doxorubicin (HL-60/ADR cells: >124-fold and S1-M1-3.2 cells: 52-fold) and mitoxantrone (HL-60/ADR cells: 32-fold and S1-M1-3.2 cells: 535-fold).

Less Resistance to MAC-321 in Cells with β-Tubulin Mutations

In cell culture, resistance to PTX and another tubulin-polymerizing agent, Epo, has been associated with tubulin mutations (16–18). EpoA and EpoB promote microtubule polymerization (26, 27) and bind to a similar site in tubulin

compared with PTX and DTX (9). Therefore, we determined whether MAC-321 could overcome this mode of resistance by using a 1A9 human ovarian cell line selected in PTX plus the MDR1 reversal agent verapamil (PTX10, PTX22) or selected in the presence of 8 nM EpoA (EpoA8) (16, 17). Additional comparisons were done with an A549 lung carcinoma selected for resistance to EpoB (A549.EpoB40) (18). These resistant cell lines express β-tubulin containing distinct point mutations in the taxane- or Epo-binding sites and have been previously reported as: PTX10 ($270^{Phe \rightarrow Val}$), PTX22 ($364^{Ala \rightarrow Thr}$), EpoA8 ($274^{Thr \rightarrow Ile}$), and EpoB40 ($292^{Gln \rightarrow Glu}$) (16–18). However, they do not overexpress P-glycoprotein.

In all four tubulin-mutant lines tested, resistance to PTX ranged from 4- to 21-fold while MAC-321 displayed lower levels of cross-resistance (1.6- to 10.7-fold) (Table 3). Comparable levels of cross-resistance were observed between MAC-321 and DTX in the 1A9/PTX- and EpoA-tubulin mutant lines while lower resistance was detected in the A549.EpoB line (Table 3). The data suggest that MAC-321 may interact in a similar but distinct taxane-binding domain of β-tubulin compared with other agents. In contrast to tubulin polymerizing agents, no cross-resistance

Table 1. Cytotoxicity profile of MAC-321 in a panel of PTX-sensitive and -resistant tumor cell lines[a]

Cell Line	Tumor Origin	P-glycoprotein Expression[b]	IC$_{50}$ (nM)			
			MAC-321	PTX	DTX	Vinblastine
A549	NSCLC	0	1.2 ± 0.7	7.5 ± 1.8	1.9 ± 0.7	3.7 ± 0.9
HCT-116	colon	0	3.1 ± 0.7	8.2 ± 1.4	5.4 ± 0.8	2.3 ± 1.5
NCI-H838	NSCLC	0	3.3 ± 0.3	2.1 ± 0.9	6.3 ± 0.7	0.9 ± 0.6
KB-3-1	epidermoid	0	1.3 ± 0.5	3.9 ± 1.3	1.1 ± 0.6	0.8 ± 0.4
1A9 (A2780 subclone)	ovarian	0	1.7 ± 0.6	5.1 ± 0.9	1.9 ± 1.0	1.9 ± 0.2
Average ± SD			2.1 ± 1.00	5.4 ± 2.50	3.3 ± 2.40	1.9 ± 1.20
MX-1W	breast	+	2.6 ± 1.4	15.4 ± 2.9	5.78 ± 2.37	n.d.
NCI-H1299	NSCLC	++	5.3 ± 1.8	37.7 ± 2.3	12.9 ± 0.98	6.33 ± 1.24
DLD-1	colon	+++	1.2 ± 0.9	32.5 ± 2.4	14.29 ± 1.18	15.38 ± 0.62
HCT-15	colon	++++	3.3 ± 0.2	438.6 ± 1.9	54.62 ± 0.19	15.81 ± 1.97
Average ± SD			3.1 ± 1.70	131.0 ± 205.30	21.9 ± 22.10	9.4 ± 7.75
COLO 205	colon	n.d.	0.6 ± 0.1	7.0 ± 2.3	0.6 ± 0.2	1.3 ± 1.0
LOX	melanoma	n.d.	4.3 ± 2.4	17.4 ± 1.4	6.9 ± 0.6	2.8 ± 1.7
Panc-1	pancreatic	n.d.	1.2 ± 0.9	6.2 ± 1.7	1.9 ± 1.2	n.d.
AsPc-1	pancreatic	n.d.	1.2 ± 0.3	2.4 ± 0.3	1.9 ± 0.7	n.d.
Capan-1	pancreatic	n.d.	1.2 ± 1.0	2.6 ± 0.8	1.5 ± 0.9	n.d.
Average ± SD			2.7 ± 1.50	7.1 ± 6.10	2.6 ± 2.50	0.8 ± 1.20

[a]Cell toxicity was measured using the SRB protein stain as an assessment of protein content and survival after a 72-h incubation period with MAC-321, PTX, DTX, or vinblastine as described in "Materials and Methods." IC$_{50}$ ± SD values are representative of three independent experiments.
[b]P-glycoprotein expression was determined by Western blotting and quantitative real-time RT-PCR using MDR-specific antibodies and probes, respectively, as described in "Materials and Methods." Scoring code based on Western blotting: 0, not detected; + to ++++, low to high expression; n.d., not determined.

was observed for vinblastine or for dolastatin-10, which presumably bind to the *Vinca* and *Vinca*-peptide binding domains of tubulin, respectively (data not shown). The binding domain for these agents is believed to be distinct from the taxane pharmacophore based on pharmacological and biochemical data (9).

MAC-321 Is Highly Efficacious When Administered as a Single Intravenous or Oral Dose in PTX-Sensitive Tumor Xenografts *in Vivo*

The activity of MAC-321 was assessed in several nude mouse xenograft models that are known to be sensitive or resistant to treatment with PTX and DTX. The first two experiments were performed using the PTX-sensitive Lox melanoma xenograft model. We have previously established that this tumor model is highly responsive to PTX such that greater than 95% inhibition of tumor growth is sustained between 7 and 30 days after administration of 60 mg/kg PTX given on days 1, 5, and 9 (23). Animals bearing small established Lox melanoma xenografts were treated with 10–70 mg/kg MAC-321 administered as a single i.v. dose on day 1 (defined heretofore as the day after tumor weight of approximately 100 mg was achieved) (Fig. 6A). Similarly in a second experiment, animals were treated with 10–200 mg/kg MAC-321 administered as a single oral dose on day 1 (Fig. 6B).

As noted in Fig. 6A, a clear dose response was observed in the animals receiving MAC-321 by the i.v. route of administration. In this experiment, as low as 10 mg/kg MAC-321 given i.v. was capable of significantly inhibiting tumor growth 6 days after administration. Moreover,

increasing the dose of MAC-321 extended the duration of growth inhibition. Tumors were not detected in nine of nine animals 30 days after administration of 70 mg/kg
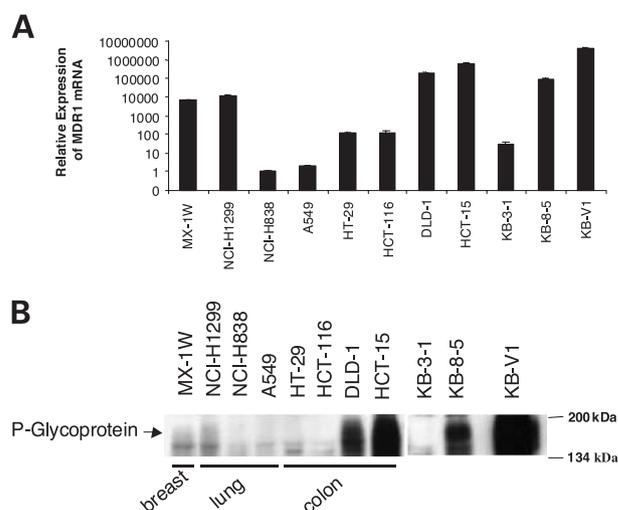
Figure 4. Expression of MDR1 mRNA and protein in a panel of tumor cell lines. RNA (**A**) or membrane proteins (**B**) was prepared from non-selected breast, lung, colon, or ovarian cell lines or from parental (*KB-3-1*) cell lines selected for resistance to colchicine (*KB-8-5*) or vinblastine (*KB-V1*). **A,** levels of MDR1 mRNA were assessed by real-time RT-PCR. *Columns,* mean relative expression from two experiments of MDR1 mRNA normalized to β-actin mRNA as described in "Materials and Methods"; *bars,* SD. Expression is represented on a logarithmic scale with the lowest expressing cell line, NCI-H838, set at 1. **B,** P-glycoprotein was detected by immunoblotting methods using an anti-MDR1 antibody.

Table 2. Resensitization of MAC-321 in PTX-resistant cell lines with MDR inhibitors[a]

Compound	IC$_{50}$ (nM)		
	KB-3-1	KB-8-5	KB-V1
PTX	2.2 ± 0.3	41.9 ± 5.5	3100.0 ± 141.4
PTX + CL-329,753	2.2 ± 0.1	1.3 ± 0.7	6.0 ± 1.4
MAC-321	0.6 ± 0.04	0.8 ± 0.1	64.6 ± 2.1
MAC-321 + CL-329,753	0.5 ± 0.1	0.5 ± 0.1	0.8 ± 0.1

[a]PTX-resistant KB-8-5 and KB-V1 cells and their KB-3-1 parental counterpart lines were grown in the presence of PTX or MAC-321 alone or in combination with 5 μM of the MDR reversal agent, CL-329,753 for 3 days. Growth was assessed by SRB staining as described in "Materials and Methods." Data are reported as average IC$_{50}$ ± SD for three experiments as described above.

MAC-321 when given as a single i.v. dose. Consistent with previously published data (23), PTX was also highly effective. When given 60 mg/kg PTX administered on days 1, 5, and 9, growth of LOX tumors was inhibited greater than 95% up to day 35 and approximately 60% of the animals did not have detectable tumors at this time (data not shown). Little or no weight loss was observed at all doses of MAC-321 except for 70 mg/kg where a 9% decrease in weight was detected. However, lethality with MAC-321 can be obtained when given i.v. at the maximum tolerated dose of 140 mg/kg in the Lox xenograft models. In addition, Lox tumors weighing approximately 1 g before treatment with 70 mg/kg MAC-321 decreased by 80% 3 weeks after initial dosing (data not shown).

MAC-321 was also highly efficacious in the Lox melanoma model when given p.o. (Fig. 6B). However, a higher amount of drug was needed to achieve the same results obtained with i.v. administration. The minimum efficacious dose of MAC-321 was 20 mg/kg while a dose of 200 mg/kg was needed to completely inhibit tumor growth in four of five animals (single death unrelated to drug). As in the i.v. dosing, little or no weight loss was observed in any of the p.o. dose groups. The greatest amount of weight loss, 9%, was observed 4 days after administration of 200 mg/kg MAC-321. However, in this group, the weight of the animals recovered 9 days after drug administration.

Two additional xenograft models that are known to be sensitive to treatment with PTX were used to confirm the activity of MAC-321 *in vivo* (Fig. 6, C and D). Tumors were staged and animals were treated with vehicle, 70 mg/kg MAC-321 i.v., or 70 mg/kg MAC-321 p.o. on day 1. In the KB-3-1 tumor model, comparisons were made with a single 60 mg/kg dose of PTX given i.v. (administered on day 1). Alternatively, 20 mg/kg PTX, given i.v., was dosed on days 1, 5, 9, and 13 (Fig. 6C). In the A375SM tumor model, comparisons were made with 60 mg/kg PTX, given i.v. on days 1, 5, and 9 (Fig. 6D). Tumors responded well to all agents. Minimal (KB-3-1) or no (A375SM) tumor growth was observed after 30 days when 70 mg/kg MAC-321 was administered as a single i.v. dose. Greater than 90% inhibition was observed by day 15 when PTX was given on any schedule in the KB-3-1 and A375SM models.

However, tumor growth resumed 15 days after a single dose of 60 mg/kg PTX in KB-3-1 or after the last dose of PTX in A375SM xenograft models (Fig. 6, C and D). Thus, a single i.v. dose of MAC-321 eliminates tumor growth in xenograft models that require multiple doses of PTX or DTX to achieve the same effect. MAC-321 also has oral activity in both the KB-3-1 and A375SM model. Pronounced activity was observed in the A375SM model as no tumors were observed in 10 of 10 animals treated with oral MAC-321 (Fig. 6D).

We have also observed that in MX-1 PTX-sensitive tumors, 25 mg/kg of DTX (q4d × 3) and 70 mg/kg MAC-321 (qd × 1) administered i.v. have comparable activity because cures was evident for both drugs (data not shown). Similar to i.v. dosing of PTX, p.o. administration of MAC-321 also resulted in greater than 90% tumor growth inhibition.

MAC-321 Has Superior Activity in PTX/DTX-Resistant Xenograft Animal Models

Experiments were performed in xenograft models that were inherently resistant to treatment with PTX and DTX, such as the colon carcinoma cell line DLD-1. As shown above, this cell line overexpressed MDR1 to equivalent levels found in KB-8-5 cells (Fig. 4) and was approximately 6-fold resistant to DTX and PTX compared with the five P-glycoprotein-negative cell lines described in Table 1. In tumors derived from DLD-1, 25 mg/kg DTX given i.v. on days 1, 5, and 9 did not inhibit the growth of tumors (Fig. 7A). The latter observation was significant because DTX given in the same manner was highly efficacious in other tumor models (data not shown) and is in accordance with previous studies (28). Moreover, we have previously reported that 60 mg/kg PTX given on the same schedule did not significantly inhibit tumor growth (23). The results were markedly different for MAC-321 because tumor growth was inhibited by greater than 95% with a single i.v. dose of 60 mg/kg (Fig. 7A). The minimum efficacious dose of MAC-321 was 20 mg/kg in the DLD-1 model compared with 10 mg/kg in the Lox melanoma model.
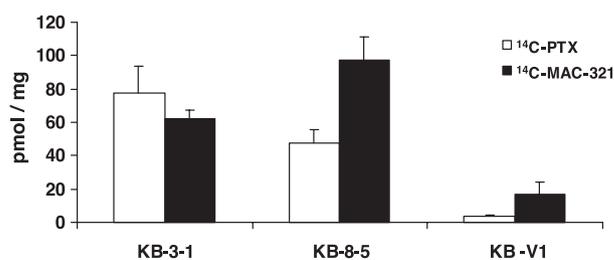

Figure 5. Accumulation of MAC-321 and PTX in KB-3-1 cells. The intracellular accumulation of MAC-321 in KB-3-1, KB-8-5, and KB-V1 cells was performed as described in "Materials and Methods." Cells were incubated with [^{14}C]-MAC-321 (104 mCi/mmol) and [^{14}C]-PTX (74 mCi/mmol) at a final concentration of 500 nM (0.051 and 0.039 μCi/ml, respectively) for 2 h at 37°C. Radioactivity in the cell lysates was determined by liquid scintillation counting and was normalized for protein content. Data are reported as mean pmol drug/mg of total protein from triplicate wells.

Table 3. Cytotoxicity profile of MAC-321 *versus* standard chemotherapeutics in PTX- and Epo-resistant cell lines that contain mutations in the taxane-binding site of β-tubulin[a]

Compound	IC$_{50}$ (nM)*				Relative Resistance[b]		
	1A9	1A9 PTX10	1A9 PTX22	1A9 EpoA8	1A9 PTX10	1A9 PTX22	EpoA8
MAC -321	1.6 ± 0.6	5.4 ± 2.7	8 ± 1.4	2.6 ± 1.6	3.4	5.0	1.6
PTX	5.1 ± 1.0	26.6 ± 16.3	89.2 ± 55.6	20.9 ± 10.8	5.2	17.5	4.1
DTX	1.8 ± 0.6	7.5 ± 0.3	7.9 ± 0.2	2.2 ± 1.2	4.2	4.4	1.2
EpoA	1.8 ± 0.5	16.0 ± 49.0	4.1 ± 3.3	31.8 ± 14.0	8.9	2.3	17.7

Compound	IC$_{50}$ (nM)**		Relative Resistance[b]
	A549	A549.EpoB40	A549.EpoB40
MAC-321	1.1 ± 0.6	11.3 ± 1.2	10.7
PTX	7.5 ± 1.3	156.9 ± 16.9	20.8
DTX	1.9 ± 0.7	31.8 ± 1.6	16.6
EpoB	1.0 ± 0.1	91.5 ± 3.9	95.3

[a]Parental cells and their resistant cell counterpart were grown in the presence of the indicated agents for 3 days and growth assessed by SRB staining (*) or ATP-binding (**) as described in "Materials and Methods." Data are reported as average IC$_{50}$ ± SD.
[b]Average relative resistance is defined as the ratio of the IC$_{50}$ of the resistant cell model to that of the respective sensitive/parental cell counterpart.

The oral activity of MAC-321 was also explored in the DLD-1 model. Animals were treated with vehicle or 50–400 mg/kg MAC-321 administered as a single oral dose on the day after staging (Fig. 7B). The minimum efficacious dose of MAC-321 was 100 mg/kg. However, no clear dose-response relationship was obtained. Complete inhibition of tumor growth was achieved within 7 days after administration at doses of 100–400 mg/kg, but tumor growth resumed after day 7. Furthermore, there was no difference in tumor growth inhibition between the doses of 100 and 400 mg/kg. This may be explained by the lack of solubility at high doses because the solutions appeared turbid when prepared at concentrations above 70 mg/kg of MAC-321 and no weight loss was noted at the highest dose tested.

To further explore the utility of MAC-321 in PTX-resistant models, the MDR1-positive cervical carcinoma cell line KB-8-5, which was approximately 19-fold resistant to PTX or DTX, was used (Table 2). This level of resistance *in vitro* translates to resistance in animals as well (Fig. 7C). KB-8-5 xenografts were treated with either vehicle, 70 mg/kg MAC-321 i.v., or 70 mg/kg MAC-321 p.o. given the day after
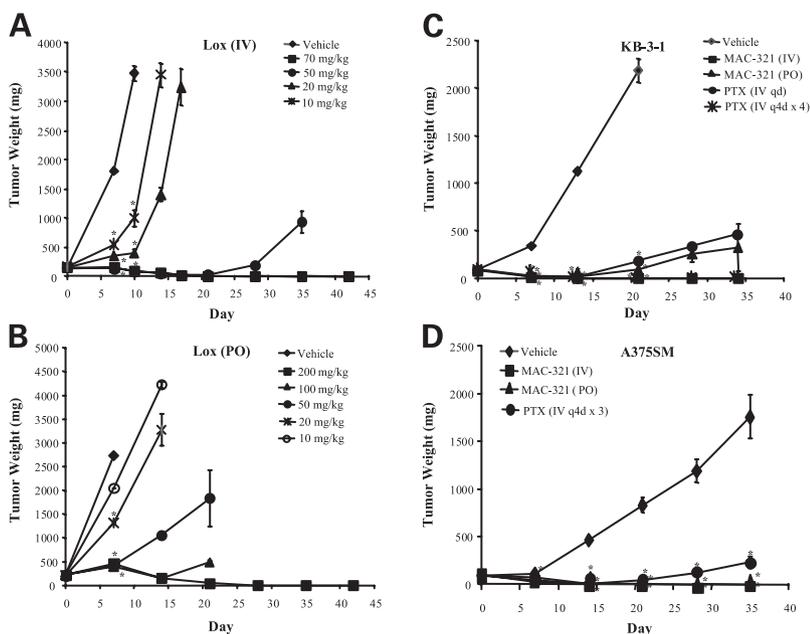

Figure 6. Efficacy of MAC-321 in PTX-sensitive xenograft tumor models *in vivo* by i.v. and p.o. administration. Female nu/nu mice were given injections of 1.5 × 10^6 Lox melanoma cells, 5 × 10^6 KB-3-1 cells, or 8 × 10^6 A375SM melanoma cells. Animals bearing Lox melanoma tumors approximately 100 mg in size were treated i.v. with vehicle or 10–70 mg/kg MAC-321 (**A**) or 10–200 mg/kg p.o. (**B**) on day 1. Tumor growth in all cases was determined approximately every 7 days. **C** and **D,** animals bearing KB-3-1 or A375SM staged tumors were treated with vehicle, 70 mg/kg MAC-321 p.o., or 70 mg/kg MAC-321 i.v. on day 1. In KB-3-1 xenografts, PTX was given i.v. at 60 mg/kg on day 1 or 20 mg/kg on days 1, 5, 9, and 13 (**C**). In A375SM xenografts, PTX was dosed i.v. at 60 mg/kg on days 1, 5, and 9 only (**D**). Statistical significance was determined by Student's two-tailed *t* test for all doses and test agents compared with the vehicle control group. *, $P \leq 0.01$

Figure 7. Activity of MAC-321 (i.v. and p.o.) in PTX-resistant xenograft tumor models *in vivo*. Female nu/nu mice were given injections of 6 × 10^6 DLD-1 colon carcinoma cells, 5 × 10^6 KB-8-5 cells, or five fragments of MX-1W breast carcinoma. Animals bearing DLD-1 staged tumors were treated i.v. (**A**) or p.o. (**B**) with vehicle or at the doses of MAC-321 indicated on the day after reaching approximately 100 mg in size. In addition, DLD-1 xenografts were also treated i.v. with 25 mg/kg of DTX on days 1, 5, and 9. Animals bearing KB-8-5 (**C**) or MX-1W (**D**) staged tumors were treated with either vehicle, 70 mg/kg MAC-321 p.o., or 70 mg/kg MAC-321 i.v. on day 1. Sixty milligrams per kilogram PTX were administered i.v. on days 1, 5, and 9 (**C** and **D**). *, $P \leq 0.01$ and #, $P \leq 0.02$ as determined by Student's two-tailed t test.
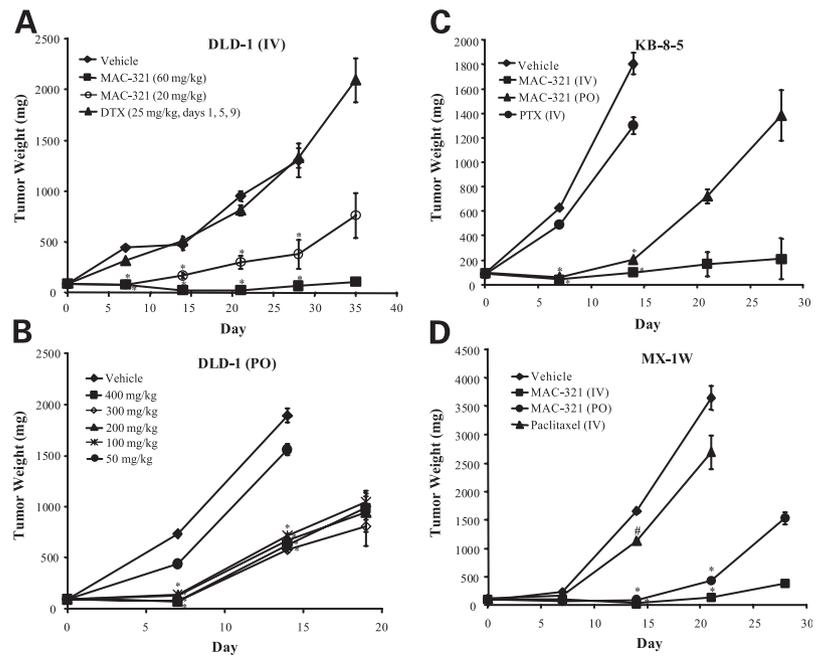
staging (single dose) or 60 mg/kg PTX given on days 1, 5, and 9 (Fig. 7C). The growth of tumors treated with PTX was not inhibited on this schedule. However, a single dose of MAC-321 significantly inhibited tumor growth when administered either i.v. or p.o. (Fig. 7C). As noted in the PTX-sensitive tumor models, i.v. administration of MAC-321 resulted in better tumor growth inhibition compared with oral administration (90% *versus* 45%, respectively, by day 21). Tumors remained detectable after both routes of delivery and regrowth of tumors occurred after oral administration.

Similar results were obtained using the human breast carcinoma cell line MX-1W (a variant of the MX-1 cell line obtained from the National Cancer Institute) which had lower levels of MDR-1 overexpression compared with KB-8-5 cells (Fig. 4). As a result, approximately 3-fold less PTX was needed to inhibit the growth of MX-1W cells compared with KB-8-5 cells in tissue culture (15.4 ± 2.9 nM *versus* 41.9 ± 5.5 nM, respectively). While tumors derived from the original MX-1 cell line were sensitive to treatment with PTX (data not shown) and consistent with previous reports (29), the MX-1W cells were resistant to treatment with PTX *in vivo* (Fig. 7D). Conversely, MAC-321 given by the i.v. or p.o. route was highly effective in MX-1W tumors with a response profile that was comparable to KB-8-5 tumors.

While MAC-321 markedly inhibited the growth of most tumors that were resistant to PTX *in vivo*, the HCT-15 xenograft model was exceptional, because 70 mg/kg MAC-321 administered as a single i.v. dose were only partially effective (Fig. 8). Indeed, in tissue culture, these colon carcinoma cells were inherently resistant to both PTX and DTX due to a very high level of overexpression of MDR1 (Fig. 4 and Table 1) and may account for decreased efficacy of MAC-321. Moreover, it has been previously shown that

HCT-15 tumors are completely resistant to PTX (23). To explore the utility of multi-dose regimens, 20 mg/kg MAC-321 were given i.v. on day 1 or on days 1, 7, and 14. (Lower doses of MAC-321 were required on the multi-dose schedule, because repeated high doses of 70 mg/kg were toxic.) While a single 20 mg/kg dose of MAC-321 was ineffective, multi-dosing resulted in greater than 90% inhibition (Fig. 8). The latter regimen resulted in a transient 7% weight loss.

Discussion

PTX and DTX are novel anticancer cytotoxic agents that block microtubule-mediated chromosome separation by
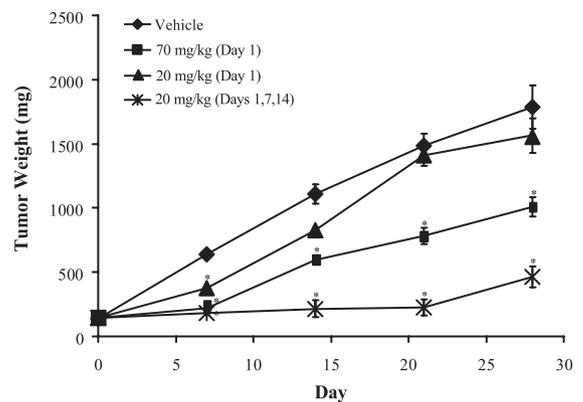
Figure 8. Multiple-dose scheduling of MAC-321 in PTX-resistant HCT-15 colon tumor xenografts. Female nu/nu mice were given injections of 7 × 10^6 HCT-15 colon carcinoma cells and animals bearing staged tumors were treated with vehicle or MAC-321 i.v. on day 1 or on days 1, 7, and 14 at the doses indicated. Tumor growth was measured weekly up to 21 days. *, $P \leq 0.02$ as determined by Student's two-tailed t test.

promoting the assembly of tubulin dimers and stabilizing microtubules (2). Despite wide use in the clinic, those patients that initially respond to these taxanes frequently develop resistance thereby minimizing therapeutic efficacy. Moreover, some tumors that typically have endogenous P-glycoprotein expression, such as colon carcinomas (30), are not responsive to either agent. In addition to these limitations, the compounds are poorly soluble, require the use of toxic vehicles, and induce numerous adverse reactions including profound neutropenia and leukopenia (both agents) or fluid retention (unique to DTX). Thus, new taxanes that overcome many of the adverse effects of PTX and DTX without compromising superior efficacy are being sought.

MAC-321 is a DTX analogue that shares some biochemical properties with PTX and DTX, because all these agents induce polymerization of tubulin in a cell free system and induce bundling of microtubules in tumor cells. In addition, MAC-321, DTX, and PTX inhibit cell cycle progression in the G_2-M phase, resulting in apoptosis and cytotoxicity.[1] However, MAC-321 has a number of unique preclinical pharmacological properties that are superior to PTX or DTX.

The first advantage of MAC-321 compared with PTX or DTX is its ability to overcome resistance to either agent in tissue culture or animal models. The basis for this resistance is due, in part, to a weak interaction of MAC-321 with P-glycoprotein compared with DTX or PTX. In support of this finding, most tumor cells that overexpress P-glycoprotein (either inherently or acquired as a result of selective pressure by certain chemotherapeutic agents such as colchicine or vinblastine) retain sensitivity to MAC-321 compared with PTX or DTX. However, MAC-321 is still likely to be substrate for P-glycoprotein, because KB-V1 cells, which were approximately 1400-fold resistant to PTX and highly overexpress P-glycoprotein, were also 80-fold resistant to MAC-321. Indeed, the hypothesis that MAC-321 is an MDR1 substrate is further corroborated by noting that resistance in KB-V1 cells is reversible with an MDR1 inhibitor and low drug accumulation occurs in the presence of both agents. However, resistance in cells such as KB-V1 that express very high levels of MDR is not typically observed clinically (30) and thus may not be of therapeutic relevance. Therefore, the lack of resistance to MAC-321 *in vitro* or *in vivo* in cell lines such as KB-8-5, DLD-1, or HCT-15 that overexpress levels of P-glycoprotein in a clinically relevant range suggests that MAC-321 may have utility in patients who have failed previous taxane therapy due to P-glycoprotein overexpression. However, because P-glycoprotein is present and functional in normal tissues (15), including progenitor cells of the hematopoietic system (31) and endothelial cells within the blood-brain barrier (15), it remains to be determined if enhanced efficacy of MAC-321 in a P-glycoprotein-expressing tumor will also be associated with increased toxicity in humans.

Resistance to anti-microtubule agents can also be mediated by altered expression of tubulin and/or MAPs as well as tubulin mutations (14). In recent years, several PTX- and DTX-resistant tumor cell lines harboring single-point mutations resulting in amino acid substitutions in β-tubulin have been identified. Interestingly, less MAC-321 cross-resistance was observed in tumor lines that were either resistant to PTX (16, 17) or Epos (17, 18) and contain β-tubulin mutations with no elevation in MDR1. This might be expected because resistance to the reference agent used to derive the mutant cell line would be greater than the test agent such as MAC-321. This suggests that the binding site for MAC-321 within β-tubulin may be similar but distinct compared with PTX and Epos. The clinical relevance of MAC-321 activity in PTX- and DTX-resistant models, while suggestive of an improved taxane, is not entirely clear because, experimentally, resistance has been attributed to a variety of mechanisms such as MDR overexpression, modifications in the apoptosis cascade, or tubulin mutations. The contribution of each of these drug resistance mechanisms to the clinical response to taxanes is either controversial (*e.g.*, MDR1) or has not been substantiated (*i.e.*, tubulin mutations or apoptotic mechanisms) (15, 32).

The second advantage of MAC-321 lies in its ability to be formulated in a vehicle that is not expected to induce a hypersensitivity reaction. Previous taxanes have been administered in polyoxyl 35 castor oil (Cremophor EL) or polysorbate 80 (Tween 80). In both cases, patients must be pre-medicated to avoid a hyperallergenic response that has been attributed to the vehicle rather than the taxane (33, 34). Because the experimental vehicle used for i.v. administration of MAC-321 does not contain polyoxyl 35 castor oil or polysorbate 80, it may not induce a hypersensitive response in patients. Furthermore, MAC-321, unlike PTX, is highly soluble in ethanol (approximately 275 mg/ml *versus* 39 mg/ml, respectively), thus eliminating the need for polyoxyl 35 castor oil or polysorbate 80. However, it is important to note that MAC-321 is equally efficacious in polyoxyl 35 castor oil. Therefore, it is likely that the superior activity observed for MAC-321 is due to the compound and not the formulation.

The third advantage of MAC-321 is that it is effective when administered p.o. Previously, it has been shown that PTX and DTX are ineffective when given p.o. and both agents have poor bioavailability (35–37). Because PTX and DTX are excellent substrates for MDR1 (10, 14, 15), this effect is likely due to high levels of MDR1 that are present in the gastrointestinal tract (38, 39). Consistent with this hypothesis, the oral bioavailability of PTX is improved in $MDR^{-/-}$ mice as well as in mice and patients co-administered a MDR inhibitor (40, 41). The efficacy of p.o. administered MAC-321 is comparable to i.v. administered PTX in sensitive tumor models when given as a single dose. However, less efficacy of oral MAC-321 in PTX-resistant models may be due to P-glycoprotein expression in both the tumor and gastrointestinal tract. The efficacy and tolerability of oral MAC-321 makes it feasible to consider daily low-dose therapy as a viable alternative to a high-dose infrequent therapy. This

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[1] J. E. Kesterson and R. E. Longley, unpublished observations.

metronomic approach, which produces little side effects but is highly efficacious with other agents (42), may be effective with MAC-321 as well. In addition, it would make an alternative regimen amendable to testing in combination with other standard therapies or those based on signal transduction inhibitors (*i.e.*, estrogen or epidermal growth factor receptor inhibitors) where oral dosing is efficacious.

A fourth advantage of MAC-321 may involve the dosing schedule. In our experiments, a single dose of MAC-321 can be highly effective, and therefore, dividing the dose was not usually necessary. This is similar to DTX where an equivalent total dose of the compound has been reported to be equally efficacious when given on an intermittent schedule (days 1 and 6 or days 1, 5, 9, and 13) or 3 times a day for 5 days (36). By comparison, PTX often requires repetitive dosing (*i.e.*, daily doses on days 1 through 5 or days 1, 5, and 9) to demonstrate efficacy in numerous models (29). Multiple dose therapy with MAC-321 may still be appropriate in PTX-resistant models where a single dose of MAC-321 is only partially effective.

Many investigators have identified novel naturally derived and synthetic anti-microtubule agents that overcome various modes of resistance to PTX and DTX *in vitro* and *in vivo* (24, 43). Novel non-taxane agents that stabilize microtubules include Epos, eleutherobin, sarcodictyins, discodermolides, and laulimalide (27, 44–47). Other taxanes that have similar potency to MAC-321 in cell culture, overcome MDR1-mediated resistance, and are active by i.v. administration in animal models include IDN 5109 (48, 49), BMS-275183 (50), BMS-185660 (35), RPR109881A (51), and Epo analogues such as BMS-247550 (52). Presently, these agents are in Phases 0–III for clinical evaluations. Further comparisons between these new chemical entities in patients will be warranted to clearly distinguish the efficacy of these next-generation anti-microtubule agents.

In conclusion, MAC-321 is a novel taxane that can be distinguished from taxanes currently used to treat cancer because it overcomes PTX and DTX resistance mediated by P-glycoprotein. The fact that MAC-321 can be administered p.o., as well as i.v. in a non-Cremophor EL vehicle, may provide substantial benefits for a wide variety of patients including those whose cancer are refractory to the currently used taxanes or who have developed resistance to such agents. On the basis of these results, MAC-321 is being evaluated in Phase II clinical trials for the treatment of cancer in humans.

Acknowledgments

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