Anti-Cancer Drugs 1999, **10**, pp. 655–661

Preclinical study

Comparison of DX-8951f and topotecan effects on tumor colony formation from freshly explanted adult and pediatric human tumor cells

Richard A Lawrence, Elzbieta Izbicka, Robert L De Jager,[1] Akiko Tohgo,[2] Gary M Clark,[3] Steven D Weitman, Eric K Rowinsky and Daniel D Von Hoff

CTRC Research Foundation, Institute for Drug Development, San Antonio, TX 78245, USA. [1]Daiichi Pharmaceutical Corp, Fort Lee, NJ 07024, USA. [2]Daiichi Pharmaceutical Corp, 16-13 Kitakasai 1-Chome Edogawa-Ku, Tokyo 134, Japan. [3]University of Texas Health Science Center at San Antonio, San Antonio, TX 78284, USA.

DX-8951f, which shows great therapeutic potential, was tested in the human tumor cloning system in adult and pediatric tumor types against which topotecan has been active. In 47 tumors from adults, DX-8951f had definite cytotoxic activity in a concentration-dependent manner with both 1 h and continuous exposures. Topotecan was minimally effective using a 1 h exposure and showed concentration-dependent inhibition with continuous exposure. In head-to-head comparisons at 1 h exposure against adult tumors, DX-8951f was significantly more effective at 0.1 and 1.0 μg/ml than topotecan. In head-to-head comparisons (continuous exposure), 1.0 μg/ml DX-8951f was more effective than topotecan at 1.0 μg/ml in adult tumors, including three of four head and neck, one of two kidney, two of five liver, six of 10 non-small cell lung, five of eight ovarian, four of eight prostate tumors, and in single specimens of breast, mesothelioma, colon and small cell lung tumors. With continuous exposure, DX-8951f and topotecan were equally effective at equimolar concentrations. The maximum tolerated dose for DX-8951f is 3 times that of topotecan, so higher doses of DX-8951f could be administered to patients. DX-8951f is a promising new antineoplastic agent with significant activity against tumors taken directly from patients. [© 1999 Lippincott Williams & Wilkins.]

Key words: Camptothecin, DX-8951f, human tumor cloning, topoisomerase I inhibitors, topotecan.

Introduction

DNA topoisomerases assist in cell division by altering DNA topology to permit DNA synthesis, recombination and transcription. Type I topoisomerase generates transient single-strand DNA breaks and relieves torsional stress by untwisting the DNA helix. Type II topoisomerase introduces transient double-strand DNA breaks and can resolve DNA molecule tangles, as well as untwist the helix.[1] Inhibition of topoisomerases has been shown to arrest cells in G_2/M and to induce apoptosis.[2–5] Since rapidly proliferating cells require topoisomerase activity, topoisomerase inhibitors have been developed and tested for antitumor activity. Inhibition of topoisomerases has been shown to induce differentiation in human leukemia cell lines, and to induce apoptosis with DNA cleavage in thymocytes, promyelocytic cells, breast cancer cells and lung cancer cells.[2–5] One such inhibitor is camptothecin. Derivatives of this compound are currently in clinical trials.

DX-8951f, a water-soluble derivative of camptothecin, has shown *in vitro* activity against malignant cell lines as well as against human tumor xenografts, gastric adenocarcinoma SC-6 and human breast cancer MDA-MB-231. The *in vitro* data, combined with greater *in vivo* activity of DX-8951f versus CPT-11 (another camptothecin derivative), suggested that DX-8951f has great potential as a therapeutic agent.[6,7]

The purpose of this study was to further investigate the activity of DX-8951f against tumor types for which topotecan, another water-soluble derivative of camptothecin with a similar mechanism of action, has been shown to be active. DX-8951f and topotecan were tested head to head against adult and pediatric tumors so that direct comparisons could be made.

Through the initial work of Hamburger and Salmon, it is now possible to culture human tumors using a

Correspondence to Elzbieta Izbicka, Institute for Drug Development, CTRC Research Foundation, 7979 Wurzbach Road, Suite 337, San Antonio, TX 78229, USA.
Tel: (+1) 210 616-5892; Fax: (+1) 210 616-5948;
E-mail: eizbicka@saci.org

two-layer soft agar[8,9] system. This culture system, called the human tumor cloning system, was initially utilized to select the most appropriate anticancer agent for an individual patient's tumor. The Tucson group demonstrated that if a patient's tumor was sensitive to a drug *in vitro*, the chance of the patient responding clinically to the drug was 81%, while if the patient's tumor was resistant to the drug *in vitro*, the chance of the patient not responding to the drug was 99%.[10] Based on those encouraging results, our laboratory studied *in vitro-in vivo* correlations, retrospectively, in a series of 800 patients. In those 800 patients, we found a positive predictive value (PPV) (i.e. the assay predicted the patient's tumor would respond and their tumor did respond) of 70%, while the negative predictive value (NPV) for the assay was 98%.[11] In a more recent prospective clinical trial of the cloning system involving 604 patients, we found the PPV for the assay was 64%, while the NPV for the assay was 86%.[12] We have reported a clinical trial in which patients were randomized either to an agent selected by the cloning assay or an agent by the clinician caring for the patient. The clinical response rate for patients treated with the cloning assay choice was 21%; while the clinical response rate for patients treated with the clinician's choice was 4%.[13] Thus, *in vitro* predictive assays can improve the response rate of patients.

Based on these encouraging *in vitro-in vivo* correlations, the cloning system was utilized to screen for antitumor activity of new compounds.[14,15] Our laboratory has used this system to screen for activity of new agents and to pinpoint the tumor types, which should receive particular attention in phase II clinical trials with a new agent.[15]

There have been many technical improvements in the cloning assay. These changes have both enabled more patients' tumors to be grown in soft agar and have improved the quality control of the assay.[14] In this study the 35 mm Petri dish system was utilized. The effects of DX-8951f versus topotecan on the formation of human tumor colony-forming units (TCFUs) were assayed by utilizing fresh human tumors taken directly from pediatric and adult patients, and cultured in soft agar.

Materials and methods

Materials

McCoy's 5A medium (Cellgro) and fetal calf serum were purchased from Fisher Scientific (Pittsburgh, PA). CMRL medium, asparagine, glutamine and penicillin/streptomycin were purchased from Gibco (Grand Island, NY). Agar (Difco), horse serum, insulin and HEPES were purchased from Sigma (St Louis, MO). DX-8951f was supplied by Daiichi Pharmaceuticals (Tokyo, Japan) and topotecan was obtained from Smith Kline-Beecham (Philadelphia, PA).

Collection and preparation of tumor cells

After obtaining informed consent in accordance with federal and institutional guidelines, malignant effusions, ascites and bone marrow aspirates containing tumor cells, as well as solid tumor specimens were collected from patients undergoing procedures done as part of their diagnostic workup or as part of treatment for their disease. No procedures were done solely for research purposes. Solid tumors or lymph nodes were minced into 2–5 mm fragments in the operating room and immediately placed in McCoy's 5A medium containing 10% heat-inactivated newborn calf serum, 10 mM HEPES and 1% penicillin/streptomycin. Within 4 h, these solid tumors were mechanically disassociated with scissors, forced through no. 100 stainless steel mesh, through 25 gauge needles, and then washed with McCoy's medium containing horse serum (5%), fetal calf serum (10%), sodium pyruvate (2 mM), glutamine (2 mM), penicillin (90 U/ml), streptomycin (90 μg/ml) and L-serine (35 μg/ml) as previously described.[8–13] Ascitic, pleural and pericardial fluids, and bone marrows were obtained by standard techniques. The fluid or marrow was placed in sterile containers containing 10 U of preservative-free heparin per ml of malignant fluid or marrow. After centrifugation at 150 *g* for 10 min, the cells were harvested and washed with McCoy's plus 10% heat-inactivated fetal calf serum. The viability of cell suspensions was determined on a hemacytometer with Trypan blue.

DX-8951f and topotecan preparations

DX-8951f and topotecan were diluted in sterile distilled water to create stock concentrations of 20 μg/ml. Aliquots of 0.5 ml of each stock solution were labeled and stored at −70°C. Aliquots were thawed for each new tumor sample tested. Sterile distilled water served as a vehicle control. The final concentrations tested were 0.001, 0.1 and 1.0 μg/ml. These concentrations were based on IC_{50} values determined *in vitro* in tumor cell lines.[6] All concentrations of DX-8951f are expressed as those of the monomethanesulfonate dihydrate.

Culture of cells

Cells harvested as described above were suspended in 0.3% agar in enriched CMRL 1066 supplemented with 15% heat-inactivated horse serum, penicillin (100 units/ml), streptomycin (2 mg/ml), glutamine (2 mM), insulin (3 units/ml), asparagine (0.6 mg/ml) and HEPES buffer (2 mM). For the continuous exposure the compound was added to the above mixture. Cells were plated in 35 mm Petri dishes using the two-layer system described by Hamburger and Salmon to prevent growth of fibroblasts with some modifications. Base layers consisted of agar (0.5%) in McCoy's 5A medium supplemented with soy broth (0.6%) and asparagine (100 μg/ml). Cells were plated at a density of 5×10^5/dish in a mixture of 0.3% agar in CMRL 1066 medium containing horse serum (15%), fetal calf serum (2%), vitamin C (5 mg%), penicillin (90 U/ml), streptomycin (90 μg/ml), non-essential amino acids (0.1 mM), glutamine (2 mM), insulin (2 U/ml) and hydrocortisone (4 ng/ml). Immediately before use each bottle of CMRL was further supplemented with asparagine (100 μg/ml) and sodium pyruvate (2 mM). Three plates were prepared for each data point. The plates were placed in a 37°C incubator, removed on day 14 and the number of colonies in each plate was counted. The number of colonies (defined as 50 or more cells) formed in the three compound-treated plates were compared to the number of colonies formed in the three control plates and the number of colonies surviving at each concentration of compound was expressed as a percentage of the number in the vehicle control plates.

For the 1 h exposure studies, the cells were incubated with the compound for 1 h, washed and plated in the top layer of agar just as was done for the continuous exposure studies.

Test definition and quality control measures

A test was defined as an experiment, performed on a unique tumor tissue sample, that included a vehicle control, a positive control (orthosodium vanadate) and three specified compound concentration levels. An evaluable test was a test in which an average of 20 or more colonies were present on day 14 in the vehicle control plates and the number of colonies in the positive control plates was less than 30% of the number in the vehicle control plates.

To assure the presence of an excellent single-cell suspension on the day of plating, positive and untreated controls were used. For each tumor tissue sample tested, three positive control plates were used in which the cells were exposed to the non-specific cellular toxin, orthosodium vanadate, at 200 μg/ml. Three vehicle control plates were also prepared on day 0. On the day of culture reading (day 14) the number of colonies in the positive control plates should be 30% or less of the number of colonies in the vehicle control plates. If there was no effect on colony formation, then the single-cell suspension on day 0 was poor (since orthosodium vanadate does not affect clumps) and the tumor sample test was considered non-evaluable.

Statistics

Dose-response relationships and comparisons between DX-8951f and topotecan require head-to-head comparisons. McNemar's test for head-to-head, paired proportions was used for these comparisons.

Results

A total of 125 specimens were processed. Forty-seven fresh adult and six pediatric tumor specimens were evaluable for the 1 h exposure, whereas 49 adult and seven pediatric tumor specimens were evaluable for the continuous exposure. The remaining specimens were not evaluable because of insufficient growth in the negative control, overgrowth of the colonies preventing accurate counting or contamination in the original sample. Data on non-evaluable specimens was eliminated from the data analysis.

Table 1 summarizes data for evaluable adult specimens tested against DX-8951f. A concentration-response effect of the drug against human TCFUs was indicated by comparing the percentages of colonies responding among successively higher concentrations using McNemar's test (see Table 4A). Tumor types showing the greatest response to DX-8951f included non-small cell lung, head and neck, and liver tumors at a 1 h exposure. In continuous exposure, tumor types with the greatest response to DX-8951f were colon, head and neck, non-small cell lung, ovarian, and prostate tumors.

Table 2 summarizes data for evaluable adult specimens tested against topotecan. With a 1 h exposure, there was no statistically significant concentration-response effect for this drug and topotecan inhibited colony formation in few of the tumors tested. In contrast, a definite concentration-response effect is evident with continuous exposure ($p < 0.05$, McNe-

mar's test). Tumor types showing the greatest effect of topotecan treatment were colon, head and neck, non-small cell lung, ovarian, and pancreatic tumors.

Tables 3-6 compare tumor specific responses of DX-8951f with topotecan in adult tumors at 1 h and continuous exposures, respectively. As shown in

Table 1. *In vitro* responses[a] to DX-8951f for adult tumors in a human tumor cloning system

Tumor type	1 h exposure concentration (μg/ml)			14 day continuous concentration (μg/ml)		
	0.001	0.10	1.00	0.001	0.10	1.00
Bladder	0/1	0/1	0/1	–	–	–
Breast	0/1	0/1	1/1	0/9	2/9	5/9
Colon	0/1	0/1	1/1	0/6	3/6	5/6
Head and neck	0/4	2/4	3/4	0/2	1/2	2/2
Kidney	0/2	0/2	1/2	–	–	–
Liver	0/5	2/5	4/5	–	–	–
Lung (non-small cell)	0/11	2/11	6/11	1/6	2/6	4/6
Lung (small cell)	0/1	1/1	1/1	0/1	0/1	1/1
Mesothelioma	0/1	0/1	1/1	0/1	1/1	1/1
Ovary	0/8	2/8	5/8	2/11	6/11	9/11
Pancreas	0/1	0/1	0/1	0/2	1/2	1/2
Prostate	1/9	2/9	4/9	0/8	2/10	6/8
Stomach	–	–	–	0/1	1/1	1/1
Unknown	1/2	1/2	2/2	–	–	–
	2/47 (4%)	12/47 (26%)	29/47 (62%)	3/47 (6%)	19/49 (39%)	35/47 (74%)

[a]Inhibition=50% or less survival.

Table 2. *In vitro* responses[a] to topotecan (SKF104) for adult tumors in a human tumor cloning system

Tumor type	1 h exposure concentration (μg/ml)			14 day continuous concentration (μg/ml)		
	0.001	0.10	1.00	0.001	0.10	1.00
Bladder	0/1	1/1	1/1	–	–	–
Breast	0/1	0/1	0/1	0/9	2/9	4/9
Colon	0/1	0/1	0/1	0/6	2/6	6/6
Head and neck	1/5	1/5	1/5	0/2	1/2	2/2
Kidney	1/3	1/3	2/3	–	–	–
Liver	0/5	1/5	3/5	–	–	–
Lung (non-small cell)	0/10	0/10	2/10	0/6	2/6	5/6
Lung (small cell)	0/1	0/1	0/1	1/1	1/1	1/1
Mesothelioma	0/1	0/1	0/1	0/1	0/1	1/1
Ovary	0/8	0/8	1/8	0/11	4/11	9/11
Pancreas	0/1	0/1	0/1	0/2	0/2	2/2
Prostate	1/7	1/8	1/8	1/8	2/10	5/8
Stomach	–	–	–	0/1	0/1	0/1
Unknown	0/2	1/2	1/2	–	–	0/1
	3/46 (7%)	6/47 (13%)	12/47 (26%)	2/47 (4%)	14/49 (29%)	35/48 (73%)

[a]Inhibition=50% or less survival.

Table 3. *In vitro* responses[a] to DX-8951f and topotecan for adult tumors in a human tumor cloning system: 1 h exposure

Tumor type	DX-8951f concentration (μg/ml)			Topotecan concentration (μg/ml)		
	0.001	0.10	1.00	0.001	0.10	1.00
Bladder	0/1	0/1	0/1	0/1	1/1	1/1
Breast	0/1	0/1	1/1	0/1	0/1	0/1
Colon	0/1	0/1	1/1	0/1	0/1	0/1
Head and neck	0/4	2/4	3/4	1/5	1/5	1/5
Kidney	0/2	0/2	1/2	1/3	1/3	2/3
Liver	0/5	2/5	4/5	0/5	1/5	3/5
Lung (non-small cell)	0/11	2/11	6/11	0/10	0/10	2/10
Lung (small cell)	0/1	1/1	1/1	0/1	0/1	0/1
Mesothelioma	0/1	0/1	1/1	0/1	0/1	0/1
Ovary	0/8	2/8	5/8	0/8	0/8	1/8
Pancreas	0/1	0/1	0/1	0/1	0/1	0/1
Prostate	1/9	2/9	4/9	1/7	1/8	1/8
Unknown	1/2	1/2	2/2	0/2	1/2	1/2
	2/47 (4%)	12/47 (26%)	29/47 (62%)	3/46 (7%)	6/47 (13%)	12/47 (26%)

[a]Inhibition=50% or less survival.

Table 4. *In vitro* responses[a] to DX-8951f and topotecan for adult tumors in a human tumor cloning system: 14 day continuous exposure

Tumor type	DX-8951f concentration (μg/ml)			Topotecan concentration (μg/ml)		
	0.001	0.10	1.00	0.001	0.10	1.00
Breast	0/9	2/9	5/9	0/9	2/9	4/9
Colon	0/6	3/6	5/6	0/6	2/6	6/6
Head and neck	0/2	1/2	2/2	0/2	1/2	2/2
Lung (non-small cell)	1/6	2/6	4/6	0/6	2/6	5/6
Lung (small cell)	0/1	0/1	1/1	1/1	1/1	1/1
Mesothelioma	0/1	1/1	1/1	0/1	0/1	1/1
Ovary	2/11	6/11	9/11	0/11	4/11	9/11
Pancreas	0/2	1/2	1/2	0/2	0/.2	2/2
Prostate	0/8	2/10	6/8	2/10	2/10	5/8
Stomach	0/1	1/1	1/1	0/1	0/1	0/1
Unknown	–	–	–	–	–	0/1
	3/47 (6%)	19/49 (39%)	35/47 (74%)	2/47 (4%)	14/49 (29%)	35/48 (73%)

[a]Inhibition=50% or less survival.

Table 5. Sensitivity/resistance comparisons of DX-8951f and topotecan for adult tumors: 1 h exposure

Drug A	Concentration	Drug B	Concentration	SS	SR	RS	RR	Total	*p*-value by McNemar's Test
Topotecan	0.001	topotecan	0.1	0	1	4	39	44	0.18
Topotecan	0.1	topotecan	1.0	2	2	8	33	45	0.058
Topotecan	0.001	topotecan	1.0	0	1	10	33	44	0.007
DX8951f	0.001	DX8951f	0.1	2	0	10	35	47	0.002
DX8951f	0.1	DX8951f	1.0	12	0	17	18	47	0.001
DX8951f	0.001	DX8951f	1.0	2	0	27	18	47	0.001
DX8951f	0.001	topotecan	0.001	0	1	1	42	44	1.000
DX8951f	0.1	topotecan	0.1	2	9	2	32	45	0.035
DX8951f	1.0	topotecan	1.0	2	23	5	12	45	0.001

SS=sensitive to A/sensitive to B; SR=sensitive to A/resistant to B; RS=resistant to A/sensitive to B; RR=resistant to A/resistant to B.

Table 6. Sensitivity/resistance comparisons of DX-8951f and topotecan for adult tumors: 14 day continuous exposure

Drug A	Concentration	Drug B	Concentration	SS	SR	RS	RR	Total	*p*-value by McNemar's Test
Topotecan	0.001	topotecan	0.1	2	0	12	33	47	0.001
Topotecan	0.1	topotecan	1.0	14	0	21	12	47	0.001
Topotecan	0.001	topotecan	1.0	2	0	33	12	47	0.001
DX8951f	0.001	DX8951f	0.1	3	0	16	28	47	0.001
DX8951f	0.1	DX8951f	1.0	19	0	16	12	47	0.001
DX8951f	0.001	DX8951f	1.0	3	0	32	12	47	0.001
DX8951f	0.001	topotecan	0.001	0	3	2	42	47	0.66
DX8951f	0.1	topotecan	0.1	9	10	5	25	49	0.20
DX8951f	1.0	topotecan	1.0	26	9	9	3	47	1.00

SS=sensitive to A/sensitive to B; SR=sensitive to A/resistant to B; RS=resistant to A/sensitive to B; RR=resistant to A/resistant to B.

Tables 3 and 5, at the 1 h exposure, DX-8951f was significantly more effective against most tumors than topotecan at the 0.1 and 1.0 μg/ml concentrations as assessed by McNemar's test (0.001 μg/ml, p=1.00; 0.1 μg/ml, p=0.035; 1.0 μg/ml, p=0.001). With continuous exposure, however, head-to-head comparisons between DX-8951f and topotecan revealed no significant differences at any of the concentrations tested (Tables 5 and 6). Therefore, in future testing at a continuous exposure, lower concentrations of DX-8951f (e.g. 0.1, 0.01, 0.001 or 0.0001 μg/ml) may be appropriate.

In direct head-to-head comparisons of DX-8951f and topotecan with 1 h exposure in individual tumor specimens, DX-8951f at 1.0 μg/ml was more effective than topotecan at 1.0 μg/ml in three of four head and neck tumors, one of two kidney tumors, two of five liver tumors, six of 10 non-small cell lung tumors, five of eight ovarian tumors, three of eight prostate tumors, and in the single specimens of breast, mesothelioma, colon and small cell lung tumors. Topotecan was equally or more effective than DX-8951f in one of two kidney tumors, two of five liver tumors, one of 10 non-small cell lung tumors, one of eight ovarian tumors, one of eight prostate tumors and in the single specimen of bladder tumor (data not shown).

Tables 5 and 6 provide statistical information on adult tumors that were evaluated head-to-head for sensitivity and resistance to DX-8951f and topotecan at 1 h and continuous exposures, respectively. There is a 10-fold increase in the percentage of specimens sensitive to DX-8951f as the concentration of DX-8951f increases. Table 5 indicates that an increasing number of specimens are S/R (sensitive to DX-8951f but resistant to topotecan at increasing concentrations of DX-8951f) which strongly suggests incomplete cross-resistance between these two drugs at a 1 h exposure. At the 1 h exposure, DX-8951f was more

effective than topotecan at the 0.1 μg/ml (p=0.035) and 1.0 μg/ml (p=0.001) concentrations. Table 5 presents data at continuous exposure, which suggests a pattern of incomplete cross-resistance between DX-8951f and topotecan, but the differences were not statistically significant.

DX-8951f was effective in four of seven pediatric tumors tested including one of two kidney, one of one neuroblastoma, two of two ovary tumors at 1 μg/ml and one of two sarcomas at 0.1 μg/ml using the 14 day continuous exposure (data not shown). While the sample size is insufficient for statistical analysis, there was a clear concentration-response effect of the drug against human TCFUs. These preliminary data warrant further investigation of this agent against tumors that occur in childhood.

Discussion

Extracts from the bark of the Chinese tree *Camptotheca accuminata* have been traditionally used as a herbal medicine in Asia for the treatment of gastrointestinal cancer.[16] As part of a large natural product screening effort in 1966, the active principle, camptothecin, was shown to have anti-tumor activity against the L1210 and P388 murine leukemia models.[17] It was rapidly introduced into clinical trials but the mechanism of action was not elucidated until the early 1980s. Because of severe and unpredictable side effects and poor solubility in water, camptothecin was not further developed at that time.[18-20] Water-soluble derivatives with greater therapeutic indices were prepared and tested. The development of these derivatives has been recently reviewed.[21]

DX-8951f, like CPT-11 (irinotecan) and topotecan, is a water-soluble derivative of camptothecin believed to act through inhibition of topoisomerase I. It is effective *in vitro* against malignant cell lines as well as against 16 cell lines including colon, lung, breast and gastric tumors in the human tumor xenograft model *in vivo*[22] and is currently in phase I clinical trials. DX-8951f exhibited greater activity against the xenografts *in vivo* than CPT-11. DX-8951f is also effective against a SN-38-resistant cell line[23] and against tumors resistant to topotecan.[22] This greater level of activity may be attributable in part to better penetration of tumor cells.[24] In the present study, its activity was compared to that of topotecan in a variety of human tumor specimens using the human tumor cloning system. In head-to-head comparisons, DX-8951f was more effective than topotecan with 1 h exposure and equally effective with continuous exposure at equimolar concentrations. Since the maximum tolerated dose (MTD) of DX-8951f has been reported to be 3 times as high as that of topotecan, higher doses may be used to enhance its effectiveness in the clinic. At equitoxic doses, therefore, DX-8951f may be as much as 3 times more effective than topotecan. Although most topoisomerase-interactive drugs are only active with prolonged exposure, DX-8951f is unique in that it is effective even at 1 h exposure and its effectiveness is maintained on prolonged exposure in the human tumor cloning system (response to 1 h exposure not significantly different from response to 14 day continuous exposure at any concentration by McNemar's test).

DX-8951f given every fourth day *in vivo* in human tumor xenografts, for a total of four injections at a total dose of 25 mg/kg, was effective against two of two gastric tumors, six of six colon tumors, five of five lung tumors and three of three breast tumors.[23] In the tumors taken directly from patients, activity was seen with the same tumor types: one of one gastric tumors, five of six colon tumors, five of seven lung tumors and five of nine breast tumors, suggesting that these tests will be good predictors of clinical activity. No activity was found against the A-498 kidney tumor tested in the xenograft model, whereas one of two kidney tumors responded in the cloning system. In addition, we found high activity in the present study in head and neck (two of two), ovarian (10 of 12) and prostate (six of eight) tumor types which were not tested in the xenograft model. These results indicate tumor types that may be sensitive to DX-8951f in clinical trials.

The types of tumors found to be most sensitive to DX-8951f in the present study suggest that this agent has broad-spectrum activity. This spectrum of activity is similar to that of topotecan as would be expected for compounds with similar mechanisms of action. DX-8951f may prove to require shorter treatment times than other topoisomerase inhibitors since unlike topotecan and most other topoisomerase inhibitors, we found that tumor specimens were sensitive to a 1 h exposure to DX-8951f. Furthermore, the concentrations used in the present studies were in the range of a peak plasma level of 0.046 μg/ml found in clinical trials with an administered dose of 0-0.2 mg/m^2/day.[25]

Current topoisomerase I inhibitors like topotecan are active against pediatric tumors such as neuroblastoma and rhabdomyosarcoma, which are historically resistant to chemotherapy. As a potential pediatric chemotherapy, DX-8951f represents an advantage over similar agents because of its greater potency, because of indications that it is not MDR modulated and because it has fewer side effects, such as diarrhea, which can be rapidly life-threatening in children. Although too few pediatric specimens have been

analyzed to allow for rigorous statistical analysis, we are reporting preliminary data for testing of seven pediatric tumors.

Conclusion

In head-to-head comparisons at 1 h exposure against adult tumors, DX-8951f was significantly more effective at 0.1 and 1.0 μg/ml than topotecan. With continuous exposure, DX-8951f and topotecan were equally effective at equimolar concentrations. The MTD for DX-8951f is 3 times that of topotecan, so higher doses of DX-8951f could be administered to patients. The results also suggest that DX-8951f would be effective against pediatric specimens and more thorough studies are warranted. DX-8951f is a promising new antineoplastic agent with significant activity against tumors taken directly from patients.

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(Received 4 May 1999; accepted 11 May 1999)