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The activity profile of the hexacyclic camptothecin derivative DX-8951f in experimental human colon cancer and ovarian cancer

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Abstract

DX-8951f or exatecan mesylate ((1*S*,9*S*)-1-amino-9-ethyl-5-fluoro-2,3-dihydro-9-hydroxy-4-methyl-1*H*,12*H*-benzo[*de*]pyrano[3′,4′:6,7]indolizino[1,2-*b*]quinoline-10-13(9*H*,15*H*)-dione methanesulfonate dihydrate), is a new water-soluble derivative of camptothecin. We determined the activity of DX-8951f in experimental human colon cancer and ovarian cancer, being tumor types sensitive to camptothecins. With the use of the MTT assay, DX-8951f was more potent than SN-38 in four out of five human colon cancer cell lines and three out of four human ovarian cancer cell lines ($P < 0.05$). DX-8951f was considerably more potent than topotecan in all cell lines tested ($P < 0.05$). Prolonged exposure to DX-8951f resulted in a greater increase in inhibition of cell proliferation as compared to that obtained with SN-38 or topotecan ($P < 0.05$). Overexpression of Pgp, MRP1, and LRP did not affect the *in vitro* activity of DX-8951f. DX-8951f administered daily × 5 or weekly × 2 resulted in growth inhibition <50% in two human colon cancer xenografts grown s.c. in nude mice. In three human ovarian cancer xenografts, however, >50% growth inhibition was observed at both schedules. In the OVCAR-3 human ovarian cancer model, DX-8951f showed considerably greater activity than topotecan ($P < 0.01$). DX-8951f combined with cisplatin or paclitaxel did not indicate the presence of a pharmacological interaction. In OVCAR-3 xenografts the combination was clearly more effective than DX-8951f alone, as the number of complete remissions increased substantially. In conclusion, this study shows that DX-8951f is highly potent *in vitro* and highly effective in experimental human ovarian cancer *in vivo*. Prolonged exposure to DX-8951f *in vitro* greatly increased the antiproliferative effects, which may be a rationale for testing a continuous infusion schedule in the clinic. Addition of cisplatin or paclitaxel improved the *in vivo* antitumor effects of DX-8951f.

Keywords: DX-8951f; Topoisomerase I inhibitor; Human tumor xenografts; Colon cancer; Ovarian cancer

1. Introduction

The novel DNA topoisomerase I inhibitor, DX-8951f (Fig. 1), also known as exatecan mesylate, is a water-soluble, fluorinated, hexacyclic derivative of camptothecin that, unlike CPT-11, does not require metabolic activation. The potency of DX-8951f in various human malignant cell lines has been reported to be 6- and 28-fold greater than that of SN-38 and topotecan, respectively [1]. DX-8951f has shown efficacy in a variety of human tumor xenografts [2]. DX-8951f has recently entered the clinic and phase I, and pharmacokinetic trials in patients with advanced solid malignancies have been completed [3]. Based on the favorable data obtained in phase I clinical development, disease-oriented phase II studies of DX-8951f have been initiated and are currently in progress in Europe and the United States.

The camptothecins CPT-11 and topotecan have been approved for treatment of colon cancer and ovarian cancer, respectively. CPT-11 generates response rates of 18–32% in both previously treated and untreated advanced colon cancer patients. The main dose-limiting toxicity of CPT-11 consists of delayed-onset diarrhea. Treatment of advanced ovarian cancer patients with topotecan results in response rates of

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Abbreviations: Pgp, P170-glycoprotein; MRP1, multidrug resistance-associated protein 1; LRP, lung resistance protein; BCRP, Breast Cancer Resistance Protein; MTT, (3-(4,5-dimethylthiazol-2-yl)-2,6-dimethyl-morpholino)-2,5-diphenyl-tetrazolium bromide; RF, resistance factor; MTD, maximum tolerated dose; CR, complete remission; GI%, maximum percentage of growth inhibition; Recurr, Recurrences.

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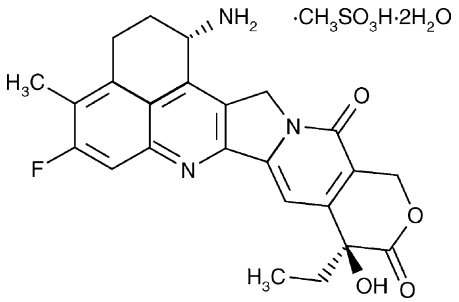

Fig. 1. Structural formula of DX-8951f.

16–38% with neutropenia as the dose-limiting toxicity [4]. Because of its promising preclinical antitumor effects, DX-8951f may be considered candidate for an improved therapeutic index in these and other malignancies. Therefore, it would be helpful to obtain more insight in the potential activity of DX-8951f in colon cancer and ovarian cancer as well as in possible mechanisms of resistance.

It has been suggested that DX-8951f is less affected by multidrug resistance mechanisms described for other camptothecins. For example, DX-8951f has shown significant antitumor effects against cell lines and xenografts in which CPT-11 was not effective [2,5]. It may also overcome P170-glycoprotein (Pgp)-mediated multidrug resistance, as it is highly effective against cell lines overexpressing the Pgp transporter both *in vitro* and *in vivo* [1,2]. At this moment, however, little is known on the influence of the expression of the multidrug resistance-associated protein 1 (MRP1) or the lung resistance protein (LRP) on the activity of DX-8951f. We have already demonstrated that, unlike SN-38 and topotecan, DX-8951f is a poor substrate for the Breast Cancer Resistance Protein (BCRP) [6].

In the present study, we determined whether DX-8951f is effective in experimental human colon cancer or ovarian cancer. To that end, we first compared its potency with that of CPT-11, SN-38, and topotecan in human malignant cell lines derived from these tumor types. We assessed whether the duration of drug exposure shows differences in the kinetic activity of the three compounds. The influence of Pgp, MRP1, and LRP on the *in vitro* potency of DX-8951f was also determined. We then compared the efficacy of DX-8951f in two equitoxic schedules in human colon cancer and ovarian cancer xenografts. As DX-8951f showed activity in human ovarian cancer xenografts, we selected the OVCAR-3 model to assess whether a combination with cisplatin or paclitaxel would improve antitumor effects.

2. Materials and methods

2.1. Drugs

For *in vitro* experiments DX-8951f (Daiichi Pharmaceutical Co, Ltd.) was dissolved in DMSO (Riedel-de Haën) at a concentration of 3.17 mM (as methanesulfonate dihydrate). CPT-11 was provided by Aventis Pharma as a solution of 20 mg/mL. Its metabolite SN-38 (Daiichi Pharmaceutical Co, Ltd.) was dissolved in DMSO to a final concentration of 3.9 mM. Solutions in water were obtained from doxorubicin (Amersham Pharmacia) at a concentration of 3.45 mM and from topotecan (Glaxo-SmithKline) at a final concentration of 2.18 mM. Drugs were further diluted in tissue-culture medium when investigated for their antiproliferative effects *in vitro*. The final concentration of DMSO in culture did not exceed 1% (v/v) which was nontoxic to the cells.

For *in vivo* experiments DX-8951f and topotecan were dissolved in water to a final concentration of 1 mg/mL (as methanesulfonate dihydrate) and 0.5 mg/mL, respectively. Cisplatin (Bristol Myers-Squibb) was available as a stock solution of 0.5 mg/mL in NaCl 0.9% and the 6 mg/mL solution of paclitaxel (Bristol Myers-Squibb) was diluted to 1.5 mg/mL using NaCl 0.9%.

2.2. Cell lines

The human colon cancer cell lines COLO205, COLO320, LS174T, SW1398, and WiDr have been described previously for their sensitivity to CPT-11 and SN-38 [7]. The human ovarian cancer cell lines used in this study were A2780 [8], H134 (established in our laboratory), IGROV-1 [9], and OVCAR-3 [10]. In addition, four pairs of human malignant cell lines were used with sublines expressing Pgp, MRP1, and/or LRP, being the Pgp-positive subline BRO/mdr1.1 and its parental melanoma cell line BRO, the Pgp-positive subline 2780AD, and the ovarian cancer cell line A2780, the non-Pgp multidrug-resistant subline GLC4/ADR of the small cell lung cancer cell line GLC4 and the non-Pgp multidrug-resistant subline 2R120 of the non-small cell lung cancer cell line SW1573/S1. In these cell lines, expression of Pgp, MRP1, and LRP has been confirmed previously by immunocytochemistry and resistance factors (RFs) for CPT-11 and SN-38 have been reported [11]. All cell lines were cultured in Dulbecco's modified Eagle's medium (GibcoBRL) supplemented with 10% heat-inactivated fetal calf serum (FCS; GibcoBRL), 50 IU/mL penicillin, and 50 μg/mL streptomycin (ICN) in an incubator with a humidified atmosphere containing 5% CO_2 at 37°.

2.3. In vitro growth inhibition studies

Drug activity studies *in vitro* were performed using the MTT assay. In short, 2500–5000 cells per well in 100 μL of medium were plated in 96-well microtiter plates and grown for 24 hr. Cells were exposed continuously for an additional 96 hr to varying concentrations of the drugs in a total volume of 200 μL. In separate experiments, shorter drug exposure times of 2, 4, and 24 hr were included, whereafter the cells were washed with medium and incubated for the remaining period of the 96 hr. At the time of analysis, the medium was removed and 50 μL of the tetrazolium salt of

MTT (Sigma) in PBS (0.4 mg/mL) were added. The plates were incubated for 2 hr and formazan crystals were dissolved in 200 μL of DMSO/0.5% FCS. The absorbance was measured at 540 nm using a Labsystems Multiscan Bichromatic plate reader (Labsystems). The results were expressed as the IC_{50}, which is the concentration of the drug(s) inducing 50% inhibition of cell growth of treated cells as compared to the growth of control cells. The ratio of the IC_{50} subline vs. IC_{50} parental cell line was calculated and expressed as the RF. In control cultures, cell growth was exponential during the test period. All concentrations were tested in four replicate wells and each experiment was performed at least three times.

2.4. Xenografts

Female nude mice (Hsd: athymic nude-*nu*) were purchased at the age of 6 weeks (Harlan). The animals were maintained in cages with paper filter covers under controlled atmospheric conditions. Cages, bedding, food, and water were changed and sterilized weekly. Animals were handled in a sterile manner in a laminar down-flow hood. For the animal experiments, ethical approval was obtained from the 'University Committee on Experimental Animals'. The xenografts were established from cell lines grown in tissue-culture medium. The solid tumors arising at the inoculation site were transferred as tissue fragments with a diameter of 2–3 mm through a small skin incision into both flanks of further recipients.

2.5. Maximum tolerated doses (MTD)

Doses of DX-8951f for the weekly × 2 and daily × 5 schedules and of topotecan for the daily × 5 schedule were administered i.p. according to the MTD for nontumor-bearing mice. This MTD was based on the occurrence of a mean weight loss of approximately 10% of the initial weight within the first 2 weeks after the start of treatment. Recovery of weight loss should be completed on day 14. Consequently, mice were weighed on weekdays for 2 weeks and, thereafter, twice a week. The MTD was assessed in groups of three nontumor-bearing nude mice per dose level. Doses were adjusted in tumor-bearing animals, if required.

After testing DX-8951f as a single agent, the efficacy of DX-8951f combined with cisplatin or paclitaxel in a weekly × 2 i.v. schedule was evaluated in OVCAR-3 human ovarian cancer xenografts. As described earlier, DX-8951f was administered weekly × 2 i.v. at MTD when given as a single dose. The MTD of cisplatin as a single agent was defined earlier and found to be 5 mg/kg i.v. weekly × 2 [12]. Determination of the MTD of paclitaxel for weekly × 2 injections had to be assessed first in groups of three nontumor-bearing nude mice. To determine the MTD for the combination schedules, it was decided to start with 70% of the MTD for single doses of DX-8951f, cisplatin, and paclitaxel, and adjust doses if required.

2.6. In vivo antitumor efficacy

The human tumor xenografts grown in nude mice were measured twice a week in three dimensions with vernier calipers. The volume was calculated by the equation length × width × thickness × 0.5, and expressed in mm^3. At the start of treatment (designated as day 0), groups of four to seven tumor-bearing mice were formed to provide a mean tumor volume of approximately 100–150 mm^3 in each group.

For the evaluation of drug efficacy, the tumor volume was expressed by the formula V_T/V_0, where V_T is the volume on any given day and V_0 is the volume on day 0. The ratio of the mean relative volume of treated tumors over that of control tumors multiplied by 100% ($T/C\%$) was assessed on each day of measurement. Antitumor effects were expressed as the maximum percentage of growth inhibition ($GI\% = 100\% - T/C\%$). The drug was considered to be active when the growth inhibition obtained in a given xenograft model was ≥50%, very active when ≥75%, and inactive when growth inhibition was <50% [11]. Complete remission (CR) was defined as the disappearance of a tumor for a period of at least 1 month after treatment.

2.7. Statistics

Differences in *in vitro* drug sensitivities and *in vivo* antitumor effects of two independent samples were evaluated using the two-tailed Students' *t*-test with unequal variances. Comparison of one group with multiple other groups was performed using the Dunnett's test. *In vivo* and *in vitro* data were normally distributed after log transformation.

3. Results

3.1. Antiproliferative effects of DX-8951f in vitro

The antiproliferative effects of DX-8951f were compared with those of CPT-11, SN-38, and topotecan in five human colon cancer cell lines and four human ovarian cancer cell lines. The results were expressed as IC_{50} values (Table 1). As was shown previously by Jansen *et al.* [13], all cell lines displayed a much higher sensitivity to SN-38 than to CPT-11; the ratios of the IC_{50} CPT-11:IC_{50} SN-38 varied within approximately 3 logs. DX-8951f was more potent than SN-38 in four out of five human colon cancer cell lines and in three out of four human ovarian cancer cell lines ($P < 0.05$). In comparison with topotecan, DX-8951f was significantly more potent in all cell lines tested ($P < 0.05$).

Different drug exposure times were used to study the kinetics of cell growth inhibition in five human colon cancer cell lines. Cells were exposed to DX-8951f, SN-38, and topotecan for 2, 4, 24, or 96 hr. The IC_{50} values are demonstrated in Fig. 2A–C. At all exposure times,

Table 1
Antiproliferative effects of CPT-11, SN-38, topotecan (TPT), and DX-8951f in human colon cancer and ovarian cancer cell lines after a drug exposure time of 96 hr

Cell line	CPT-11[a] (±SEM)	SN-38[a] (±SEM)	TPT[a] (±SEM)	DX-8951f[a] (±SEM)
Colon cancer				
COLO205	$3.9\ (\pm0.0) \times 10^{-6}$	$3.6\ (\pm0.7) \times 10^{-9}$ [b]	$2.8\ (\pm0.3) \times 10^{-8}$ [b]	$6.1\ (\pm3.5) \times 10^{-10}$
COLO320	$3.0\ (\pm0.2) \times 10^{-6}$	$1.9\ (\pm0.4) \times 10^{-9}$ [b]	$9.3\ (\pm3.7) \times 10^{-9}$ [b]	$5.2\ (\pm2.3) \times 10^{-10}$
LS174T	$2.6\ (\pm1.1) \times 10^{-6}$	$1.3\ (\pm0.6) \times 10^{-9}$	$5.8\ (\pm1.2) \times 10^{-9}$ [b]	$2.8\ (\pm1.4) \times 10^{-10}$
SW1398	$3.1\ (\pm0.5) \times 10^{-6}$	$5.0\ (\pm1.0) \times 10^{-9}$ [b]	$3.3\ (\pm0.5) \times 10^{-8}$ [b]	$2.7\ (\pm0.6) \times 10^{-10}$
WiDr	$3.6\ (\pm0.2) \times 10^{-6}$	$9.4\ (\pm2.7) \times 10^{-9}$ [b]	$3.2\ (\pm0.3) \times 10^{-8}$ [b]	$7.5\ (\pm3.0) \times 10^{-10}$
Ovarian cancer				
A2780	$1.5\ (\pm0.4) \times 10^{-6}$	$1.7\ (\pm0.4) \times 10^{-9}$ [b]	$2.8\ (\pm0.1) \times 10^{-8}$ [b]	$1.2\ (\pm0.3) \times 10^{-10}$
H134	$2.7\ (\pm0.1) \times 10^{-6}$	$7.4\ (\pm1.5) \times 10^{-9}$ [b]	$2.6\ (\pm0.1) \times 10^{-8}$ [b]	$2.2\ (\pm0.6) \times 10^{-10}$
IGROV-1	$2.3\ (\pm0.2) \times 10^{-6}$	$6.5\ (\pm1.3) \times 10^{-9}$ [b]	$2.7\ (\pm0.2) \times 10^{-8}$ [b]	$2.7\ (\pm1.1) \times 10^{-10}$
OVCAR-3	$9.8\ (\pm1.0) \times 10^{-6}$	$2.8\ (\pm0.4) \times 10^{-9}$	$2.7\ (\pm0.3) \times 10^{-8}$ [b]	$7.6\ (\pm2.0) \times 10^{-9}$

[a] Mean ± SEM of at least three separate experiments.
[b] SN-38 or topotecan less potent than DX-8951f ($P < 0.05$) by Dunnett's test.

DX-8951f was more potent than topotecan in all cell lines ($P < 0.05$) and it was also more potent than SN-38 ($P < 0.05$) except in COLO320 after a 2- and 4-hr drug exposure time and in LS174T after a 2-, 4-, and 24-hr drug exposure time. To better visualize the differences in the kinetics to achieve cell growth inhibition for the three compounds, the IC_{50} values were expressed as a ratio of the value calculated after a 2-hr drug exposure. For each drug the mean of all ratios per exposure time obtained in the five cell lines was calculated. The results are depicted in Fig. 2D. It is clearly visible in the graph that prolonged exposure to DX-8951f resulted in an 82-fold reduction of the amount of drug required for 50% cell growth inhibition, whereas this decrease was much smaller for both SN-38 (31-fold) and topotecan (23-fold) ($P < 0.05$).

3.2. Overexpression of Pgp, MRP1, and LRP

The expression of Pgp, MRP1, and LRP in the four pairs of cell lines with drug-resistant sublines was previously visualized by immunocytochemistry [11]. In brief, A2780 cells did not express any of the multidrug resistance proteins tested, whereas 2780AD cells showed strong positive staining for Pgp and weak LRP staining. The cell lines BRO and BRO/mdr1.1 both expressed LRP and BRO/mdr1.1 was strongly positive for Pgp. GLC4 cells were negative for all three proteins, but GLC4/ADR expressed high levels of MRP1 and some LRP. The cell lines SW1573/S1 and 2R120 both contained LRP, while in 2R120 also low levels of MRP1 could be detected.

The antiproliferative effects of doxorubicin and DX-8951f in these cell lines, expressed as IC_{50} values, were determined and the results are shown in Fig. 3. Doxorubicin was used as a positive control to confirm that all sublines exhibited high resistance against this drug (RFs from 37 to 814). Only the highly Pgp-overexpressing subline, 2780AD, showed a significant difference in sensitivity to DX-8951f as compared to its parental cell line (RF = 5.5; $P < 0.05$). Of interest, the cell lines have also been tested for sensitivity to SN-38 and showed that SN-38 was less effective in 2780AD (RF = 8.7; $P < 0.001$) as well as in BRO/mdr1.1 (RF = 5.7; $P < 0.05$) [11].

3.3. MTDs of single agents and drug combinations

Starting doses for determination of the MTD of DX-8951f were derived from earlier experiments [2]. For the daily × 5 i.p. schedule, a range of decreasing concentrations starting at 4.0 mg/kg were tested in nontumor-bearing animals. Eventually, the MTD of daily × 5 i.p. was defined as 1.5 mg/kg in tumor-bearing animals. In a similar approach, for the weekly × 2 i.p. schedule DX-8951f was adjusted from 20 to 17.5 mg/kg for treatment. Topotecan, starting at 3.0 mg/kg daily × 5 i.p. was adjusted to 1.5 mg/kg i.p. for treatment. Cisplatin was used weekly × 2 at a dose of 5 mg/kg i.v. as defined earlier [12]. For paclitaxel, it was found that doses >20 mg/kg i.v. caused death of the animals within 24 hr. Therefore, paclitaxel weekly × 2 was given at a dose of 20 mg/kg i.v. throughout the experiments.

After testing DX-8951f as a single agent, DX-8951f combined with cisplatin or paclitaxel was evaluated for efficacy in OVCAR-3 human ovarian cancer xenografts. A weekly × 2 i.v. schedule was proposed in which drugs were given on the same day or with an interval of 24 hr. In tumor-bearing mice using combination schedules, generally 60–70% of the full dose of each agent can be administered [14]. Therefore, DX-8951f was reduced from 17.5 to 12.5 mg/kg, cisplatin from 5 to 3.5 mg/kg and paclitaxel was given at a dose of 20 mg/kg. Table 2 shows the six experimental groups of nontumor-bearing animals. Maximum weight loss was approximately 10% in all groups and there were no toxic deaths. Although reversal of weight loss was not complete on day 14, it was decided to use these schedules for the subsequent treatment experiments.

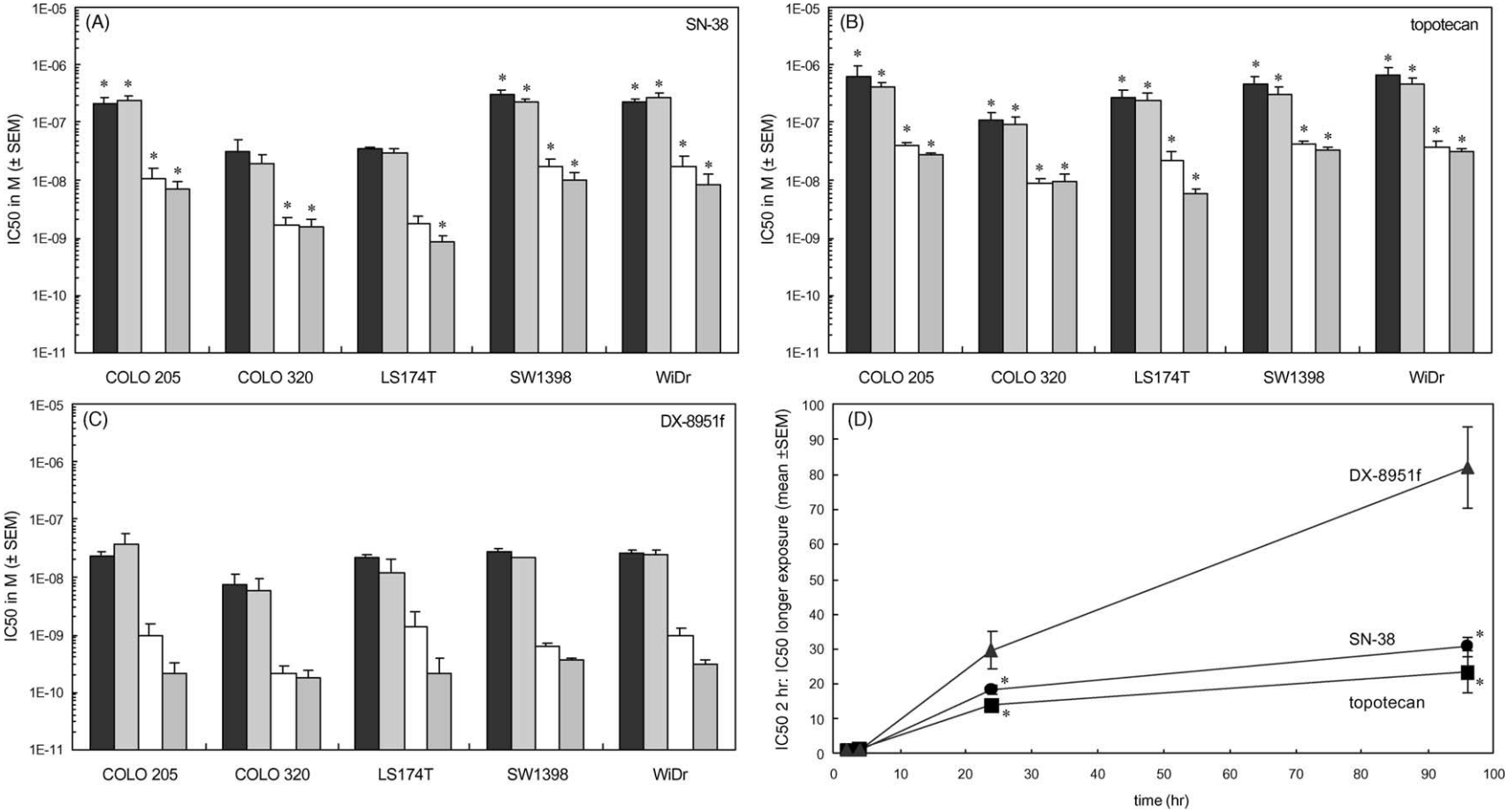

Fig. 2. Antiproliferative effects of (A) SN-38, (B) topotecan, and (C) DX-8951f after an exposure time of 2 hr (black), 4 hr (striped), 24 hr (white), and 96 hr (gray) in human colon cancer cell lines. Bars represent SEM; (D) mean ± SEM of the ratios IC_{50} values after 2-hr drug exposure:IC_{50} values after longer exposure times obtained in all cell lines. (∗) $P < 0.05$ with reference to DX-8951f by Dunnett's test.

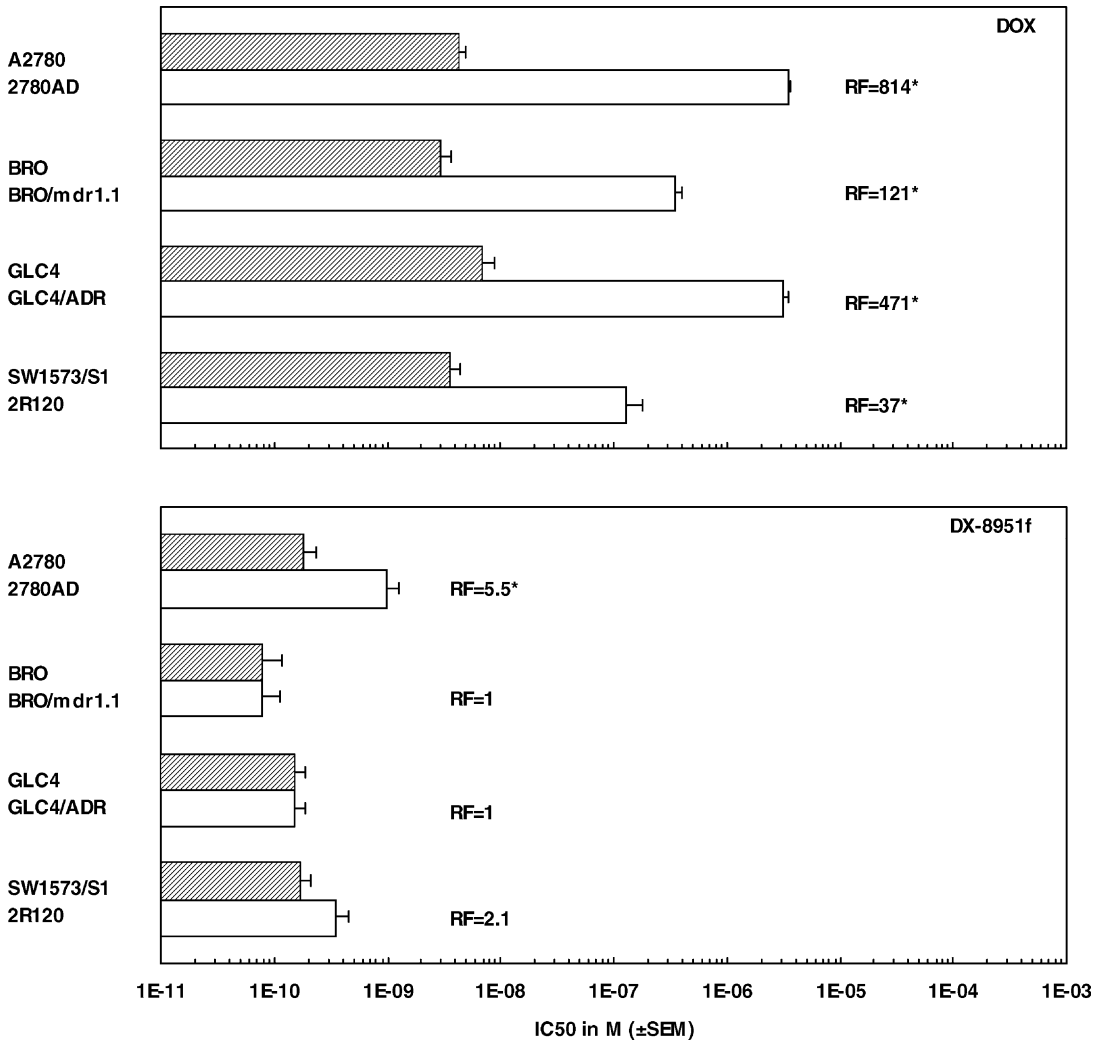

Fig. 3. Antiproliferative effects of doxorubicin and DX-8951f after a 96-hr exposure time in cell lines and their multidrug-resistant sublines. RF expressed as the ratio of the IC_{50} subline vs. IC_{50} parental cell line. Bars represent SEM. (*) $P < 0.05$ by two-tailed Students' *t*-test.

3.4. Antitumor activity of single agents

To determine whether there was a difference in efficacy between administration routes of DX-8951f, both the 1.5 mg/kg daily × 5 and the 17.5 mg/kg weekly × 2 schedules were compared when given i.p. or i.v. to groups of three mice bearing OVCAR-3 xenografts (Table 3). When DX-8951f was administered i.p. or i.v. daily × 5, a maximum tumor growth inhibition was reached of 90 and 88%, respectively. These percentages were not significantly different when comparing the mean relative tumor volumes on day 25 and 32 of the experiment. The weekly × 2 i.p. and i.v. routes each resulted in 97% inhibition of growth. DX-8951f was found to be highly effective in these OVCAR-3 xenografts, and 4 out of 10 CRs were observed in the weekly × 2 schedules. As no difference

Table 2
MTD of DX-8951f combined with cisplatin or paclitaxel given i.v. to nontumor-bearing nude mice

Drug	Dose[a] (mg/kg)	Days	Drug	Dose[a] (mg/kg)	Days	Maximum weight loss[b]	Weight day 14[b]	Toxic deaths
DX-8951f	12.5	0, 7	Cisplatin	3.5	0, 7	14.5 ± 5.8	90.2 ± 6.7	0/3
DX-8951f	12.5	0, 7	Cisplatin	3.5	1, 8	11.3 ± 6.2	95.2 ± 4.8	0/3
Cisplatin	3.5	0, 7	DX-8951f	12.5	1, 8	13.2 ± 2.2	93.5 ± 6.8	0/3
DX-8951f	12.5	0, 7	Paclitaxel	20	0, 7	9.8 ± 3.3	97.3 ± 3.9	0/3
DX-8951f	12.5	0, 7	Paclitaxel	20	1, 8	13.4 ± 0.7	92.8 ± 1.1	0/3
Paclitaxel	20	0, 7	DX-8951f	12.5	1, 8	11.3 ± 5.2	93.1 ± 6.1	0/3

[a] Doses of DX-8951f are expressed as the methanesulfonate dihydrate.
[b] Percentage of weight (loss) as compared to the weight on day 0 (±SD).

Table 3
Comparison of i.p. and i.v. administration of DX-8951f in nude mice bearing OVCAR-3 xenografts

Drug	Dose[a] (mg/kg)	Route	Days	Maximum weight loss[b]	Toxic deaths	GI%[c] (day)	CR[d]
OVCAR-3							
Control	–	–	–	–	0/3	n.a.[e]	n.a.
DX-8951f	1.5	i.p.	0–4	10.4 ± 2.1	0/3	90.2 (32)	0/4
DX-8951f	1.5	i.v.	0–4	14.3 ± 1.4	0/3	87.9 (25)	0/6
DX-8951f	17.5	i.p.	0, 7	5.0 ± 5.7	0/3	97.0 (32)	3/5
DX-8951f	17.5	i.v.	0, 7	8.6 ± 3.4	0/3	97.2 (32)	1/5

[a] Doses of DX-8951f are expressed as the methanesulfonate dihydrate.
[b] Percentage of weight (loss) as compared to the weight on day 0 (±SD).
[c] GI%, maximum percentage of growth inhibition.
[d] CR, complete remission.
[e] n.a., not applicable.

between i.p. and i.v. administration of DX-8951f was observed, the i.p. administration route was used in the following experiments facilitating daily × 5 injections.

The two schedules of DX-8951f administered daily × 5 or weekly × 2 were evaluated for efficacy in two human colon cancer xenografts, COLO320 and WiDr, and three human ovarian cancer xenografts, OVCAR-3, A2780, and FMa. The results are listed in Table 4. In general, maximum weight loss from all schedules used was between 5 and 10%, because of which treatments may be considered as equitoxic as required for analysis of differences in efficacy. No toxic deaths occurred in any of the schedules. Treatment of the COLO320 xenografts with DX-8951f daily × 5 and weekly × 2 resulted in a growth inhibition of 42 and 48%, respectively. For WiDr xenografts, the daily × 5 schedule led to a maximum growth inhibition of 26% and the weekly × 2 schedule to 49%. Both COLO320 and WiDr xenografts were thus not sensitive to DX-8951f as the percentage of maximum growth inhibition was <50%. Comparison of the schedules in the human ovarian cancer xenografts showed that all three xenografts were responsive to DX-8951f. The growth inhibition percentages in A2780 xenografts of 76 and 85% and in H134 xenografts of 51 and 52% for the respective daily × 5 and weekly × 2 schedules were not significantly different. In OVCAR-3 xenografts, the daily × 5 treatment with DX-8951f was significantly

Table 4
Comparison of two schedules of DX-8951f in nude mice bearing human tumor xenografts

Drug	Dose[a] (mg/kg)	Route	Days	Maximum weight loss[b]	Toxic deaths	GI%[c] (day)	CR[d]
COLO320							
Control	–	–	–	0.1 ± 3.4	0/6	n.a.[e]	n.a.
DX-8951f	1.5	i.p.	0–4	8.4 ± 3.8	0/6	42 (18)	0/12
DX-8951f	17.5	i.p.	0, 7	7.8 ± 6.6	0/6	48 (18)	0/12
WiDr							
Control	–	–	–	–	0/6	n.a.	n.a.
DX-8951f	1.5	i.p.	0–4	14.8 ± 5.2	0/6	26 (26)	0/11
DX-8951f	17.5	i.p.	0, 7	15.0 ± 12.4	1/6	49 (26)	0/10
A2780							
Control	–	–	–	1.7 ± 4.5	0/6	n.a.	n.a.
DX-8951f	1.5	i.p.	0–4	13.8 ± 8.0	0/6	76 (11)	0/10
DX-8951f	17.5	i.p.	0, 7	12.0 ± 5.7	0/6	85 (18)	0/10
H134							
Control	–	–	–	–	0/5	n.a.	n.a.
DX-8951f	1.5	i.p.	0–4	10.7 ± 7.6	0/6	51 (14)	0/10
DX-8951f	17.5	i.p.	0, 7	8.5 ± 7.4	0/6	52 (14)	0/12
OVCAR-3							
Control	–	–	–	2.6 ± 4.5	0/6	n.a.	n.a.
DX-8951f	1.5	i.p.	0–4	13.2 ± 6.0	0/6	85 (28)	0/10
DX-8951f	17.5	i.p.	0, 7	7.0 ± 1.9	0/6	94 (28)[f]	3/11
Topotecan	1.5	i.p.	0–4	4.2 ± 1.4	0/6	68 (22)[f]	0/10

[a] Doses of DX-8951f are expressed as the methanesulfonate dihydrate.
[b] Percentage of weight (loss) as compared to the weight on day 0 (±SD).
[c] GI%, maximum percentage of growth inhibition.
[d] CR, complete remission.
[e] n.a., not applicable.
[f] DX-8951f weekly × 2 is more and topotecan daily × 5 is less effective than DX-8951f daily × 5 ($P < 0.01$) by two-tailed Students' t-test.

Table 5
Efficacy of the combination of DX-8951f with cisplatin or paclitaxel in human ovarian cancer OVCAR-3 xenografts

Group	Dose[a] (mg/kg)	Days	Toxic deaths	GI%[b] (day)	CR[c]	Recurrence[d]
DX-8951f	17.5	0, 7	0/6	97.2 (28)	2/10	2/2[e]
Cisplatin	5	0, 7	0/6	99.0 (28)[f]	9/11	1/9[e]
DX-8951f + cisplatin	12.5 + 3.5	0, 7 + 0, 7	1/7	99.5 (28)[f]	10/10	2/10[e]
DX-8951f → cisplatin	12.5 → 3.5	0, 7 → 1, 8	0/7	99.2 (28)[f]	10/12	1/10[e]
DX-8951f	12.5	0, 7	0/5	93.5 (28)	5/9	0/5[g]
Cisplatin	3.5	0, 7	0/4	85.9 (28)	1/7	0/1[g]
DX-8951f + paclitaxel	12.5 + 20	0, 7 + 0, 7	0/6	99.1 (29)	11/11	0/11[e]
DX-8951f → paclitaxel	12.5 → 20	0, 7 → 1, 8	0/6	98.2 (29)	10/11	1/10[e]
Paclitaxel → DX-8951f	20 → 12.5	0, 7 → 1, 8	0/6	98.8 (29)	11/11	0/11[e]
Paclitaxel	20	0, 7	0/6	99.6 (28)	10/11	4/10[e]

[a] Doses of DX-8951f are expressed as the methanesulfonate dihydrate.
[b] GI%, percentage of growth inhibition.
[c] CR, complete remission.
[d] Recurrence, complete remission once defined, but tumor recurred within the observation periods.
[e] Recurrence, complete remission once defined, but tumor recurred within the observation periods ≥146 days.
[f] More effective than DX-8951f 17.5 mg/kg weekly × 2 ($P < 0.05$) by Dunnett's test.
[g] Recurrence, complete remission once defined, but tumor recurred within the observation periods 71 days.

less effective than the weekly × 2 schedule resulting in growth inhibition of 85 and 99%, respectively ($P < 0.01$).

In the OVCAR-3 model, topotecan was included using the schedule of 1.5 mg/kg daily × 5 i.p. (Table 4). Topotecan induced a maximum growth inhibition of 68% as compared to 85% for the daily × 5 administration of DX-8951f. When calculating the differences between the mean relative tumor volumes of the two treatment groups on day 22 and 28 of the experiment, topotecan was significantly less effective than DX-8951f ($P < 0.01$).

3.5. Antitumor activity of drug combinations

The tumor growth inhibition of DX-8951f in combination with cisplatin was evaluated in OVCAR-3 xenografts, in which drugs were given on the same day or DX-8951f preceded cisplatin with an interval of 24 hr. The combination schedules were compared with DX-8951f and cisplatin given alone at MTD (Table 5). One toxic death occurred in the group in which both drugs were given on the same day. High activity was observed for all four treatments. Growth inhibition on day 28 was slightly less in the group treated with DX-8951f as compared to the other three treatment groups ($P < 0.05$). CRs were substantial in the groups treated with cisplatin alone or with the combinations, but less in the case of DX-8951f. Within the observation period of 155 days, all DX-8951f CRs recurred in contrast to the other groups. In a separate experiment, doses of both DX-8951f and cisplatin were given at 70% of MTD. For cisplatin, a dose–response relationship was evident, because the growth inhibition of 99.0% observed at MTD reduced to 85.9%. For DX-8951f, a dose–response relationship was less apparent, as the growth inhibition reduced from 97.2 to 93.5%. It is clear, however, that 70% of MTD of DX-8951f and cisplatin could easily be combined resulting in an increased extent of growth inhibition and a substantial number of sustained CRs.

The results of the combination of DX-8951f with paclitaxel in three different schedules in OVCAR-3 xenografts are depicted in Table 5. As it was not possible to determine the MTD of paclitaxel on the basis of weight loss this schedule was omitted. The three schedules could easily be given and did not result in toxic deaths. Again, high activity was seen in all treatment groups resulting in a high number of CRs (Table 5). Within the observation period of 181 days, only in the case of DX-8951f preceding paclitaxel with an interval of 24 hr, 1 of 10 CRs recurred. Addition of paclitaxel to DX-8951f was clearly better effective than DX-8951f alone as the number of CRs increased substantially. Treatment with paclitaxel alone at 20 mg/kg i.v. weekly × 2, however, resulted in growth inhibition of 99.6% and 10 of 11 CRs. Within the observation period of 146 days, there were 4 of 10 tumors that recurred after paclitaxel treatment. Taking the recurrences into consideration, addition of DX-8951f to paclitaxel resulted in a higher number of sustained CRs.

4. Discussion

Camptothecins, i.e. CPT-11 and topotecan, are among the most promising anticancer agents recently introduced in the clinic. Although camptothecins are very potent and selective inhibitors of topoisomerase I, they suffer from limitations, such as rapid reversibility of the topoisomerase I–DNA cleavable complexes, pH-dependent opening of the E-ring resulting in the inactive carboxylate, sensitivity for several drug transporters, drug-specific side-effects, and requirement of activation to SN-38 in the case of CPT-11. The development of new derivatives has been facilitated by

the knowledge of the pharmacological behavior of camptothecins available today, the detailed data on structure and function of the target enzyme topoisomerase I and the relative easiness to synthesize camptothecin derivatives [15]. Semi-synthetic and synthetic analogs of camptothecin have been developed aiming at better efficacy and reduced toxicity as compared to topotecan and CPT-11. Among these analogs is DX-8951f.

In this study, we first determined the activity of DX-8951f in experimental human colon cancer and ovarian cancer *in vitro*. In our human colon cancer cell lines, this new camptothecin derivative was slightly, but significantly, more potent than SN-38 in four out of five cell lines (3.7- to 19-fold) and considerably more potent than topotecan in all cell lines (18- to 122-fold). Previously, Mitsui *et al*. [1] have demonstrated in six human colon cancer cell lines that the activity of DX-8951f was 3.7- to 5.5-fold greater as compared to that of SN-38 and 16- to 36-fold as compared to that of topotecan; these data are in agreement with our findings. In our study, we focused more extensively on human ovarian cancer cell lines and observed that DX-8951f was also more potent than SN-38 (14- to 34-fold) and considerably more potent than topotecan (3.6- to 233-fold). We can thus confirm that DX-8951f has higher potency than SN-38 and topotecan.

In addition, we assessed whether the duration of drug exposure showed differences in the kinetic activity patterns of the three compounds *in vitro*. It was clearly visible that prolonged exposure to DX-8951f up to 96 hr resulted in more reduction of the amount of drug required for 50% cell growth inhibition (82-fold) in comparison to that of SN-38 (31-fold) or topotecan (23-fold). This interesting observation could be based on differences between compounds related to the mechanism of action or to their cellular pharmacology. When the mechanism of action is taken into consideration, it has already been published that DX-8951f is more potent than SN-38 (3-fold) and topotecan (10-fold) in inhibiting the topoisomerase I catalytic activity [1]. Our finding supports the greater potency of DX-8951f. This could indicate that DX-8951f has a greater affinity for the topoisomerase I–DNA complex, resulting in less efficient reversal of the stabilized cleavable complexes after removal of the drug leading to more DNA strand breaks. Another possibility may be that the preferential binding site of DX-8951f to the topoisomerase I–DNA complex, such as a receptor site at the enzyme–DNA interface, is responsible for the higher potency [16]. Furthermore, it could be hypothesized that sublethal DNA damage induced by DX-8951f is repaired less efficiently. Aspects related to cellular pharmacology may also account for the differences in the kinetic activity patterns between the three compounds, such as the stability of the compounds in tissue-culture medium, protein binding, cellular uptake, and retention time. Perhaps a more important pharmacological aspect to consider is a difference in the lactone–carboxylate equilibrium of the drugs, but a better lactone stability for DX-8951f as compared to that for SN-38 or topotecan has not been observed in patients [4,17]. The enhanced efficacy of DX-8951f upon prolonged exposure may be a rationale for testing a continuous infusion schedule in the clinic.

The presence of multidrug resistance proteins has been regarded as an obstacle to successful anticancer therapy. Pgp belongs to the ABC transporter superfamily and is the best-known protein for transport of cytotoxic agents out of the tumor cell. In contrast to the parental compound camptothecin, accumulation and cytotoxicity of topotecan and SN-38 were reduced in Pgp-positive $CH^{R}C5$ Chinese hamster ovary cells relative to the parental AuxB1 cells [18,19]. We have also demonstrated cross-resistance against CPT-11 and SN-38 in Pgp-positive sublines *in vitro* [11,13]. Only low resistance against DX-8951f, however, was observed in the highly Pgp-positive cell line, 2780AD, indicating the drug to be a poor substrate for Pgp. Overexpression of Pgp *in vivo* does not seem to affect the activity of camptothecins as we have confirmed for the CPT-11 activity in Pgp-overexpressing xenografts [13]. Based on these findings, we do not expect an influence of Pgp expression on the *in vivo* activity of DX-8951f.

Another member of the ABC transporter superfamily is MRP1. The resistance profile of this protein includes anthracyclines, *Vinca* alkaloids, and etoposide (reviewed in [20]). Investigation of the role of MRP1 in resistance against camptothecins has led to conflicting data. In *MRP1*-transfected cell lines or in selected MRP1-overexpressing non-Pgp cell lines, it has been reported that cross-resistance against CPT-11 and SN-38 was not evident [13,21]. Chen *et al*. [22], however, have demonstrated in another *MRP1*-transfected cell line that MRP1 can extrude CPT-11 and SN-38 from the cells and is involved in drug resistance. Nevertheless, in our study no influence was found of MRP1 on DX-8951f efficacy as the MRP1-positive subline GLC4/ADR was as sensitive as its parental cell line GLC4.

LRP, also known as the human major vault protein, has been described to be a marker for drug resistance *in vitro*, both for MDR-related anticancer agents (doxorubicin and vincristine) and for other agents (cisplatin, carboplatin, and mephalan) [23]. LRP has been evaluated for a possible role in resistance against several camptothecin derivatives, including CPT-11 and SN-38 [11]. The LRP-overexpressing cell line 2R120 exhibited low, but significant cross-resistance against CPT-11, but not against SN-38. Data on the influence of LRP overexpression are currently not available for topotecan. In our study, LRP overexpression *in vitro* revealed no significant influence on the activity of DX-8951f.

In contrast to the previous drug resistance proteins mentioned, BCRP seems to be an important transporter involved in *in vitro* resistance against derivatives of camptothecin, including topotecan and SN-38 [24,25]. Unlike topotecan and SN-38, DX-8951f is a poor substrate for this

transporter as has been demonstrated *in vitro* or *in vivo* [26–28]. We have shown, however, that DX-8951f is able to induce overexpression of BCRP as a mechanism of resistance [28]. Of importance, the growth inhibition induced by CPT-11 in BCRP-overexpressing xenografts was more affected than that of DX-8951f [28].

In our experiments in two human colon cancer xenografts, we did not find growth inhibition >50% when DX-8951f was given daily × 5 or weekly × 2 at MTD. In the past, COLO320 xenografts were described to be sensitive to CPT-11 while WiDr was not [7]. In contrast, Kumazawa *et al*. [2] have reported high activity of DX-8951f in six human colon cancer xenografts. DX-8951f was more effective than CPT-11 in two of these xenografts, including WiDr. The schedules used consisted of four injections given every 4 days and on the basis of maximum body weight loss a total dose of 50 mg/kg (anhydrous free base) could easily be administered. The differences in tolerability of DX-8951f may partially be explained by the use of a different strain of mice. In our human ovarian cancer xenografts, however, high activity was observed both at the daily × 5 and weekly × 2 schedules. Of interest in OVCAR-3 xenografts, the drug DX-8951f showed considerably greater activity than topotecan.

For the near future it is anticipated that DX-8951f will be incorporated in combination schedules with other cytotoxic agents. For ovarian cancer this means that DX-8951f might be combined with cisplatin or paclitaxel, being agents with high efficacy in this disease [29]. Upon combination of cytotoxic agents, drug interactions may be expected caused by interference with drug metabolism or excretion. In this respect, cisplatin is known to be a nephrotoxic agent and could possibly reduce the clearance of drugs that use the kidney for elimination. In the case of topotecan, renal clearance accounts for approximately 40% (range 26–80%) of total drug disposition [30]. When cisplatin preceded topotecan in a phase I clinical study, significantly worse neutropenia and thrombocytopenia were observed than induced by the alternate sequence [31]. It was hypothesized that cisplatin could cause acute, subclinical renal tubular damage that impaired the systemic clearance of topotecan. For DX-8951f, a range of 10–25% of the intact drug was recovered in the urine of patients participating in phase I trials [3]. By combining DX-8951f with cisplatin in mice, we did not observe additional toxicity when cisplatin preceded DX-8951f. The low renal clearance of DX-8951f may well be of advantage for future combination chemotherapy in which cisplatin is included.

Studies on the metabolism of DX-8951f by liver microsomes have indicated that CYP3A4 and CYP1A2 of the P450 liver enzyme system play a key role and the biliary route has been confirmed as the major route of excretion in mice, rats, and dogs [3]. Formation of 3′-*p*-hydroxypaclitaxel from paclitaxel is believed to occur by use of the CYP3A4 enzyme [32] because of which a drug interaction with DX-8951f may be expected. As DX-8951f is not clearly affected by Pgp, biliary excretion will likely not be influenced by paclitaxel. Combination of DX-8951f and paclitaxel in mice did not result in excessive toxicity of a particular sequence. These data suggest that an interaction between DX-8951f and paclitaxel may not play an important role in combination chemotherapy.

All combination schedules of DX-8951f and paclitaxel examined in this study were tolerated well. It was shown that 70% of MTD of the single agents DX-8951f and paclitaxel could easily be combined. Addition of cisplatin or paclitaxel to DX-8951f was clearly more effective than DX-8951f alone as the number of CRs increased substantially. With respect to the 70% MTD of cisplatin, addition of DX-8951f clearly resulted in a higher number of sustained CRs. Improved efficacy for paclitaxel was less pronounced when combined with DX-8951f as the number of sustained CRs increased from 6 to 9 of 11 tumors. A human ovarian cancer model that is less sensitive to each of the three drugs may give an indication whether the increased efficacy of the drugs combined is based on addition or synergism of antitumor effects.

In conclusion, DX-8951f offers pharmacological advantages over CPT-11 and topotecan. It has a favorable preclinical antitumor activity profile *in vitro* as well as *in vivo*, most pronounced in ovarian cancer. Moreover, prolonged exposure to DX-8951f resulted in a great increase in the inhibition of cell proliferation, being a rationale for testing a continuous infusion schedule in the clinic. DX-8951f can easily be combined with cisplatin or paclitaxel *in vivo*, which resulted in increased antitumor effects of DX-8951f. The high activity of DX-8951f in experimental human ovarian cancer calls for a clinical trial including the compound in combination chemotherapy to improve the outcome in patients with advanced ovarian cancer.

References

[1] Mitsui I, Kumazawa E, Hirota Y, Aonuma M, Sugimori M, Ohsuki S, Uoto K, Ejima A, Terasawa H, Sato K. A new water-soluble camptothecin derivative, DX-8951f, exhibits potent antitumor activity against human tumors in vitro and in vivo. Jpn J Cancer Res 1995;86:776–82.
[2] Kumazawa E, Jimbo T, Ochi Y, Tohgo A. Potent and broad antitumor effects of DX-8951f, a water-soluble camptothecin derivative, against various human tumors xenografted in nude mice. Cancer Chemother Pharmacol 1998;42:210–20.
[3] DeJager R, Cheverton P, Tamanoi K, Coyle J, Ducharme M, Sakamoto N, Satomi M, Suzuki M. DX-8951f: summary of phase I clinical trials. Ann NY Acad Sci 2000;922:260–73.
[4] Takimoto CH, Wright J, Arbuck SG. Clinical applications of the camptothecins. Biochim Biophys Acta 1998;1400:107–19.
[5] Takiguchi S, Kumazawa E, Shimazoe T, Tohgo A, Kono A. Antitumor effect of DX-8951, a novel camptothecin analog, on human pancreatic tumor cells and their CPT-11-resistant variants cultured in vitro and xenografted into nude mice. Jpn J Cancer Res 1997;88:760–9.
[6] Boven E, Van Hattum AH, Hoogsteen I, Schlüper HMM, Pinedo HM. New analogues of camptothecins. Activity and resistance. Ann NY Acad Sci 2000;922:175–7.

[7] Jansen WJ, Zwart B, Hulscher ST, Giaccone G, Pinedo HM, Boven E. CPT-11 in human colon-cancer cell lines and xenografts: characterization of cellular sensitivity determinants. Int J Cancer 1997;70: 335–40.
[8] Rogan AM, Hamilton TC, Young RC, Klecker Jr RW, Ozols RF. Reversal of adriamycin resistance by verapamil in human ovarian cancer. Science 1984;224:994–6.
[9] Bénard J, Da Silva J, De Blois MC, Boyer P, Duvillard P, Chiric E, Riou G. Characterization of a human ovarian adenocarcinoma line, IGROV1, in tissue culture and in nude mice. Cancer Res 1985;45: 4970–9.
[10] Hamilton TC, Young RC, McKoy WM, Grotzinger KR, Green JA, Chu EW, Whang-Peng J, Rogan AM, Green WR, Ozols RF. Characterization of a human ovarian carcinoma cell line (NIH:OVCAR-3) with androgen and estrogen receptors. Cancer Res 1983;43:5379–89.
[11] Van Hattum AH, Pinedo HM, Schluper HM, Hausheer FH, Boven E. New highly lipophilic camptothecin BNP1350 is an effective drug in experimental human cancer. Int J Cancer 2000;88:260–6.
[12] Boven E, van der Vijgh WJ, Nauta MM, Schluper HM, Pinedo HM. Comparative activity and distribution studies of five platinum analogues in nude mice bearing human ovarian carcinoma xenografts. Cancer Res 1985;45:86–90.
[13] Jansen WJ, Hulscher TM, van Ark-Otte J, Giaccone G, Pinedo HM, Boven E. CPT-11 sensitivity in relation to the expression of P170-glycoprotein and multidrug resistance-associated protein. Br J Cancer 1998;77:359–65.
[14] Bissery MC, Vrignaud P, Lavelle F. Preclinical profile of docetaxel (taxotere): efficacy as a single agent and in combination. Semin Oncol 1995;22:3–16.
[15] Kohn KW, Pommier Y. Molecular and biological determinants of the cytotoxic actions of camptothecins. Perspective for the development of new topoisomerase I inhibitors. Ann NY Acad Sci 2000;922:11–26.
[16] Tanizawa A, Kohn KW, Kohlhagen G, Leteurtre F, Pommier Y. Differential stabilization of eukaryotic DNA topoisomerase I cleavable complexes by camptothecin derivatives. Biochemistry 1995;34: 7200–6.
[17] Minami H, Fujii H, Igarashi T, Itoh K, Tamanoi K, Oguma T, Sasaki Y. Phase I and pharmacological study of a new camptothecin derivative, exatecan mesylate (DX-8951f), infused over 30 minutes every three weeks. Clin Cancer Res 2001;7:3056–64.
[18] Hendricks CB, Rowinsky EK, Grochow LB, Donehower RC, Kaufmann SH. Effect of P-glycoprotein expression on the accumulation and cytotoxicity of topotecan (SK&F 104864), a new camptothecin analogue. Cancer Res 1992;52:2268–78.
[19] Mattern MR, Hofmann GA, Polsky RM, Funk LR, McCabe FL, Johnson RK. In vitro and in vivo effects of clinically important camptothecin analogues on multidrug-resistant cells. Oncol Res 1993;5:467–74.
[20] Hipfner DR, Deeley RG, Cole SP. Structural, mechanistic and clinical aspects of MRP1. Biochim Biophys Acta 1999;1461:359–76.
[21] Hasegawa S, Abe T, Naito S, Kotoh S, Kumazawa J, Hipfner DR, Deeley RG, Cole SP, Kuwano M. Expression of multidrug resistance-associated protein (MRP), MDR1 and DNA topoisomerase II in human multidrug-resistant bladder cancer cell lines. Br J Cancer 1995;71:907–13.
[22] Chen ZS, Furukawa T, Sumizawa T, Ono K, Ueda K, Seto K, Akiyama SI. ATP-dependent efflux of CPT-11 and SN-38 by the multidrug resistance protein (MRP) and its inhibition by PAK-104P. Mol Pharmacol 1999;55:921–8.
[23] Izquierdo MA, Scheffer GL, Flens MJ, Schroeijers AB, van der Valk P, Scheper RJ. Major vault protein LRP-related multidrug resistance. Eur J Cancer 1996;32A:979–84.
[24] Yang CJ, Horton JK, Cowan KH, Schneider E. Cross-resistance to camptothecin analogues in a mitoxantrone-resistant human breast carcinoma cell line is not due to DNA topoisomerase I alterations. Cancer Res 1995;55:4004–9.
[25] Brangi M, Litman T, Ciotti M, Nishiyama K, Kohlhagen G, Takimoto C, Robey R, Pommier Y, Fojo T, Bates SE. Camptothecin resistance: role of the ATP-binding cassette (ABC), mitoxantrone-resistance half-transporter (MXR), and potential for glucuronidation in MXR-expressing cells. Cancer Res 1999;59:5938–46.
[26] Maliepaard M, van Gastelen MA, Tohgo A, Hausheer FH, van Waardenburg RC, de Jong LA, Pluim D, Beijnen JH, Schellens JH. Circumvention of breast cancer resistance protein (BCRP)-mediated resistance to camptothecins in vitro using non-substrate drugs or the BCRP inhibitor GF120918. Clin Cancer Res 2001;7:935–41.
[27] Ishii M, Iwahana M, Mitsui I, Minami M, Imagawa S, Tohgo A, Ejima A. Growth inhibitory effect of a new camptothecin analog, DX-8951f, on various drug-resistant sublines including BCRP-mediated camptothecin derivative-resistant variants derived from the human lung cancer cell line PC-6. Anticancer Drugs 2000;11:353–62.
[28] Van Hattum AH, Schlüper HMM, Pinedo HM, Boven E. Preclinical activity of DX-8951f (exatecan mesylate) and induction of Breast Cancer Resistance Protein (BCRP) as a mechanism of resistance. Proc Am Assoc Cancer Res 2001;42:815.
[29] McGuire WP, Hoskins WJ, Brady MF, Kucera PR, Partridge EE, Look KY, Clarke-Pearson DL, Davidson M. Cyclophosphamide and cisplatin compared with paclitaxel and cisplatin in patients with stage III and stage IV ovarian cancer. N Engl J Med 1996;334:1–6.
[30] Herben VM, ten Bokkel Huinink WW, Beijnen JH. Clinical pharmacokinetics of topotecan. Clin Pharmacokinet 1996;31:85–102.
[31] Rowinsky EK, Kaufmann SH, Baker SD, Grochow LB, Chen TL, Peereboom D, Bowling MK, Sartorius SE, Ettinger DS, Forastiere AA, Donehower RC. Sequences of topotecan and cisplatin: phase I, pharmacologic, and in vitro studies to examine sequence dependence. J Clin Oncol 1996;14:3074–84.
[32] Desai PB, Duan JZ, Zhu YW, Kouzi S. Human liver microsomal metabolism of paclitaxel and drug interactions. Eur J Drug Metab Pharmacokinet 1998;23:417–24.