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Differential activity of topotecan, irinotecan and SN-38 in fresh human tumour cells but not in cell lines

E. Jonsson [a,*], S. Dhar [a], B. Jonsson [a], P. Nygren [a], W. Graf [b], R. Larsson [a]

[a] *Department of Medical Sciences, Clinical Pharmacology, University Hospital, SE-751 85 Uppsala, Sweden*
[b] *Department of Surgery, Uppsala University Hospital, Uppsala, Sweden*

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Abstract

The topoisomerase I inhibitors topotecan irinotecan (CPT-11) and its metabolite SN-38 were studied in a panel of cell lines and in primary tumour cells from patients, using a non-clonogenic cytotoxicity assay. All three substances showed similar activity patterns in the panel of cell lines established to classify the drugs mechanistically. In the patient tumour cells the drugs had different effects. In haematological and ovarian cancer samples, SN-38 was much more potent than topotecan, followed by irinotecan, while in colorectal cancer samples only irinotecan showed substantial activity. This *in vitro* activity pattern seems to agree with clinical experiences to date. The inactivity of SN-38 in colorectal cancer suggests irinotecan may also have some other role in addition to being a prodrug to SN-38. This study raises questions as to the role and relevance of early preclinical model systems in anticancer drug development, and suggests that important information can be obtained from studies using primary cultures of human tumour cells. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Cytotoxicity assay; Cytotoxic drug; Human tumour cell; Topoisomerase I inhibitor; Drug development; Tumour cell line; Camptothecin derivative

1. Introduction

Topotecan and irinotecan (CPT-11) are the first substances in the clinic postulated to exert their cytotoxic action through the inhibition of topoisomerase I. They are both water-soluble derivatives of camptothecin, which is extracted from the Chinese tree *Camptotheca acuminata*. They both consist of the same ring structure containing a lactone moiety necessary for cytotoxicity (Fig. 1). Irinotecan is a prodrug that is hydrolysed to its active metabolite SN-38 by carboxyesterases [1].

Despite their chemical similarity, irinotecan and topotecan seem to have different pharmacodynamic profiles and are not completely cross-resistant [2]. Their activity in clinical phase II studies is also different. For irinotecan, clinical studies have focused on colorectal cancer, where clinical response rates in the range 15–36% have been achieved in untreated patients and 13–25% in patients with prior chemotherapy [3]. Increased survival and quality of life has been shown in large phase III studies when giving irinotecan as second line chemotherapy compared with 5-fluorouracil (5-FU) or supportive care alone, suggesting that irinotecan has a place in the treatment of colorectal cancer [4,5]. In non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) response rates in the range 11–36% and 37–47%, respectively, were observed in previously untreated patients. For topotecan, the early clinical development has focused on ovarian cancer and SCLC. As second-line treatment for ovarian cancer, the response rate is 13–25%, and the drug is active even in patients that have already received platinum treatment [3]. Topotecan does not appear to have substantial activity against colorectal cancer [6–8].

Some preclinical studies observed differences between irinotecan and topotecan. In a clonogenic assay on human tumour cells, non-cross-resistance between the two drugs was shown in 38% of the samples [2]. In a mouse study with human tumour xenografts, irinotecan was as active against two xenografts selected for topotecan resistance as it was against the parental tumours [9].

* Corresponding author. Tel.: +46-18661-4944; fax: +46-1851-9237.
E-mail address: elin.jonsson@medsci.uu.se (E. Jonsson).

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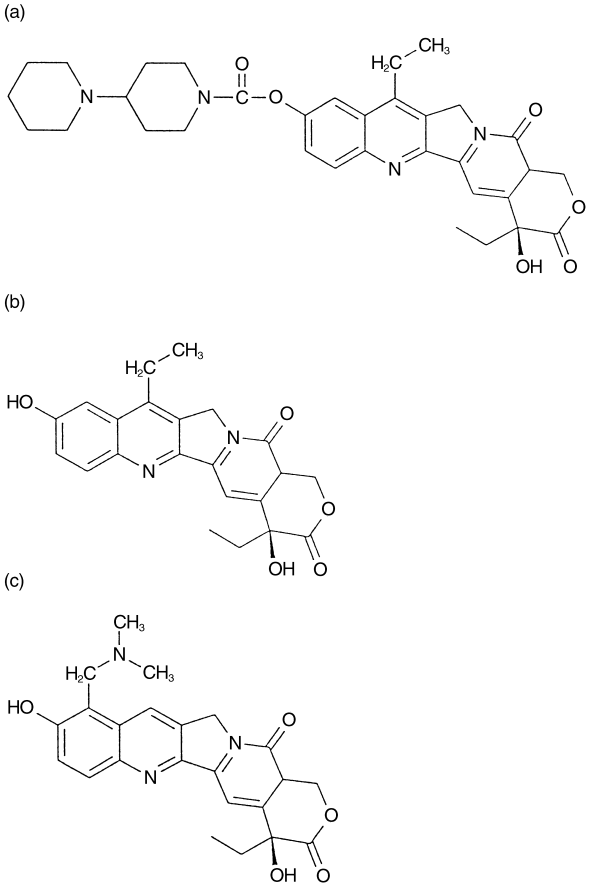

Fig. 1. Chemical structure of (a) irinotecan; (b) SN-38; and (c) topotecan.

Irinotecan is postulated to be a prodrug for SN-38, a metabolite with more than 1000 times higher topoisomerase I-inhibiting properties than its mother compound [10]. Irinotecan is converted to SN-38 by carboxyesterases. These enzymes are abundant in plasma and liver, but also in tumour cells. Some investigators have found a correlation between the activity of irinotecan and the amount of carboxyesterase activity in the tumour cells [11,12], whereas in some experiments it appears that the enzyme activity present in the tumour tissue is low compared with the normal tissue and plays a minor role in the efficacy of irinotecan [13]. It has also been shown that irinotecan is more selective for solid tumours than SN-38 and that irinotecan is more active *in vivo* than SN-38, which indicates that there could be other mechanisms for the antitumour activity of irinotecan than just being a prodrug for SN-38 [14–17]. In humans, the AUC (area under the concentration–time curve) ratio of SN-38 to irinotecan is approximately 2–4% [18], while in mice this ratio seems to be much higher, suggesting a higher esterase activity in the mouse [16,19]. Topotecan, in contrast, is not a prodrug.

The differences that obviously exist between very similar drugs and that are detected by some model systems and not by others, raise the issue of the choice of and relevance of the different model systems for preclinical drug testing. In the present study, two different model systems were compared with respect to response to topotecan, irinotecan and SN-38. The drugs were tested in a panel of 10 human cell lines and on primary human tumour cells from patients with different diagnoses. As a comparison, two anthracycline drugs, doxorubicin and idarubicin, were tested in the same model systems.

2. Materials and methods

2.1. Cell lines

To obtain a sensitivity pattern for the drugs, a panel of 10 human tumour cell lines was used. The panel contains both sensitive parental cell lines and cell lines selected for drug resistance, and has been previously described in detail [20]. Basic information on each cell line together with references are given in Table 1.

2.2. Patient samples

For investigating the activity profile of irinotecan, cell samples from 156 cancer patients with different diagnoses were used (Table 2). Because of the limited number of cells, only 141 of these samples were also tested for topotecan and 84 for doxorubicin and idarubicin. In 17 of the samples, five different concentrations of topotecan, irinotecan and SN-38 could be tested to obtain a

Table 1
Cell lines used in this study, together with references

Parental cell line	Resistant sub-line	Tumour origin	Selecting agent	Ref.
CCRF-CEM	CEM/VM-1	Leukaemia	Teniposide	[21]
NCI-H69	H69AR	SCLC	Doxorubicin	[22]
RPMI 8226/S	8226/Dox40	Myeloma	Doxorubicin	[23]
RPMI 8226/S	8226/LR-5	Myeloma	Melphalan	[24]
U-937 GTB	U-937-Vcr	Lymphoma	Vincristine	[25]
ACHN		Renal		[26]

SCLC, small cell lung cancer; RPMI, Roswell Park Memorial Institute.

Table 2
Number of successfully analysed patient samples included in the study

Diagnosis	Irinotecan (50 μg/ml) *n* (%)	Topotecan (2–2.5 μg/ml) *n* (%)
ALL	14 (9)	14 (10)
AML	10 (6)	10 (7)
CLL	6 (4)	6 (4)
NHL	5 (3)	5 (4)
Breast	36 (23)	27 (19)
Colorectal	13 (8)	11 (8)
Renal	4 (3)	4 (3)
Ovarian	28 (18)	28 (20)
NSCLC	5	5
Sarcoma	5 (3)	5 (4)
Childhood solid	8 (5)	7 (5)
Other	22 (14)	19 (13)
Total	156 (100)	141 (100)

ALL, acute lymphocytic leukaemia; AML, acute myelocytic leukaemia; CLL, chronic lymphocytic leukaemia; NHL, non-Hodgkin's lymphoma; NSCLC, non-small cell lung cancer.

concentration–effect relationship (3 chronic lymphocytic leukaemia, 2 acute myelocytic leukaemia, 4 colorectal cancers, 1 gastric cancer, 1 gastrointestinal stroma cell tumour, 3 ovarian cancer, 1 uterine cancer and 2 renal cancers).

The tumour samples were obtained by bone marrow/peripheral blood sampling, routine surgery or diagnostic biopsy, and this sampling was approved by the ethics committee at the Uppsala University Hospital. Briefly, the leukaemic cells were isolated from bone marrow or blood by 1.077 g/ml Ficoll-Paque (Pharmacia Biotech, Uppsala, Sweden) density gradient centrifugation [27]. Tumour tissue from solid tumour samples was minced into small pieces and tumour cells were then isolated by collagenase dispersion followed by Percoll (Pharmacia Biotech) density gradient centrifugation. In some cases, cells were cryopreserved in 90% heat-inactivated fetal calf serum (FCS; HyClone, Cramlington, UK) and 10% dimethylsulphoxide (DMSO; Sigma, St Louis, MO, USA) and stored in liquid nitrogen or at −150°C, which does not affect their sensitivity to the drugs [28].

2.3. Reagents and drugs

Topotecan (SmithKline Beecham, King of Prussia, PA, USA), irinotecan and SN-38 (Rhône-Poulenc Rorer, Vitry sur Seine, France) were dissolved in sterile water and diluted further in phosphate-buffered saline (PBS; HyClone). Doxorubicin (Pharmacia & Upjohn, Stockholm, Sweden) and idarubicin (Zavedos; Pharmacia & Upjohn) were purchased from the local pharmacy and diluted in PBS. In the panel of cell lines, all drugs were tested at five different concentrations in 10-fold serial dilutions from the maximum 100 μg/ml for irinotecan, doxorubicin and idarubicin and 10 μg/ml for topotecan and SN-38. The patient samples were tested at 50 μg/ml irinotecan, 2–2.5 μg/ml of topotecan, 2.5 μg/ml doxorubicin and 0.5 μg/ml idarubicin, concentrations that gave a sufficient scatter of survival indices (SI) in the material. To determine a concentration–effect relationship for topotecan, irinotecan and SN-38 in the patient cells, 5 different concentrations in 5-fold serial dilutions were used, beginning at 50, 50 and 10 μg/ml, respectively.

Ninety-six-well microtitre plates were prepared with 20 μl per well of drug solution with 10 times the desired drug concentration, and stored at −70°C before use. The drugs were tested in triplicate wells, six untreated wells served as controls and six wells containing culture medium only served as blanks.

A culture medium consisting of Roswell Park Memorial Institute (RPMI)-1640 medium (HyClone) supplemented with 10% FCS, 2 mM L-glutamine, 50 μg/ml streptomycin and 60 μg/ml of penicillin was used both for the cell lines and for the patient cells.

2.4. The FMCA procedure

The fluorometric microculture cytotoxicity assay (FMCA) measures fluorescence generated by cells with intact plasma membranes when they hydrolyse the non-fluorescent probe fluorescein diacetate (FDA) to fluorescent fluorescein. The assay procedure has been previously described in detail [27], and will only be described briefly here. The cells, suspended in culture medium, were seeded into the 96-well plates prepared with the drugs. The cell density needed to obtain an optimal fluorescent signal in the FMCA analysis varied depending on the cell type; 5000–20 000 cells per well for the cell lines and 10 000 to 100 000 cells per well for the patient cells. The plates were incubated for 72 h at 37°C, washed with PBS and then 100 μl FDA solution (10 μg/ml; Sigma) was added to each well. After another 45-min incubation, the generated fluorescence was measured at 538 nm in each well with a scanning fluorometer (Fluoroscan, Labsystems Oy, Helsinki, Finland). The fluorescence in each well is proportional to the number of viable cells.

For the patient samples, cytocentrifuge preparations were made and more than 70% tumour cells was required in the cell preparation both prior to and after the incubation. Additional quality criteria were a signal in the control wells of five times the signal in the blank wells and a coefficient of variation in the six control wells and the treated wells below 30%.

2.5. Quantification of results

Cell survival from the FMCA was presented as survival indices (SI;%) defined as the percentage fluorescence in the experimental wells of that in control wells with blank signal subtracted.

The similarities between the drugs as detected by the two model systems was calculated by measuring the degree of linear relationship between the SI values for the respective drugs at a certain concentration in all patient samples or in all cell lines with Pearson's correlation coefficient.

The concentration–effect curves of the drugs in the different diagnoses were presented as the mean SI +/− standard error of the mean (SEM) for the different experiments and the curves were fitted with a non-linear regression to a sigmoid Emax-model with a fixed Emax (SI = 0%) (Graphpad Prism Software, Inc., San Diego, CA, USA).

The disease-specific activities of irinotecan and topotecan were expressed as relative activity indices (RAI), representing the fraction of the samples within a group of one diagnosis showing a SI below the median SI for the whole group of samples.

3. Results

For the cell lines, the SI values for each cell line when incubated with irinotecan 10 μg/ml and topotecan 0.1 μg/ml were highly correlated (Fig. 2a; $r = 0.97$). The correlation between the SI for irinotecan 50 μg/ml and topotecan 2–2.5 μg/ml in the 141 patient samples was much lower, with a correlation coefficient of 0.31 (Fig. 2b). As a comparison, high correlations between the effects in both the cell lines (Fig. 2c) and the patient samples ($n = 84$; Fig. 2d) were shown for doxorubicin and idarubicin, 0.97 and 0.87, respectively. All correlations were significantly different from zero ($P < 0.01$).

When identifying samples contributing to the low correlation observed between topotecan and irinotecan, it was observed that most colorectal and renal tumour samples were highly sensitive to irinotecan, but not to topotecan. Conversely, many haematological samples showed a high sensitivity towards topotecan, but to a lesser extent towards irinotecan. When topotecan and irinotecan were correlated only in the patient samples where anthracycline sensitivity was determined ($n = 84$), the correlation coefficient was somewhat higher than in the larger group of samples ($r = 0.46$; data not shown). Very few samples from gastrointestinal and renal tumours were tested against the anthracyclines.

Table 3 shows a similar correlation analysis between topotecan, irinotecan and SN-38 in the panel of cell lines and in the 17 samples where all three drugs were tested. The correlation was very high (0.86–0.97; $P < 0.001$) between all three drugs in the panel of cell lines, but in the patient samples, only topotecan and SN-38 showed a high correlation (0.95, $P < 0.001$), whereas irinotecan was clearly different ($r = 0.17$ to topotecan and 0.26 to SN-38, NS).

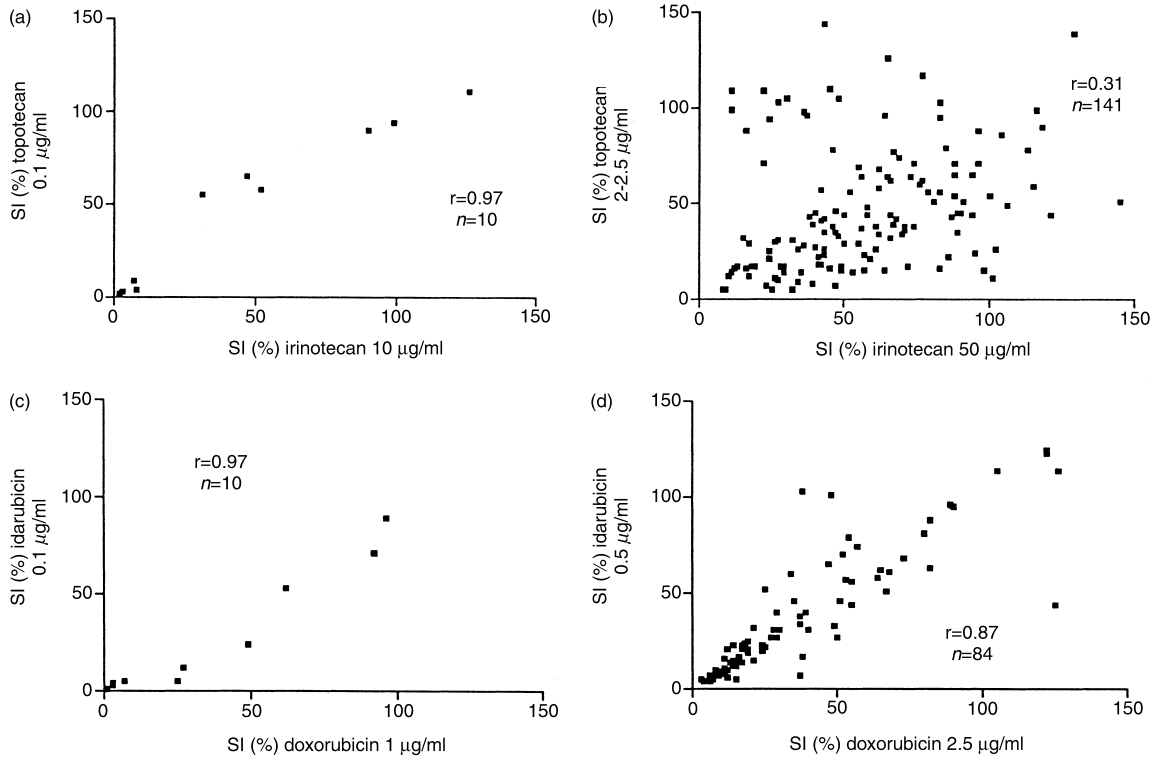

Fig. 2. Relationship between the effect of topotecan and irinotecan in: (a) the panel of cell lines and (b) the patient material and between the effect of doxorubicin and idarubicin in (c) the panel of cell lines and (d) the patient material. For the cell line panel, each point represents one cell line and for the patient material each point represents one patient sample.

Table 3
Correlation between the SI values for topotecan, irinotecan and SN-38 in the panel of cell lines (a; $n=10$) and in the patient material (b; $n=17$)

(a)	Irinotecan 10 μg/ml	Topotecan 0.1 μg/ml	(b)	Irinotecan 50 μg/ml	Topotecan 2 μg/ml
Topotecan 0.1 μg/ml	0.97		Topotecan 2 μg/ml	0.17	
SN-38 0.1 μg/ml	0.91	0.86	SN-38 0.4 μg/ml	0.26	0.95

Concentration–effect relationships for irinotecan, SN-38 and topotecan for three tumour types are shown in Fig. 3. In haematological and ovarian cancer samples, SN-38 showed the highest activity, followed by topotecan and irinotecan, which showed little activity except at the highest concentration (50 μg/ml; Fig. 3a and b). The estimated IC_{50}s for all three drugs were significantly different ($P<0.05$). Colon/gastric cancer samples showed the opposite pattern, with a toxic effect on the cells due to irinotecan, but little or no effect on the cells treated with topotecan or SN-38 (Fig. 3c). In this cell type, an IC_{50} could only be determined for irinotecan.

Table 4 shows this diagnosis-specific activity pattern in another way. Irinotecan showed a relative activity in the colorectal cancer group (RAI = 0.69) which was similar to the RAI obtained in the haematological tumour groups (RAI = 0.60–0.64). Topotecan showed a more classical activity pattern with the haematological malignancies being the most sensitive (RAI = 0.71–0.90), reasonable activity in some solid diagnoses, i.e. ovarian and breast cancer (RAI = 0.32 and 0.37), and no response in the colorectal cancer samples.

4. Discussion

Irinotecan and topotecan are both derivatives of camptothecin and are postulated to have the same mechanism of action, topoisomerase I inhibition. This easily leads to the conclusion that they should have similar pharmacodynamic properties, as is often the case for derivatives in a group of drugs. In our panel of 10 different cell lines the activity of irinotecan and topotecan was very similar, implying similar mechanisms of action. The National Cancer Institute (NCI) made similar observations with a high correlation when

Table 4
RAI (relative activity index) for irinotecan (50 μg/ml) and topotecan (2 μg/ml) for different diagnoses[a]

Diagnosis	RAI (*n*)	
	Irinotecan	Topotecan
ALL	0.64 (14)	0.71 (14)
AML	0.60 (10)	0.90 (10)
Breast	0.22 (36)	0.37 (27)
Colorectal	0.69 (13)	0 (11)
Ovarian	0.29 (28)	0.32 (28)
Childhood solid	0.50 (8)	0.43 (7)

ALL, acute lymphocytic leukaemia; AML, acute myelocytic leukaemia.

[a] RAI was defined as the fraction of samples within a diagnosis having a SI below the median SI for all of the samples included. Only diagnoses with seven or more samples were included in the table.

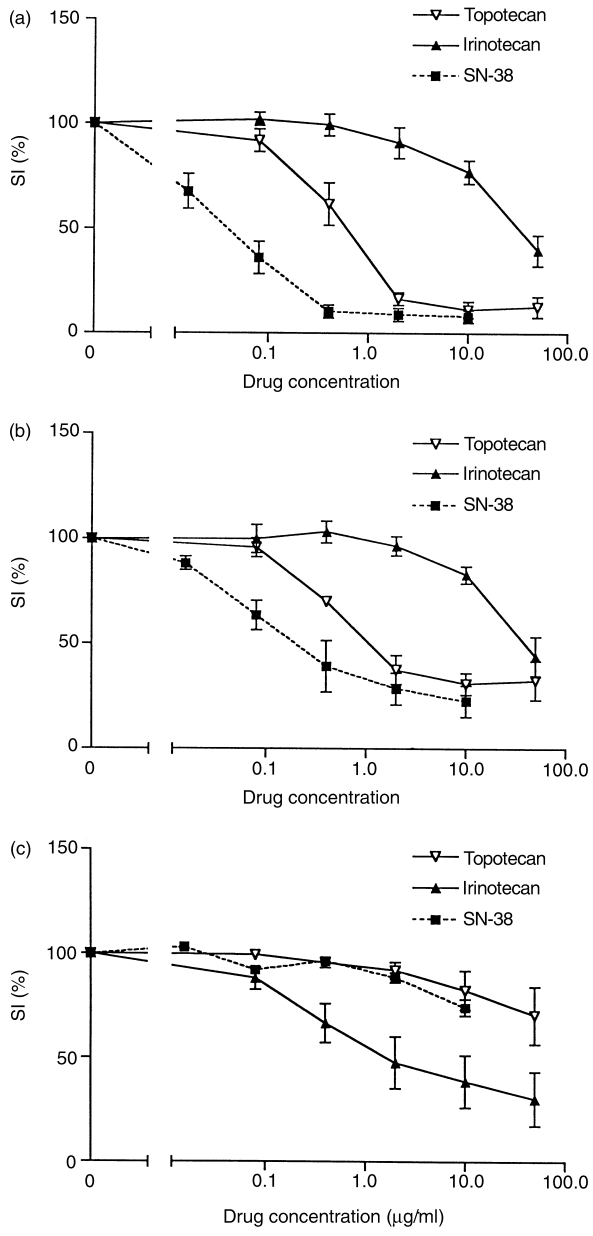

Fig. 3. Concentration–effect curves for irinotecan, topotecan and SN-38 in: (a) haematological samples ($n=5$); (b) ovarian cancer samples ($n=3$); and (c) colorectal/gastric cancer samples ($n=5$). Effect presented as survival index (SI%) defined as the fluorescent signal in the drug-treated wells divided by the signal in the control wells with the blank values subtracted.

comparing the drugs with the COMPARE method in their panel of 60 cell lines (www.dtp.nci.nih.gov) [29].

However, in the clinic, irinotecan and topotecan seem to have different disease-specific activities. For example, irinotecan has shown its most promising activity in colorectal cancer, while topotecan is used primarily in the treatment of ovarian carcinoma [3]. The toxicity pattern is also very different, the most prominent toxicity for topotecan is myelosuppression while for irinotecan it is gastrointestinal toxicity [1]. Some preclinical studies have also indicated that there could be major differences between the drugs. A high activity of irinotecan against colon cancer was suggested by the NCI cell line data where the relative activity in the colon cancer cell lines was higher for irinotecan than for topotecan (www.dtp.nci.nih.gov). In xenograft studies, irinotecan showed a better effect than topotecan in colon cancer cell line xenografts [30], and irinotecan showed activity against two xenografts selected for topotecan resistance [9]. In a study using the clonogenic assay in 24 primary tumours from patients, non-cross-resistance was noted in 38% of specimens [2].

In addition, in our experiments using patient material, with non-proliferating or slowly proliferating tumour cells from patients with different malignancies, irinotecan and topotecan showed different activity patterns. Thus, samples were identified where topotecan had a low activity where the effect of irinotecan was high, and vice versa. One of the most interesting differences detected was the activity in colorectal cancer samples. In this diagnosis, topotecan was inactive, while irinotecan showed high activity. The opposite was true for ovarian cancer, although in these samples the RAI were similar for both drugs. Interestingly, this is in agreement with the activity pattern found in clinical trials [3]. In contrast to irinotecan and topotecan, the two related drugs doxorubicin and idarubicin behaved similarly in both panels of cell lines and in the patient material.

Some authors report correlations between irinotecan efficacy and intracellular esterase activity *in vitro*, which could suggest that sensitive tumour cells convert irinotecan to SN-38 to a larger extent [11,12]. Since the FMCA is based on esterase-dependent hydrolysis of the non-fluorescent probe FDA to fluorescein, the fluorescence signal in the control wells is considered to be an indirect measurement of the esterase activity in the cells. Solid tumour cells often have higher fluorescence signals in the assay than haematological ones, and for most drugs until now investigated in our laboratory. This was true also for topotecan while for irinotecan there was no such correlation (data not shown). This could be interpreted as being due to some influence of esterases on irinotecan activity, making solid tumour cells with high esterase levels less resistant to irinotecan than to topotecan. Our results show, however, that the metabolite SN-38 itself is almost inactive in some of these highly esterase-expressing and irinotecan-sensitive cell types, making this interpretation less likely.

For the camptothecin derivatives, there exists a chemical equilibrium between the closed lactone form which exerts the biological activity, and the inactive open carboxylate form. In this study, nothing is known about the relative amounts of the two species, but the fraction in the active lactone form is assumed to be similar between experiments. Topotecan, irinotecan and SN-38 are all shown to be substrates for P-glycoprotein (Pgp), but with a far weaker affinity than the classical multi drug-resistance (MDR)-substrates [31]. The role of drug efflux proteins like Pgp on the cellular accumulation of the different camptothecin species in this study is not known.

One important difference between irinotecan and topotecan is that topotecan is active *per se* whereas irinotecan is postulated to act, at least partly, through its active metabolite SN-38 [1]. The observation that the effect of irinotecan does not correlate with that of SN-38 in the patient material (Table 3) is interesting, and indicates that irinotecan also has an effect that is independent of SN-38 production. In Fig. 3, it is evident that SN-38 is much more potent than irinotecan in many tumour types, but in the gastrointestinal tumour group SN-38 is inactive while irinotecan has an impressive activity. This could indicate some inherent activity of the mother substance irinotecan or that some other metabolite other than SN-38 may play an important role in some tumour types. Other metabolites of irinotecan have been identified, probably the most important being the aminopentane carboxylic acid metabolite (APC) produced by CYP3A4-mediated metabolism. This metabolite, however, shows very limited antiproliferative properties and no other metabolite is to date considered to be of clinical importance [32,33].

That the effects of irinotecan are not necessarily exerted through its metabolite SN-38 has also been reported by others. On P-388 mouse leukaemia cells, the topoisomerase I inhibiting activity seems to be due to SN-38, whereas the inhibitory effects on DNA and RNA synthesis and the inhibition of thymidine and uridine uptake seems to be effects of irinotecan alone [10]. The solid cell specificity observed in mouse cells for irinotecan is not seen with SN-38, whereas SN-38 shows more myelotoxicity than irinotecan, also indicating that irinotecan may have other mechanisms of antitumour activity than being a prodrug to SN-38 [16]. This difference in activity between irinotecan and SN-38 might be a clue to the different results in the different model systems.

The different results obtained in our two different model systems, a panel of cell lines and primary tumour cells from patients, raise important questions about the relevance of early preclinical model systems for the testing of anticancer drugs. The panel of cell lines

showed a mechanistic similarity existed for the drugs but failed to detect the differences in activity that nevertheless seem to exist. In contrast, data from the patient material suggested topotecan and irinotecan act through different mechanisms, and detected interesting differences also between irinotecan and its metabolite SN-38. The difference in activity between the different diagnoses detected using the patient cells seems to accord with the as yet limited clinical experiences.

The use of primary, mainly non-proliferative, tumour cells from patients could be relevant in the testing of anticancer drugs. Knowledge about the disease-specific activity pattern of a drug would be valuable in early drug development, in order to make a better choice of which patients to treat with these drugs in early clinical trials. Fresh human tumour cells seem to predict such activity patterns better than tumour cell lines, and the use of the *in vitro* assay FMCA for the prediction of disease-specific activity was recently supported by Fridborg and co-workers [34]. The present findings suggesting different activities for irinotecan and SN-38 in different tumour types warrant further mechanistic and kinetic studies.

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