Cancer Chemother Pharmacol (2002) 49: 194–200
DOI 10.1007/s00280-001-0393-3

ORIGINAL ARTICLE

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Factors influencing the cellular accumulation of SN-38 and camptothecin

Received: 18 May 2001 / Accepted: 8 October 2001 / Published online: 22 January 2002

Abstract *Purpose:* The influence of biophysical factors (drug metabolism, transport proteins, and chemical stability) on the cellular accumulation of camptothecin (CPT) and SN-38 was examined. *Methods:* Drug transporter RNA transcript levels were measured by real-time reverse transcriptase polymerase chain reaction (RT-PCR). Intracellular drug accumulation, metabolism, and drug stability studies were all performed by HPLC. *Results:* A panel of three human cell lines exhibiting different drug resistant phenotypes was investigated. HT29 colon cells glucuronidated SN-38 but did not express P-gp or MRP1 or 2. HCT116 colon cells expressed P-gp and MRP2 but did not catalyse conjugation. A2780 ovarian cells neither catalysed drug metabolism nor contained these drug transporters. In all lines, SN-38 lactone was rapidly taken up achieving peak concentrations at the earliest time point studied (5 min, 3.3–4.1 ng/10^6 cells). Subsequently, a fall in intracellular lactone concentration occurred, stabilising after 4 h at 0.48–1.18 ng/10^6 cells. No significant differences in intracellular levels of lactone were observed between the three cell lines with one exception: a twofold increase in HCT116 cells at 24 h. Stability studies in culture medium revealed that SN-38 lactone concentrations disappeared at the same rate regardless of whether cells were present, initially falling to reach equilibrium with the hydroxy acid by 4 h. Indeed, changes in intracellular lactone concentrations followed closely chemical stability profiles in media. Similar patterns of cellular retention and chemical degradation were observed with CPT. *Conclusion:* The major determinant of drug accumulation in three diverse cell line phenotypes was lactone chemical stability in culture medium.

Keywords Camptothecin · SN-38 · Cellular accumulation · Glucuronidation · Chemical stability

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Introduction

20(S)-Camptothecin (CPT) exhibits a unique mechanism of action, interfering with the breakage/reunion reaction of the nuclear enzyme topoisomerase I (topo I), resulting in stabilisation of a ternary complex (the cleavable complex) between the drug, protein, and DNA [21]. It is selectively toxic to cells in the S phase [20], requiring DNA polymerase activity to convert the reversible cleavable complex into an irreversible double-stranded DNA break through collision with the advancing replication complex [12]. For enzyme inhibition, an intact lactone system in ring E and the 20-hydroxyl group in the *R* position are essential [23], since both have been proposed to participate in critical hydrogen-bonding interactions within the cleavable complex [13, 41].

CPT-11 (irinotecan, Campto) is a promising, new, water-soluble analogue of CPT with proven clinical activity against a number of human cancers [37, 39]. CPT-11 is a pro-drug for the active metabolite SN-38 produced in vivo by the action of ubiquitously distributed carboxylesterases [24, 35]. SN-38 can undergo glucuronidation at the free hydroxyl group of C10; this is catalysed by hepatic UDP-glucuronosyltransferase isozyme UGT1A1, which, in turn, is then eliminated by biliary excretion [2, 22].

In common with other S-phase selective agents, prolonged exposure of cancer cells to CPT and SN-38 is significantly more effective than dose intensification, both in vitro and in vivo [16, 44]. However, acquired and

intrinsic drug resistance to CPT and SN-38 has frequently been correlated to the presence of drug transport proteins including MRP 1 and 2 and BCRP [4, 8, 28, 46]. Only limited studies have been performed on the cellular accumulation of CPT and SN-38 [15, 27, 34]. Here, drug measurements were carried out by indirect methods and therefore could not distinguish between the biologically active lactone form (the parent drug) and the biologically inactive hydroxy acid form, the chemical hydrolysis (lactone ring opened) product of SN-38 [23]. Frequently, drug concentrations as high as 50–75 μM were required to compensate for the limited sensitivity of the analytical technique [25, 46]. In the present study, a new, sensitive HPLC method has been employed to quantify the lactone and hydroxy acids of both SN-38 and CPT, so that detailed analysis of cellular accumulation at a lower concentration of drug (1 μM) could be performed. Several factors that may potentially affect drug uptake, such as transport proteins (MRP 1 and 2), glucuronidation [42], and chemical stability have been investigated.

Materials and methods

Materials

CPT (lactone, 95% purity) was obtained from Sigma Chemical Company, Poole, UK. SN-38 lactone was a kind gift of Rhone Poulenc Rorer, Vitry-sur-Seine, France. Stock solutions of hydroxy acids of SN-38 and CPT were generated chemically by treatment of the parent compounds with alkali (0.1 M sodium hydroxide), then drying these solutions, and reconstituting in 0.13 M Tris base, pH 10.0. This procedure gave a 93% yield of hydroxy acid. A standard of the 10-*O*-glucuronide of SN-38 was produced by incubation of the parent compound with a bulk culture of HT29 cells. The chemical structure of the latter two compounds was confirmed by LC/MS (submitted as separate manuscripts).

Cell lines

The HT29 human colon adenocarcinoma cancer cell line was obtained from ATCC (10801 University Boulevard, Manassas, Virginia, USA) and the HCT116 human colon carcinoma cell line was obtained from ECACC (European collection of cell culture, Salisbury, U.K). A2780 human ovarian cancer cells were kindly provided by Drs. T.C. Hamilton and R.F. Ozols, Medicine Branch, Division of Cancer Treatment, NCI, Bethesda, NY, USA. Cell lines were cultured in RPMI 1640 supplemented with 5% heat-inactivated foetal calf serum containing a 1% antibiotic mixture under standard conditions and maintained at 37 °C in a humidified atmosphere of 5% CO_2 in air. Experiments were performed on cells within 10 passages of each other.

RT-PCR analysis of *MDR1*, *MRP1*, and *MRP2* expression by TaqMan assay

Total RNA was extracted from log phase cells cultured in 175-cm^2 tissue culture flasks using the Tri Reagent technique (Sigma Chemical Co., Poole, England) based on the method of Chomczynski and Sacchi [5]. Real-time detection was achieved using the TaqMan Allelic Discrimination Demonstration Kit that allows direct detection of the PCR product by the release of a fluorescent reporter during each cycle of PCR (PE Applied Biosystems, Warrington, UK).

Drug analysis techniques

Determination of the lactone and hydroxy acids forms of SN-38 and CPT in tissue culture media and cell sonicate specimens was performed by gradient elution, reversed-phase HPLC with solid-phase extraction sample preparation (SPE), based on a modification of a previously published procedure [11]. The liquid chromatograph consisted of an Alliance 2690 separations module and a 474 scanning fluorescence detector set at 380 nm for excitation and 423 nm for emission, all from Waters Ltd (Watford, UK). The stationary phase was Symmetry Shield (RP8), 5-μm particle size, packed in a 15 cm long by 3 mm internal diameter stainless steel column (Waters). The mobile phase comprised of 10-mM ammonium acetate as buffer A and methanol as solvent B. Gradient elution was employed according to the following linear programme: time 0, 20% solvent B; 15 min, 80% solvent B; 17 min, 20% solvent B. The flow rate was 0.35 ml/min, the total run time was 23 min, and the column was maintained at a temperature of 40 °C. The injection volume was 20 μl for media samples and 50 μl for cell sonicates.

Cell monolayers were lysed as previously described [11]. Biological specimens (1 ml for media, 3 ml for cell sonicates) were processed immediately by SPE using C2-bonded 40-μm silica particles packed in 1-ml capacity mini-columns with 100 mg of sorbent operating under negative pressure (Varian Associates supplied by Phenomenex, Macclesfield, UK). The mini-columns were activated with 1 ml of methanol and conditioned with 1 ml of 50 mM Tris-HCl (pH 7.4). Samples were then loaded on to the columns, which were then washed with 2 ml 50 mM Tris-HCl (pH 7.4). The compounds of interest were finally eluted in 400 μl of methanol with 1 M ammonium acetate (90:10).

Full validation of the analytical methodology was carried out in a companion study [3]. The limit of detection on the column was 3 pg for SN-38 lactone, 1 pg for SN-38 hydroxy acid and CPT lactone, and 4 pg for CPT hydroxy acid. After SPE sample preparation of 1 ml of tissue culture media, the limit of quantification was 500 pg/ml for SN-38 lactone, 200 pg/ml for CPT-lactone, and 2 ng/ml each for SN-38 and CPT hydroxy acids. Precision and accuracy normally varied by less than 10% over a broad range of concentrations (1–400 ng/ml). Analytical conditions were optimised to prevent artefactual interconversion between the lactone and hydroxy acids forms of both compounds; this was achieved by keeping the samples on ice throughout sample preparation, adjusting the pH of final eluates to 7.4, setting the auto sampler to a temperature of 4 °C, and ensuring that the full analysis cycle took less than 4 h.

Drug accumulation studies

Cells were grown in 12.5-cm^2 plastic tissue culture flasks at a density of approximately 10^6 cells per flask in a volume of 4 ml of media containing freshly made up SN-38 and CPT at a final concentration of 1 μM, corresponding to 392 ng/ml and 348 ng/ml, respectively. To minimise sample processing times before HPLC analysis, only three replicate flasks were included per time point and time points were well spaced: 5 min, 1 h, 4 h, 6 h, and 24 h. Drug uptake studies were repeated on three separate days. Additional 12.5-cm^2 flasks were utilised to count cells at different time points throughout drug treatment and intracellular drug concentrations were normalised to ng/10^6 cells.

Results

Drug resistance phenotypes in the HT29, HCT116, and A2780 cell lines

MDR1, *MRP1*, and *MRP2* RNA transcript levels were determined by quantitative RT-PCR in the three cell lines of interest (Fig. 1). Only nominal expression of all three transporters was detected in HT29 and A2780 cells

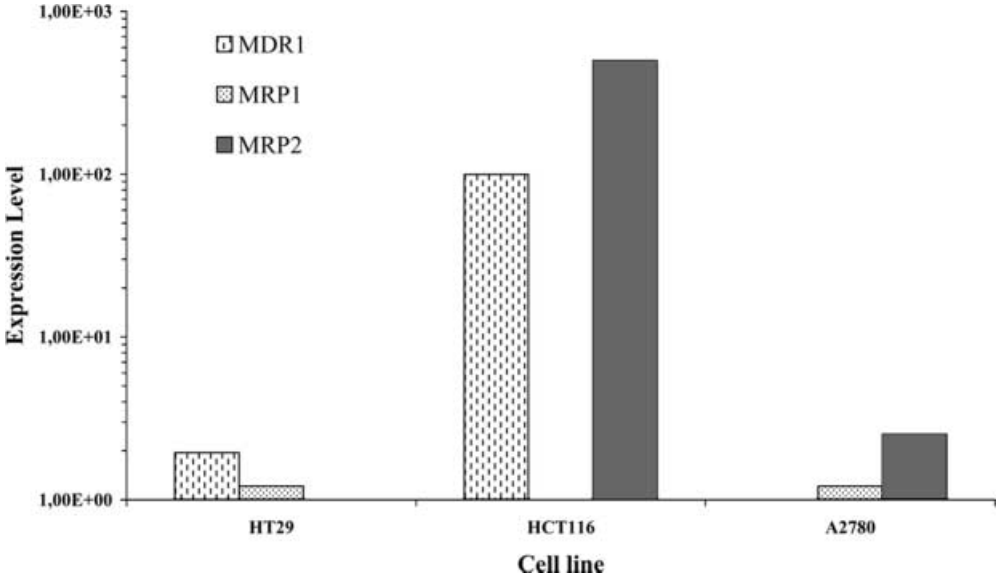

Fig. 1 Expression of *MDR1*, *MRP1* and *MRP2* mRNA determined by real-time PCR in HCT116 and HT29 colon cancer cells and A2780 ovarian cancer cells. Data are normalised against RNA content using the mean value for the 18S RNA fraction

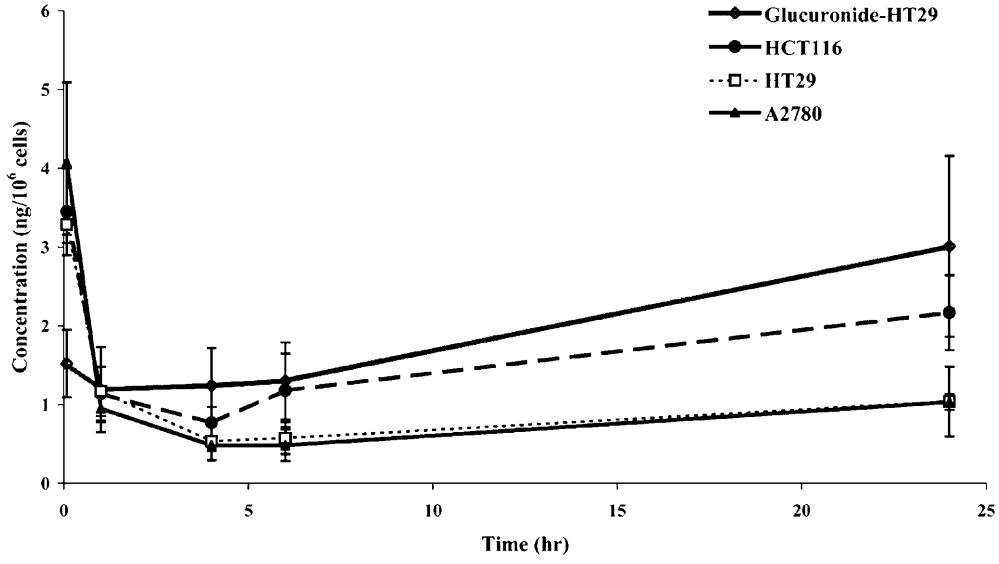

Fig. 2 Concentration versus time profiles for the cellular accumulation of SN-38 lactone in HCT116 and HT29 colon cancer cells and A2780 ovarian cancer cells after exposure to 1 μM drug as determined by HPLC with solid-phase-extraction sample preparation. In addition to the lactone, SN-38 C10 β-glucuronide was detected intracellularly. Each value represents the mean ± SD of $n = 3$ separate determinations

when the TaqMan assay was used. This is in keeping with previous studies employing a variety of techniques including Western blot analysis, RNase protection assay, RNA dot blot analysis and RT-PCR [29, 30, 45]. By contrast, HCT116 expressed *MDR1* and *MRP2* mRNA at a level two to three orders of magnitude greater than the other two cell lines, while *MRP1* mRNA was also essentially not detectable (Fig. 1), again in agreement with previous reports [32, 40]. Although CPT and SN-38 are not believed to be substrates for P-gp [8, 19], a major role for MRP2 has been described in their active efflux from cancer cells and in the expression of drug resistance [6, 7, 28].

Drug accumulation studies with SN-38

The cellular accumulation of SN-38 lactone – the active form of the drug – is shown in Figure 2. A similar profile was observed in all three lines with the highest intracellular drug concentrations (mean ± SD) being detected at the earliest time point studied (5 min): 4.1 ± 1.0 ng/10^6 cells for A2780, 3.4 ± 0.5 ng/10^6 cells for HCT116 and 3.3 ± 0.1 ng/10^6 cells for HT29. After 4 h, intracellular concentrations stopped declining and stabilised at a level that was maintained or only slightly increased over 24 h. Due to the toxicity of 1 μM SN-38 (and CPT), cells were not exposed to this concentration of drug for more than 24 h. There was no statistical difference (Student's *t* test) in intracellular lactone accumulation in the three cell lines, with the exception of the 24 h time point in HCT116, where there was a twofold increase compared to A2780 and HT29 ($P < 0.05$). Exclusively in the case of HT29 cells, the C10 β-glucuronide metabolite of SN-38 lactone was detected increasing in concentration with time, becoming the predominant intracellular form after the 1 h time point. The SN-38 lactone glucuronide is

inactive as an anticancer agent and represents a pathway of drug inactivation/detoxification [2, 43].

Cellular concentration versus time profiles for the hydroxy acid, the inactive chemical hydrolysis product of SN-38 [23], are shown in Figure 3. The hydroxy acid forms of both CPT and SN-38 carry a full negative charge and have only very limited, if any, ability to passively diffuse across the membrane without the assistance of an active organic anion transporter [17, 27]. Thus, hydroxy acid detected intracellularly is likely to be derived from in situ hydrolysis of the parent drug lactone [27]. Only nominal levels of hydroxy acid were detected in HT29 cells (peak concentration at 6 h: 0.41 ± 0.18 ng/10^6 cells), indicating that glucuronidation occurred in preference to hydrolysis as the major intracellular fate for SN-38 lactone (peak concentration of glucuronide at 24 h: 3.1 ± 1.2 ng/10^6 cells). By comparison, higher peak levels of hydroxy acid were detected in HCT116 (2.5 ± 0.86 ng/10^6 cells at 24 h) and A2780 (2.7 ± 1.5 ng/10^6 cells at 5 min). In common with the parent drug lactone, after 4 h, hydroxy acid concentrations tended to stabilise in all cell lines, with approximately twofold higher concentrations detected in HCT116.

Concentration versus time profiles of SN-38 lactone, hydroxy acid, and (in the case of HT29 cells alone) glucuronide metabolite in tissue culture media in the presence and absence of cells are shown in Figure 4. The lactone degraded with an approximate half-life of 1 h both in the presence and absence of cells, similar to the value of 1.27 h reported for the rate of chemical hydrolysis of SN-38 in a simple aqueous buffer when measured at pH 7.4 and 37 °C [1]. From 4 h onwards (as in cells), a stabilisation of lactone concentration occurred and was maintained over 24 h. This result probably reflects the chemical nature of the process under investigation, where an equilibrium is achieved between the lactone and hydroxy acid [1, 14]. As expected, the fall in SN-38 lactone concentration in tissue culture media was fully accounted for by a rise in the hydroxy acid (Fig. 4). In the tissue culture media of HT29 cells alone, the SN-38 glucuronide was detected at increasing concentrations, reaching a maximum level intermediate in value to that of the hydroxy acid and lactone at 24 h (170 ± 55 ng/ml). There was no significant difference in concentrations in media regardless of the cell line chosen or the presence or absence of cells, the only exception being at 5 min, where there was significantly less lactone present in incubations with HT29 cells ($P < 0.001$, Student's t test).

Drug accumulation studies with CPT

A similar qualitative profile to SN-38 was observed in media with CPT, characterised by a sharp decline in lactone levels, stabilising to equilibrium with the hydroxy acid after 4 h, generating similar concentrations regardless of the presence or absence of cells (data not shown). In addition to this, rapid initial uptake occurred in cells, followed by a decline to a more stable intracellular concentration, but with no significant differences in accumulation between the three lines (Fig. 5), where only low levels of hydroxy acid were generated.

Quantitatively, the intracellular accumulation of CPT lactone was two times greater than that of SN-38 (peak concentrations 5.4–6.2 ng/10^6 cells and equilibrium concentrations 0.72–1.5 ng/10^6 cells), while the levels of CPT hydroxy acid generated intracellularly were two times lower than that of SN-38 (< 0.30 ng/10^6 cells).

Discussion

In the present study, the aim was to define the importance of several biophysical factors in the cellular uptake

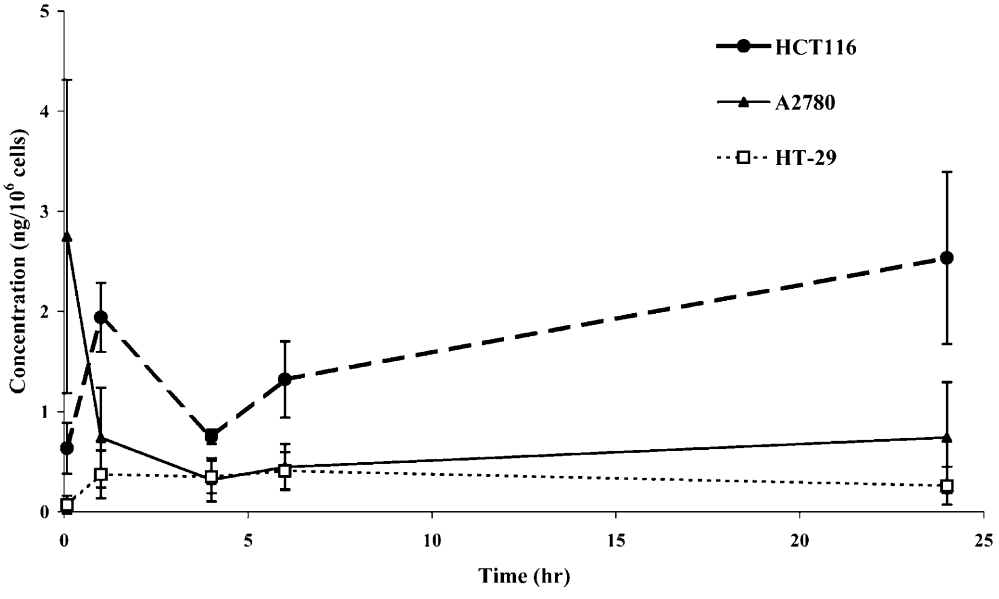

Fig. 3 Concentration versus time profiles for the intracellular generation of SN-38 hydroxy acid in HCT116 and HT29 colon cancer cells and A2780 ovarian cancer cells after exposure to 1 μM drug as determined by HPLC with solid-phase-extraction sample preparation. Each value represents the mean ± SD of $n = 3$ separate determinations

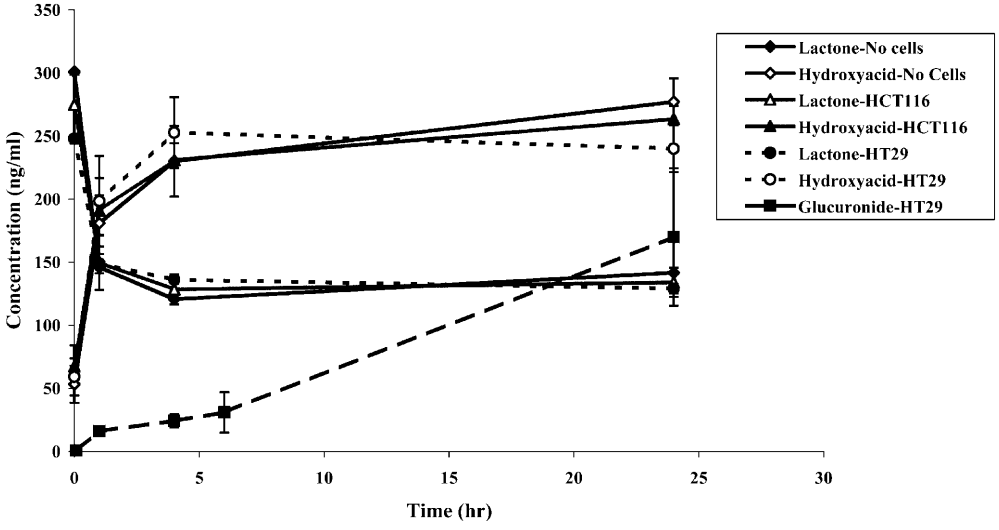

Fig. 4 Concentration versus time profiles of SN-38 lactone degradation and SN-38 hydroxy acid formation in tissue culture medium in the presence or absence of HCT116 and HT29 colon cancer cells as determined by HPLC with solid-phase-extraction sample preparation. The theoretical concentration of SN-38 lactone at time 0 was 1 μM or 392 ng/ml. Only in media in the presence of HT29 cells was the SN-38 C10 β-glucuronide detected. Each value represents the mean ± SD of $n = 3$ separate determinations

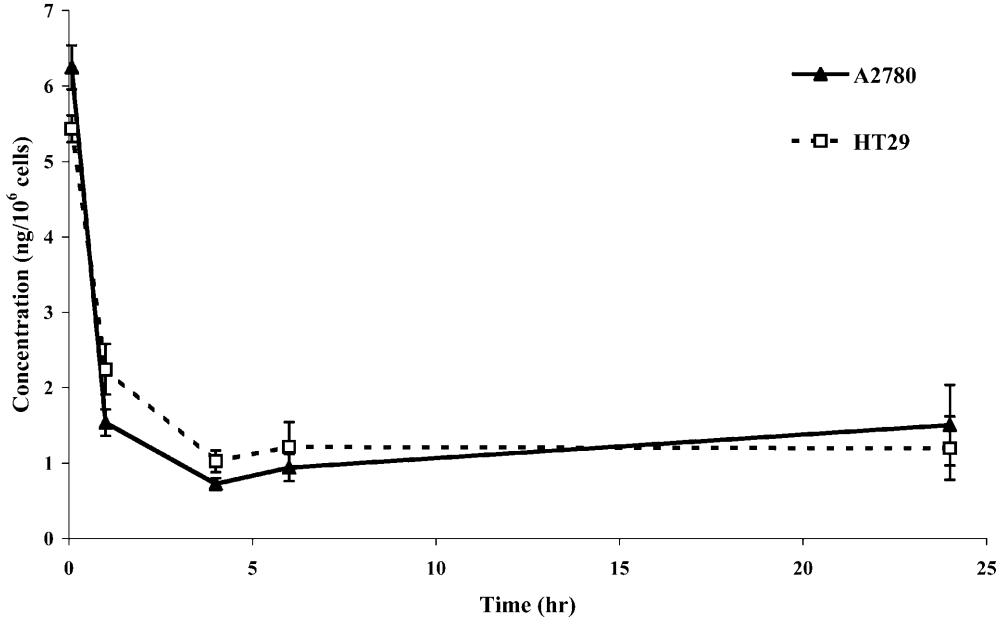

Fig. 5 Concentration versus time profiles for the cellular accumulation of CPT lactone in HT29 colon cancer cells and A2780 ovarian cancer cells after exposure to 1 μM drug as determined by HPLC with solid-phase-extraction sample preparation. Each value represents the mean ± SD of $n = 3$ separate determinations

and retention of the camptothecins CPT and SN-38. In vitro models chosen focussed on a pair of human colon cancer lines HT29 and HCT116. The former exhibits twofold intrinsic resistance to SN-38 owing to enhanced drug clearance via glucuronidation [9]. The latter is more sensitive to CPT and SN-38 [18], while displaying intrinsic resistance to most common anticancer agents owing to the expression of MRP 2, P-gp, and LRP [32]. A2780 human ovarian cancer cells were selected as a control cell line, since they neither catalyse glucuronidation of SN-38 nor express MRP 1 and 2 and P-gp [9, 30].

Interestingly, the cellular accumulation of SN-38 and CPT lactone (the active form of the drug) was almost identical in all three lines, with the exception of HCT116 where higher levels (twofold) of accumulation of SN-38 were recorded at the latest time point studied (24 h). This was unexpected, since HCT116 is known to express MRP2, and SN-38 is a substrate for this drug transporter [6, 7]. It would appear that as a critical determinant of cellular accumulation of SN-38 and CPT, at least in the three cell lines studied, MRP2 expression is of lesser importance.

Other transporters associated with drug resistance to camptothecins include the more recently described half ABC transporter BCRP/MXR/ABCP, the breast cancer resistance protein. Nonetheless, this protein is not present in HT29 and A2780 cells [36, 38]. In addition, the role of MRP3–7 in multidrug resistance in general, and to CPT and SN-38 specifically, appears to be less important, although this remains to be fully defined [30, 31].

Uptake of SN-38 and CPT lactones into cells has been demonstrated to occur by passive diffusion [27]. By contrast, a recent report on a human ovarian cancer cell line selected for resistance to topotecan and exhibiting a high level of cross resistance to SN-38 (51-fold), but not

CPT, appeared to exhibit a defect in an energy dependent uptake mechanism [33]. Thus, active uptake may play an important role in the cellular pharmacology of certain camptothecins. However, the level of SN-38 (and CPT) cellular accumulation measured in the present study was very similar to that reported in the resistant cell line ($IGROV_{T100}$), lacking active uptake [33]; this suggests a lack of active uptake in the three cell lines under investigation.

Glucuronidation was a dominant mechanism of intracellular clearance of SN-38 lactone in HT29 cells and significant levels of the glucuronide metabolite were detected in the tissue culture medium. Recent studies in Caco-2 cells have shown that SN-38 glucuronide is not capable of passive diffusion across the biomembrane [26]. Thus, it is likely that the glucuronide is actively effluxed from HT29 cells by an as yet unidentified transporter. However, as a determinant of cellular accumulation across the panel of three cell lines, glucuronidation would also appear to be of lesser general importance.

HT29 cells have been probed previously with different substrates to identify the UDP-glucuronosyl transferases (UGT) isoform(s) present. Significant activity of UGT1 family members was confirmed with highest levels of propofol activity (a UGT1A9, 1A7, and 1A10 substrate), while only nominal activity was recorded with UGT2B4, 7, and 15 substrates [10].

Perhaps the most significant result was the observation that the overall intracellular concentration versus time profiles of SN-38 and CPT lactones mirrored very closely the chemical stability profile of the drug in the tissue culture media. It may be reasonable to conclude then that stability was a major driving force behind SN-38 and CPT cellular uptake. Additionally, this would further imply that the lactones are being taken up into cancer cells predominately by passive diffusion, as previously reported for normal intestinal cells [17]. Such a model can explain why peak intracellular drug concentrations of CPT and SN-38 lactone are measured within the first few minutes of incubation in a number of cancer cell lines [15, 27], because at physiological pH the extracellular lactone concentration falls rapidly over the first 30 min of incubation, as the chemical equilibrium favours the hydroxy acid form [1, 14]. By contrast, at pH 6.2, where the lactone is more stable chemically, greater uptake of CPT and topotecan occurs and peak levels are achieved at later time points [15]. Also, this model may explain the lack of a significant effect due to the presence of MRP2 in HCT116 cells. Since the chemical degradation of the lactone is in the form of a chemical equilibrium reaction, the transport pump is always working against the equilibrium position that is set predominately due to physicochemical considerations of pH and temperature [1, 14]. In summary, the rate of chemical degradation of the parent lactone to the hydroxy acid in the extracellular culture medium appears to be the major determinant behind intracellular accumulation, perhaps because only the lactone form of the drug is able to passively diffuse into the cell.

References

1. Akimoto K, Kawai A, Ohya K (1994) Kinetic studies of the hydrolysis and lactonization of camptothecin and its derivatives, CPT-11 and SN-38, in aqueous media. Chem Pharm Bull 42:2135
2. Atsumi R, Suzuki W, Hakusui H (1991) Identification of the metabolites of irinotecan, a new derivative of camptothecin, in rat bile and its biliary excretion. Xenobiotica 21:1159
3. Boyd G, Smyth JF, Jodrell DI, Cummings J (2001) High-performance liquid chromatographic technique for the simultaneous determination of lactone and hydroxy acid forms of camptothecin and SN-38 in tissue culture media and cancer cells. Anal Biochem 297:15–24
4. Chen Z-S, Furukawa T, Sumizawa T, Ono K, Ueda K, Seto K, Akiyama S-I (1999) ATP-dependent efflux of CPT-11 and SN38 by the multidrug resistance protein (MRP) and its inhibition by Pak-104P. Mol Pharmacol 55:921
5. Chomczynski P, Sacchi N (1987) Anal Biochem 162:156
6. Chu XY, Kato Y, Niinuma K, Sudo KI, Hakusui H, Sugiyama Y (1997) Multispecific organic anion transporter is responsible for the biliary excretion of the camptothecin derivative irinotecan and its metabolites in rats. J Pharmacol Exp Ther 281:304
7. Chu XY, Kato Y, Sugiyama Y (1997) Multiplicity of biliary excretion mechanisms for irinotecan, CPT-11, and its metabolites in rats. Cancer Res 57:1934
8. Chu XY, Suzuki H, Ueda K, Kato Y, Akiyama S-I, Sugiyama Y (1999) Active efflux of CPT-11 and its metabolites in human KB-derived cell lines. J Pharmacol Exp Ther 288:735
9. Cummings J, Boyd G, Ethell BT, Macpherson JS, Burchell B, Smyth JF, Jodrell DI (2000) Glucuronidation as a de novo mechanism of drug resistance in colon cancer cells. Clin Cancer Res 6(suppl):82
10. Cummings J, Macpherson JS, Boyd G, Ethell BT, Burchell B, Smyth JF, Jodrell DI (2000) Novel mechanism of drug resistance to the topoisomerase I (topo I) inhibitor NU/ICRF 505. Proc Am Assoc Cancer Res 41:844
11. Cummings J, Meikle I, Macpherson JS, Smyth J (1995) Accumulation of anthracenyl-amino acid topoisomerase I and II inhibitors in drug sensitive and drug resistant human ovarian cancer cell lines determined by HPLC. Cancer Chemother Pharmacol 37:103
12. D'Arpa P, Beardmore C, Liu LF (1990) Involvement of nucleic acid synthesis in cell killing mechanisms of topoisomerase poisons. Cancer Res 50:6919
13. Fan Y, Weinstein JN, Kohn KW, Shi LM, Pommier Y (1998) Molecular modelling studies of the DNA-topoisomerase I ternary cleavable complex with camptothecin. J Med Chem 41:2216
14. Fassberg J, Stella VJ (1992) A kinetic and mechanistic study of the hydrolysis of camptothecin and some analogues. J Pharm Sci 81:676
15. Gabr A, Kuin A, Aalders M, El-Gawly H, Smets LA (1997) Cellular pharmacokinetics and cytotoxicity of camptothecin and topotecan at normal and acidic pH. Cancer Res 57:4811
16. Gerrits CJH, de Jonge MJA, Schellens JHM, Stoter G, Verweij J (1997) Topoisomerase I inhibitors: the relevance of prolonged exposure for present clinical development. Br J Cancer 76:952
17. Gupta E, Luo F, Lallo A, Ramanathan S, Vyas V, Rubin E, Sinko P (2000) The intestinal absorption of camptothecin, a highly lipophilic drug, across Caco-2 cells is mediated by active transporter(s). Anticancer Res 20:1013
18. Gupta M, Fan S, Zhan Q, Kohn KW, O'Connor M, Pommier Y (1997) Inactivation of p53 increases the cytoxicity of camptothecin in human colon HCT116 and breast MCF-7 cells. Clin Cancer Res 3:1653
19. Hoki Y, Fujimori A, Pommier Y (1997) Differential cytotoxicity of clinically important camptothecin derivatives in p-glycoprotein-overexpressing cell lines. Cancer Chemother Pharmacol 40:433

20. Horwitz S, Horwitz M (1973) Effects of camptothecin on the breakage and repair of DNA during the cell cycle. Cancer Res 33:2834
21. Hsiang Y-H, Hertzberg R, Hecht S, Liu LF (1985) Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I. J Biol Chem 260:14873
22. Iyer L, King CD, Whitington PF, Green MD, Roy SK, Tephly TR, Coffman BL, Ratain MJ (1998) Genetic predisposition to the metabolism of irinotecan (CPT-11). Role of uridine diphosphate glucuronosyltransferase isoform 1A1 in the glucuronidation of its active metabolite (SN-38) in human liver microsomes. J Clin Invest 101:847
23. Jaxel C, Kohn KW, Wani MC, Wall ME, Pommier Y (1989) Structure activity study of the actions of camptothecin derivatives on mammalian topoisomerase I: Evidence for a specific receptor site and a relation to antitumor activity. Cancer Res 49:1465
24. Kaneda N, Nagata H, Furuta T, Yokokura T (1990) Metabolism and pharmacokinetics of the camptothecin analogue CPT-11 in the mouse [published erratum appears in Cancer Res (1990) 50(14):4451]. Cancer Res 50:1715
25. Kanzawa F, Sugimoto Y, Minato K, Kasahara K, Bungo M, Nakagawa K, Fujiwara Y, Liu LF, Saijo N (1990) Establishment of a camptothecin analogue (CPT-11)-resistant cell line of human non-small cell lung cancer: characterization and mechanism of resistance. Cancer Res 50:5919
26. Kehrer DFS, Yamamoto W, Verweij J, deJonge MJA, deBruijn P, Sparreboom A (2000) Factors involved in prolongation of the terminal disposition phase of SN-38: Clinical and experimental studies. Clin Cancer Res 6:3451
27. Kobayashi K, Bouscarel B, Matsuzaki Y, Ceryak S, Kudoh S, Fromm H (1999) pH-Dependent uptake of irinotecan and its active metabolite, SN-38, by intestinal cells. Int J Cancer 83:491
28. Koike K, Kawabe T, Tanaka T, Toh S, Uchiumi T, Wada M, Akiyama S, Ono M, Kuwano M (1997) A canalicular multispecific organic anion transporter (cMOAT) antisense cDNA enhances drug sensitivity in human hepatic cancer cells. Cancer Res 57:5475
29. Kok JW, Veldman RJ, Klappe K, Koning H, Filipeanu CM, Muller M (2000) Differential expression of sphingolipids in MRP1 overexpressing HT29 cells. Int J Cancer 87:172
30. Kool M, de Hass M, Scheffer GL, Scheper RJ, van Eijk MJT, Juijn JA, Baas F, Borst P (1997) Analysis of expression of cMOAT (MRP2), MRP3, MRP4, and MRP5, homologues of the multidrug resistance-associated protein gene (MRP1), in human cancer cells. Cancer Res 57:3537
31. Kool M, van der Linden M, de Haas M, Scheffer GL, de Vree JM, Smith AJ, Jansen G, Peters GJ, Ponne N, Scheper RJ, Elferink RP, Baas F, Borst P (1999) MRP3, an organic anion transporter able to transport anti-cancer drugs. Proc Natl Acad Sci USA 96:6914
32. Laurencot CM, Scheffer GL, Scheper RJ, Shoemaker RH (1997) Increased LRP mRNA expression is associated with the MDR phenotype in intrinsically resistant human cancer cell lines. Int J Cancer 72:1021
33. Ma J, Maliepaard M, Nooter K, Loos WJ, Kilker HJ, Verweij J, Stoter G, Schellens JHM (1998) Reduced cellular accumulation of topotecan – a novel mechanism of resistance in a human ovarian cancer cell line. Br J Cancer 77:1645
34. Nabiev I, Fleury F, Kudelina I, Pommier Y, Charton F, Riou JF, Alix AJ, Manfait M (1998) Spectroscopic and biochemical characterisation of self-aggregates formed by antitumor drugs of the camptothecin family: their possible role in the unique mode of drug action. Biochem Pharmacol 55:1163
35. Rivory LP, Chatelut E, Canal P, Mathieu-Boue A, Robert J (1994) Kinetics of the in vivo interconversion of the carboxylate and lactone forms of irinotecan (CPT-11) and of its metabolite SN-38 in patients. Cancer Res 54:6330
36. Ross DD, Yang W, Abruzzo LV, Dalton WS, Schneider ES, Lage H, Dietel M, Greenberger L, Cole SPC, Doyle LA (1999) Atypical multidrug resistance: breast cancer resistance protein messenger RNA expression in mitoxantrone-selected cell lines. J Natl Cancer Inst 91:429
37. Rothenberg ML, Blanke CD (1999) Topoisomerase I inhibitors in the treatment of colorectal cancer. Semin Oncol 26:632
38. Scheffer GL, Maliepaard M, Pijnenborg ACLM, vanGastelen MA, deJong MC, Schroeijers AB, vanderKolk DM, Allen JD, Ross DD, vanderValk P, Dalton WS, Schellens JHM, Scheper RJ (2000) Breast cancer resistance protein is localized at the plasma membrane in mitoxantrone- and topotecan-resistant cell lines. Cancer Res 60:2589
39. Sobrero A, Kerr D, Glimelius B, Van Cutsem E, Milano G, Pritchard DM, Rougier P, Aapro M (2000) New directions in the treatment of colorectal cancer: a look to the future. Eur J Cancer 36:559
40. Stein U, Walther W, Laurencot CM, Schefer CL, Scheper RJ, Shoemaker RH (1997) Tumour necrosis factor α and expression of the multidrug resistance-associated genes LRP and MRP. J Natl Cancer Inst 89:807
41. Stewart L, Redinbo M, Qiu X, Hol W, Champoux J (1998) A model for the mechanism of human topoisomerase I. Science 279:1534
42. Takahashi T, Fujiwara Y, Yamakido M, Katoh O, Watanabe H, Mackenzie PI (1997) The role of glucuronidation in 7-ethyl-10-hydroxycamptothecin resistance in vitro. Jap J Cancer Res 88:1211
43. Takasuna K, Hagiwara T, Hirohashi M, Kato M, Nomura M, Nagai E, Yokoi T, Kamataki T (1996) Involvement of β-glucuronidase in intestinal microflora in the intestinal toxicity of the antitumor camptothecin derivative irinotecan hydrochloride (CPT-11) in rats. Cancer Res 56:3752
44. te Poele RH, Joel SP (1999) Schedule-dependent cytotoxicity of SN-38 in p53 wild-type and mutant colon adenocarcinoma cell lines. Br J Cancer 81:1285
45. Tomonaga M, Oka M, Narasaki F, Fukuda M, Nakano R, Takatani H, Ikeda K, Terashi K, Matsuo I, Soda H, Cowan KH, Kohno S (1996) The multidrug resistance-associated protein gene confers drug resistance in human gastric and colon cancers. Jap J Cancer Res 87:1263
46. Yang CH, Schneider E, Kuo ML, Volk EL, Rocchi E, Chen YC (2000) BCRP/MXR/ABCP expression in topotecan-resistant human breast carcinoma cells. Biochem Pharmacol 60:831