Cancer Chemother Pharmacol (2000) 45: 50–54

ORIGINAL ARTICLE

Peter Platzer · Theresia Thalhammer
Gerhard Hamilton · Ernst Ulsperger
Erwin Rosenberg · Rene Wissiack · Walter Jäger

Metabolism of camptothecin, a potent topoisomerase I inhibitor, in the isolated perfused rat liver

Received: 16 February 1999 / Accepted: 11 June 1999

Abstract *Purpose*: Camptothecin (CPT) is a potent topoisomerase I inhibitor that has recently been undergoing phase I clinical trials. Though CPT shows high activity against various tumor cells, its biotransformation is still unknown. To investigate the metabolism and biliary excretion of CPT, an isolated perfused rat liver system was used. *Methods*: CPT was added to the perfusion medium at a concentration of 20 μ*M*, and bile and perfusate samples were collected for 90 min. CPT (lacton and carboxylate) and three novel metabolites were identified by mass spectroscopy and quantified by reversed-phase high-performance liquid chromatography (HPLC). Kinetic parameters of CPT and its biotransformation products were then estimated in bile and effluent perfusate. *Results*: Biliary secretion of CPT and its three metabolites reached a peak secretion of 37.6 ± 16.3, 0.94 ± 0.29, 0.19 ± 0.023 and 0.302 ± 0.076 nmol/g liver/min, respectively, after 20 min. The total amount of CPT and M1–M3 excreted into bile during 90 min of perfusion was 63.5 ± 15.4%, 1.8 ± 0.37%, 0.43 ± 0.06%, and 0.72 ± 0.15% of CPT cleared from the perfusate during 90 min, respectively. In the perfusate, only one metabolite (M3) could be detected (cumulative release into the perfusion medium: 0.37 ± 0.026 μmol/liver). Analysis of the biliary metabolites by mass spectroscopy supported the existence of dihydroxy-CPT derivatives (M1 and M2), whereas M3 appears to be a monohydroxy-analog. *Conclusion*: CPT is biotransformed to three novel metabolites, mainly excreted into bile. The possible pharmacological effects of these new metabolites need to be considered.

Key words Anticancer drugs · Camptothecin · Metabolism · Topoisomerase I

P. Platzer · W. Jäger (✉)
Institute of Pharmaceutical Chemistry,
University of Vienna,
Althanstrasse 14, A-1090 Vienna, Austria
Tel.: +431-31336-8629; Fax: +431-31336-771
E-mail: wjaeger@speedy.pch.univie.ac.at

T. Thalhammer
Department of General and Experimental Pathology,
University of Vienna, AKH Wien,
Währinger Gürtel 18-20, A-1090 Vienna, Austria

G. Hamilton · E. Ulsperger
LBI of Clinical Oncology, KH Lainz, Wolkersbergerstrasse 1,
A-1130 Vienna, Austria

E. Rosenberg · R. Wissiack
Institute of Analytical Chemistry,
Vienna University of Technology,
Getreidemarkt 9/151, A-1060 Vienna, Austria

Introduction

Camptothecin (CPT), derived from the Chinese plant *Camptotheca acuminata*, is an alkaloid exhibiting antineoplastic activity by targeting the topoisomerase I [1, 2]. CPT stabilizes the cleavable complex between DNA and topoisomerase I, resulting in single-strand breaks of the DNA and finally in cell death [3, 4]. Since the drug has been shown to have maximal activity in the S-phase of the cell cycle, a prolonged exposure is required for optimal efficacy.

CPT inhibits the growth of a wide range of tumors, including leukemias and cancers of the liver, stomach, breast, and colon and has therefore undergone extensive pharmacological evaluation using human carcinoma xenograft models [5–7]. The chemical form employed for these trials was the sodium salt of CPT, chosen for its water solubility, which allowed intravenous drug administration. Testing CPT-Na showed little promise because of a high degree of toxicity and only little antitumor activity [8, 9]. Investigations into the relationship between structure and activity elucidated that the preservation of the lactone ring of CPT is crucial for its anti-tumor activity [10–13]. The lactone ring exists in a pH-dependent equilibrium with an open carboxylate form that is much less active. However, recent phase I clinical studies using CPT in its water insoluble lacton

form either as a single drug or in combination with etoposide have been successfully conducted [14–16]. Intramuscular and oral administration lead to regressions of solid tumors, e.g., in the breast, colon, and stomach.

So far, there are no data on the metabolism of CPT. To determine the role of the liver in the formation and elimination of possible CTP metabolites, a recirculating isolated perfused rat liver model was used.

Materials and methods

Chemicals and the tested drug

CPT (95% pure) was obtained from Sigma, Munich, Germany, and was free of 10 OH-camptothecin (10-OH CPT), which was verified by our high-performance liquid chromatography (HPLC) system. 10-OH CPT was purchased from Apin Chemicals, near Abingdon, United Kingdom. The methanol and water used were of HPLC grade (Merck, Darmstadt, Germany). All other chemicals and solvents were of analytical grade and used without further purification.

Liver perfusion

Livers of male Wistar rats ($n = 3$, 205–220 g), raised at the Institut für Versuchstierzucht und -haltung, University of Vienna, Himberg, Austria, were perfused in a recirculated system using the techniques described previously [17]. The animals were anesthetized with thiopental 100 mg/kg, and the liver was excised after cannulation of the common bile duct, the portal vein, and the vena cava inferior. The liver was then rapidly removed, weighed (8.8 ± 0.6 g) and transferred to a sheltered Perspex platform. The perfusions (the total volume of the reservoir was 1000 ml) were conducted using freshly prepared Krebs Henseleit buffer (118 m*M* NaCl, 4.5 m*M* KCl, 2.75 m*M* $CaCl_2$, 1.19 m*M* KH_2PO_4, 1.18 m*M* $MgSO_4$, and 25 m*M* $NaHCO_3$, equilibrated with 95% O_2/5% CO_2, pH 7.4) with a constant flow rate of 3.8 ml/g liver/min by using a peristaltic pump (Model PA-SF, IKA-Labortechnik, Straufen i. Br., Germany) through the portal vein, whereby the effluent perfusate was collected from the vena cava inferior. This flow rate was sufficient to maintain viability of the liver during the experiment, as determined by measuring oxygen consumption and the release of glutamate-oxalate-transferase, lactate-dehydrogenase, and potassium into the perfusate (data not shown). During the experiment, the perfusion pressure was continuously monitored ($6 + 1.5$ cm H_2O) and remained constant during control and after application of CPT. The temperature of the perfusion cabinet and of the perfusion medium was thermostatically controlled at 37 °C. Bile salt-independent bile flow (in the absence of bile acids) was monitored continuously by measuring the weight and frequency of bile droplets leaving the bile duct cannula. The frequency was determined by the interruption of an infrared beam as sensed by a photoelectric device. After 30 min of perfusion with control medium, 20 μ*M* CPT (from a 10-m*M* stock solution in DMSO) was added to the medium, and perfusion was continued for 90 min. To test the stability of CPT under perfusion conditions, control experiments were performed with application of the same solution (20 μ*M* CPT) to the perfusion model without a liver resulting in any detectable degradation products of CPT up for to 90 min (data not shown).

Analysis of CPT and its metabolites in bile and perfusate

Bile samples (30–50 μl) collected over a time period of 5 min were immediately frozen on dry ice and stored at −80 °C until use. Samples were centrifuged (5000 *g*, 5 min, 4 °C), and 2 μl supernatant were diluted with 998 μl distilled water. Perfusate (approximately 1.5 ml) collected from the reservoir every 5 min was stored at −80 °C. To determine the stability of bile and perfusate samples, control bile and perfusate were spiked with CPT (1 and 10 ng/ml). No significant decrease in total CPT (lactone and hydroxy-acid form) during storage for up to 14 days could be found. However, during this time there was a shift from the lactone to the hydroxy-acid form of approximately 22%. Eighty microliters of diluted bile samples and 80 μl of perfusate supernatants were injected onto the HPLC column.

A newly developed reversed-phase HPLC method was used to separate and quantify CPT and its biotransformation products. The chromatographic system included an HPLC "La Chrom" System (Merck, Darmstadt, Germany) equipped with an L-7250 injector, an L-7100 pump, an L-7300 column oven (set at 35 °C to keep the retention times constant), a D-7000 interface, and an L-7480 fluorescence detector (excitation 360 nm, emission 440 nm). Separation of CPT and its metabolites was carried out using a Hypersil BDS-C18 column (5 μm, 250×3.6 mm I.D., Astmoor, UK) preceded by a Hypersil BDS-C18 precolumn (5 μm, 10×3.6 mm I.D.) at a flow rate of 1 ml/min. The mobile phase consisted of a gradient mixed from methanol and 10 mM ammonium acetate/acetic acid buffer, pH 5.0, to elute CPT and its metabolites. Instead of converting to total CPT by addition of acid prior to HPLC analysis, pH 5.0 was chosen to separate carboxylate from lactone CPT in order to identify any possible structural modifications on the lactone ring of the CPT metabolites. These modifications would show up in the chromatogram as single peaks as they lack the ability to hydrolyze to an open carboxylate form. For any further calculations of kinetics of CPT and its biotransformation products, we then used the total amount of their corresponding lactone and hydroxy-acid forms. The column was equilibrated with 10% methanol at time 0, after injection of the sample (80 μl) the methanol content was linearly increased to 25% at 25 min. The organic content was again increased to 60% at 40 min and kept constant until 45 min. Then the percentage of methanol was decreased within 2 min to 10% to equilibrate the column for 20 min before application of the next samples. Calibration of the chromatogram was accomplished using the external standard method. As standards were not available, quantification of metabolite concentrations was based on the assumption that the unknown metabolites had a similar fluorescence intensity to CPT. Linear calibration curves were performed from the peak areas of CPT and its metabolites to the external standard CPT by spiking drug-free rat bile and perfusate with standard solutions of CPT. The standard curve for the lactone form was linear within the range 5 ng–1 μg/ml in bile and 0.1–5 ng/ml in perfusate (average correlation coefficients: > 0.97). The precision of the assay was performed with 5 different concentrations of CPT (0.1, 1, 10, 100, and 1000 ng/ml) resulting in mean inter- and intra-day variabilities of 3.1–8.7% and 2.7–10.2%, respectively, calculated as total CPT. Detection limits, defined as a signal-to-noise ratio of 3, ranged from 47 pg/ml for bile to 61 pg/ml for perfusate.

Enzymatic hydrolysis

Five microliters of the bile samples was mixed with 85 μl of 0.1 *M* sodium acetate buffer (pH 6.8), incubated with 10 μl of β-glucuronidase (10 U) or 10 μl sulfatase (5 U) at 37 °C for 1 h and the reaction was stopped by putting the vials on ice for 3 min. The samples were centrifuged at 5000 *g* for 5 min, and 80 μl of the supernatant was injected into the HPLC as described above.

Mass spectrometry

Liquid chromatography-mass spectrometry (LC/MS) measurements of CPT and its metabolites were performed using an HPLC system HP Series 1100/MSD fitted with a G1315A DAD UV-detector, a G1316A column oven (set at 35 °C), a G1313A auto sampler, a A vacuum degasser unit, and a G1311 quaternary pump (Hewlett Packard, Paolo Alto, Calif., USA). The system was coupled in line to a G1946A mass selective detector (Hewlett Packard,

Paolo Alto, Calif., USA) equipped with an atmospheric pressure chemical ionization (APCI) interface. The operating conditions were: fragmentor voltage: 50 V, gas temperature: 325 °C, vaporizer temperature 400 °C, drying gas flow: 10.0 l/min, nebulizer pressure: 20 psi, capillary voltage: 4.00 kV; corona current: 40 μA, negative ion detection. Column, mobile phase, gradient, flow rate, and injection volume were identical to those used in the analytical HPLC assay (see above).

Data analysis

Half-lives ($t_{1/2}$) and the area under the concentration-time curves (AUC) were calculated using the program PKAnalyst, Version 1.0 (MicroMath, Salt Lake City, Utah, USA). For each perfusion experiment, kinetic data were calculated as described previously [18]. The total clearance (CL) of the added drug was calculated using the following equation:

$$\mathrm{CL} = \frac{D}{\mathrm{AUC}_{(0-\infty)}} \quad (1)$$

wherein D is the dose of CPT added to the perfusate at the start of the perfusion experiment, and $\mathrm{AUC}_{(0-\infty)}$ is the corresponding area under the perfusate concentration versus time curve from time zero to infinity.

The excretion ratios (E) of CPT were calculated as the ratio of the respective total clearance to perfusate flow rate (Q):

$$E = \frac{\mathrm{CL}}{Q} \quad (2)$$

The fraction of the eliminated dose cleared by biliary excretion (BE) as CPT and its metabolite ($\mathrm{BE}_{\mathrm{CPT}}$, $\mathrm{BE}_{\mathrm{M1-M3}}$) was calculated as:

$$\mathrm{BE} = \frac{\mathrm{AUC}_{\mathrm{B}(0-90\ \mathrm{min})}}{\mathrm{CL} \times \mathrm{AUC}_{(0-90\ \mathrm{min})}} \quad (3)$$

wherein $\mathrm{AUC}_{\mathrm{B}(0-90)}$ is the AUC of CPT or its metabolites in bile elevated to 90 min, respectively, and $\mathrm{AUC}_{(0-90)}$ is the AUC of added drug in perfusate over 90 min. All data are represented as mean ± SD of three individual liver perfusions.

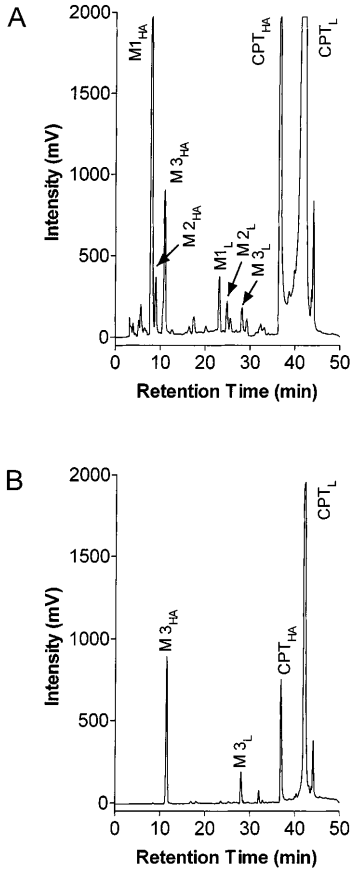

Fig. 1A, B HPLC chromatograms of camptothecin and its metabolites in bile (**A**) and perfusate (**B**), collected 60 min after continuous liver perfusion with 20 μM CPT (*M1–M3* CPT metabolites 1–3 in the lactone (*L*) and hydroxy-acid (*HA*) forms)

Results

In the isolated perfused rat liver, addition of CPT (20 μ*M*) to the perfusion medium resulted in an increase in bile flow from 0.81 ± 0.03 to 1.10 ± 0.04 mg/g liver per minute within 7 min, indicating a concentrative excretion of this anticancer drug into bile. Thereafter, bile flow slowly but continuously declined to 0.66 ± 0.03 mg/g liver per minute within 90 min. Bile samples were assessed every 5 min up to 90 min for CPT-lacton and the formation of CPT metabolites. Besides CPT, three novel metabolites (M1-M3) could be separated by HPLC (Fig. 1A). Using 10-OH CPT as a standard, we found that M1–M3 does not correspond to this putative metabolite present in *Camptotecia acuminata*. After treatment of bile samples with β-glucuronidase and sulfatase, no alteration in the HPLC profile of these metabolites was seen, ruling out phase II conjugations with glucuronic acid or sulfuric acid.

The fate of CPT and M1–M3 in the isolated perfused liver is shown in Fig. 2A, B. Biliary excretion of CPT and of M1 is rapid, reaching a maximum after 15 min (37.6 ± 16.3 and 0.94 ± 0.29 nmol/g liver per minute). Excretion rates then declined to approximately 25% after 90 min (8.7 ± 0.72, 0.25 ± 0.015 nmol/g liver per minute). The very minor metabolites M2 and M3 in bile also plateaued after 15–20 min (0.19 ± 0.023 and 0.30 ± 0.076 nmol/g liver per minute, respectively) and remained almost constant for a further 20 min. After 90 min, the excretion rate for M2 and M3 was 0.075 ± 0.004, and 0.14 ± 0.023 pmol/g liver per minute, respectively. The total amount of CPT and M1–M3 excreted in bile during the time of perfusion (90 min) represented a mean of 63.5 ± 15.4%, 1.8 ± 0.37%, 0.43 ± 0.06%, and 0.72 ± 0.15% of the dose cleared from perfusate, respectively.

During recirculating perfusion of the livers with CPT, the perfusate concentration of CPT declined in a monoexponetial manner from 19.7 ± 0.57 to 2.8 ± 0.51 μmol/l (Fig. 2D), with a mean half-life of 33.9 ± 4.2 min, showing that 85.9% of the substrate had been cleared from the perfusate within 90 min. Using Eq. 1, the total clearance of CPT from the perfusate was 20.0 ± 0.55 ml/min. Contrary to bile, metabolites M1 and M2 remained below detection limit, and only metabolite M3 could be quantified in the perfusate (Fig. 1B). Its efflux continuously increased up to 1142 ± 378 pmol/g liver per minute after 75 min

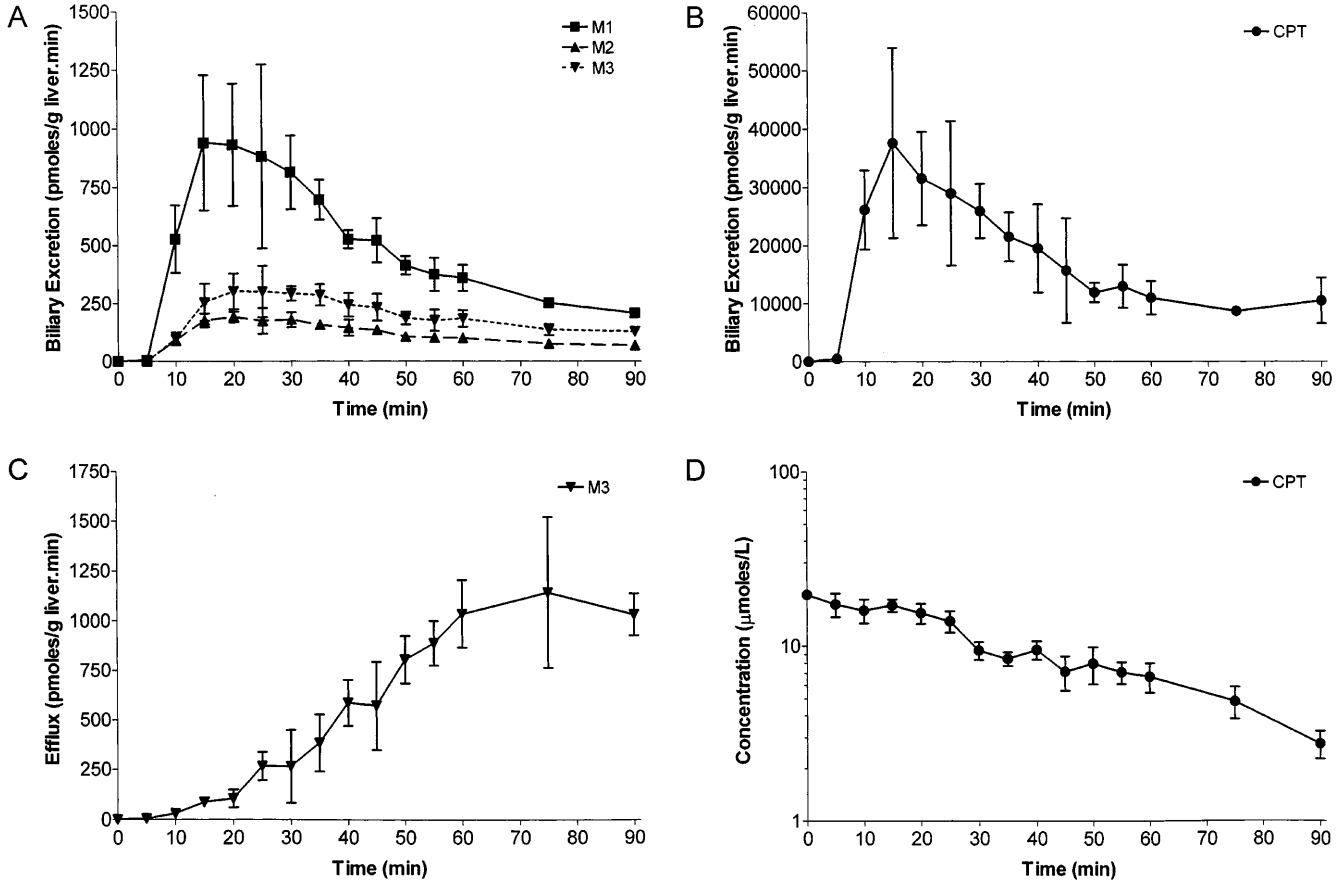

Fig. 2A–D Disposition of CPT in the isolated perfused rat liver. **A** Time course of the biliary excretion of total CPT metabolites M1–M3. **B** Time course of the biliary excretion of total CPT and **C** time course of the efflux of total CPT metabolite M3 into perfusate. **D** Mean perfusate concentration vs time profile for total CPT in the isolated liver perfusion experiments (see Materials and methods section)

(Fig. 2C). The quantity of CPT and its metabolites remaining in the liver was estimated to be 6.2 μmol and 0.3 μmol per total liver.

LC/MS analysis of bile samples showed stable molecular $[M\text{-}H]^-$-ions at m/z 347, m/z 379, m/z 379 and m/z 363 amu, in agreement with the molecular weight of CPT, dihydroxy-CPT (M1 and M2), and monohydroxy-CPT (M3). Abundant ions for CPT (m/z 303 and m/z 365), M1 (m/z 335 and m/z 397), M2 (m/z 335 and m/z 397) and M3 (m/z 319 and m/z 381), respectively, further indicate the loss of a carbon dioxide group and the hydrolysis of the lactone to the hydroxy-acid.

Discussion

The aim of our investigation was to determine the ex vitro metabolism of CPT in rat liver. The advantage of an isolated perfused liver model is that the hepatic elimination of CPT and of locally generated and preformed metabolites can be studied under controlled conditions without the confounding influence of other organs and tissues of the body. In addition, many of the physiological factors that influence the hepatic disposition of drugs and their metabolites within the intact animal, including liver blood flow and enterohepatic circulation, can be readily controlled and quantified in perfused liver systems. Using a novel HPLC system, we found that more than 63% of total CPT cleared from the perfusate during 90 min is excreted into bile in its native form together with three metabolites (M1–M3) within 10 min of starting CPT perfusion. The formation of M1–M3 in the liver is low, amounting to 2.8% of total cleared CPT. Contrary to bile, only one metabolite (M3) could be detected in the perfusate. Its cumulative efflux into the perfusate during the perfusion time, however, was about 2.5 times higher than for that of biliary excretion.

Structural identification of these biotransformation products by LC/MS analysis revealed monohydroxylation (M3) and dihydroxylation (M1–M2) of the CPT core. Hydroxylation of M3 in position 10 can be excluded, as M3 shows a different retention time in the chromatogram from 10-OH CPT. Hydroxylation of the CPT-core is in accordance with a recent study that identified hydroxylated biotransformation products of the CPT analog irinotecan in human liver microsomes [19]. However, contrary to two other minor irinotecan metabolites identified as compounds with a modified

lactone ring, all three CPT metabolites exhibited a pH-dependent equilibrium with their open carboxylate form excluding any structural alteration on this part of the molecule. Mono- and dihydroxylated CPT metabolites may be of clinical relevance as an enhanced activity, and moderate toxicity compared with CPT is observed with 10-OH CPT [20]. Contrary to SN-38, a metabolite of the CPT analog irinotecan [19], no glucuronidated metabolite of CPT could be identified after treatment of bile samples with β-glucuronidase and sulfatase. The choleresis observed during the first 15 min of CPT perfusion may result from the osmotic effect of excreted CPT. After prolonged perfusion, only a moderate decline in bile flow occurs, indicating no obvious CPT hepatotoxicity.

In conclusion, this study shows that in the rat CPT is highly excreted into bile, and phase I-metabolism only contributes to a small extent to the hepato-biliary elimination of CPT. As there are species-related differences in drug-metabolizing enzymes, particularly in those of hepatic cytochromes, the formation rate of CPT metabolites as well as the extent of their biliary excretion may be altered in man. The clinical relevance of CPT biotransformation, in particular the pharmacological or toxic effects of the novel CPT metabolites, remains to be determined.

Acknowledgements This study was supported by grants of the Austrian Academy of Science (P.P.), the OENB (5946, 6398), and the FWF (11967).

References

1. Wall ME, Wani MC, Cook CE, Palmer KH, McPhail AT, Sim, GA (1966) Plant antitumor agents. I. The isolation and structure of camptothecin, a novel alkaloidal leukemia and tumor inhibitor from *Camptotheca acuminata*. J Am Chem Soc 88: 3888
2. Pantazis P (1995) Preclinical studies of water-insoluble camptothecin congeners: cytotoxicity, development of resistance, and combination treatment. Clin Cancer Res 1: 1235
3. Ling Y-H, Perez-Soler R, Tseng MT (1993) Effect of DNA topoisomerase I inhibitor, 10-hydroxycamptothecin, on the structure and function of nuclei and nuclear matrix in bladder carcinoma MBT-2 cells. Anticancer Res 13: 1613
4. Liu LF (1995) Biochemistry of camptothecin. In: Potmesil M, Pinedo H (eds) Camptothecins: new anticancer agents. CRC Press, Boca Raton, p 9
5. Giovanella BC, Stehlin JS, Wall ME, Wani MC, Nicholas AW, Liu LF, Silber R, Potmesil M (1989) DNA topoisomerase I-targeted chemotherapy of human colon cancer in xenografts. Science 246: 1046
6. Pantazis P, Kozielski AJ, Vardeman DM, Petry ER, Giovanella BC (1993) Efficacy of camptothecin congeners in the treatment of human breast carcinoma xenografts. Oncol Res 5: 273
7. Pantazis P, Kozielski AJ, Rodriguez R, Petry E, Wall M, Giovanella BC (1994) Therapeutic efficacy of camptothecin-derivatives against human malignant melanoma xenografts. Melanoma Res 4: 5
8. Gottlieb JA, Guarino AM, Call JB, Block JB (1970) Preliminary pharmacological and clinical evaluation of camptothecin sodium (NCS 100880). Cancer Chemother Rep 54: 461
9. Muggia FM, Creaven PT, Hansen HH, Cohen MN, Selawny DS (1972) Phase I clinical trials of weekly and daily treatment with camptothecin (NSC 100880). Correlation with clinical studies. Cancer Chemother Rep 56: 515
10. 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
11. Giovanella BC, Hinz HR, Kozielski AJ, Stehlin JS, Silber R, Potmesil M (1991) Complete growth inhibition of human cancer xenografts in nude mice by treatment with 20(S)-camptothecin. Cancer Res 51: 3052
12. Potmesil M, Giovanella BC (1995) Preclinical development of 20(S)-camptothecin, 9-aminocamptothecin, and other analogues. In: Potmesil M, Pinedo H (eds) Camptothecins: new anticancer agents. CRC Press, Boca Raton, p 41
13. Potmesil M, Vardeman D, Kozielski AJ, Mendoza J, Stehlin JS, Giovanella BC (1995) Growth inhibition of human cancer metastases by camptothecins in newly developed xenograft models. Cancer Res 55: 5637
14. Stehlin JS, Natelson EA, Hinz HR, Giovanella BC, Ipolyi PD de, Fehir KM, Trezona TP, Vardeman DM, Harris NJ, Marcee AK, Kozielski AJ, Ruiz-Razura A (1995) Phase I clinical trial and pharmacokinetics results with oral administration of 20(*S*)-camptothecin. In: Potmesil M, Pinedo H (eds) Camptothecins: new anticancer agents. CRC Press, Boca Raton, p 59
15. Gupta E, Sinko P, Saleem A, Li XG, Zamek R, Goodin S, Toppmeyer D, Nathelson E, Giovanella B, Rubin E (1997) Oral multiple-dose camptothecin (CPT) pharmacokinetics: evidence of inter-patient variability. Proc Am Assoc Cancer Res 38: 75
16. Toppmeyer D, Gupta E, Saleem A, Zamek R, Much J, Ibrahim N, Bindi M, Haluska P, Li XG, Goodin S, Aisner J, Natelson E, Giovanella B (1997) A phase I pharmacokinetic and pharmacodynamic study of camptothecin (CPT) and etoposide (E) in patients with advanced malignancies. Proc Am Soc Clin Oncol 16: 203a
17. Thalhammer T, Stapf V, Gajdzik L, Graf J (1994) Bile canalicular cationic dye secretion as a model for P-glycoprotein mediated transport. Eur J Pharmacol 270: 213
18. Sallustio BC, Fairchild BA, Shanahan K, Evans AM, Nation RL (1996) Disposition of gemfibrozil and gemfibrozil acyl glucuronide in the rat isolated perfused liver. Drug Metab Dispos 42: 984
19. Lokiec F, Monegier du Sorbier B, Sanderink G-J (1996) Irinotecan (CPT-11) metabolites in human bile and urine. Clin Cancer Res 2: 1943
20. Zhang R, Li Y, Cai Q, Liu T, Sun H, Chambless B (1998) Preclinical pharmacology of the natural product anticancer agent 10-hydroxy-camptothecin, an inhibitor of topoisomerase I. Cancer Chemother Pharmacol 41: 257