Correlation between the sensitivity of tumors to treatment with CZ48 and local concentrations of the active metabolite CPT within the tumors

XING LIU, ZHISONG CAO, JOHN MENDOZA, DANA VARDEMAN and BEPPINO GIOVANELLA

Christus Stehlin Foundation for Cancer Research, Houston, TX 77025, USA

Received October 12, 2012; Accepted January 3, 2013

DOI: 10.3892/br.2013.55

Abstract. Crystalline camptothecin-20-O-propionate hydrate (CZ48) is an esterification product from the reaction of natural camptothecin with propionic anhydride. CZ48 has been tested against 29 human tumor lines grown in nude mice as xenografts. Of the tested tumor lines, 28 were found to be responsive to CZ48, by regression or significant inhibition. The total response rate was 97%. However, the effective dose required to achieve the positive response varied from 100 to 2000 mg/kg/day depending on the tumor type. Thus, the sensitivity of tumors to CZ48 treatment varied from tumor to tumor. The most sensitive CLO-breast carcinoma achieved regression when treated with 100 mg/kg/day, while PC3-prostate carcinoma required as high as 1000 mg/kg/day to achieve a definitive response. To determine the reason for these differences in sensitivities among the tumors, we treated 9 human xenografts grown in nude mice with 1000 mg/kg/day CZ48 until saturation and measured the local concentrations of the parental CZ48 as well as the corresponding metabolite camptothecin (CPT) in the tumors with the established high-performance liquid chromatography procedure. Results showed that the sensitivities of these tumors to CZ48 treatment were not affected by local concentrations of the active metabolite CPT in the tumors, but instead by the types of tumors.

Introduction

As strong inhibitors of topoisomerase 1, the derivatives of camptothecin have been thoroughly investigated by cancer researchers and clinical oncologists. Two compounds of the camptothecin family, irinotecan and topotecan, have been widely used by clinicians to treat various types of cancer (1-6). Several additional new camptothecin derivatives have been synthesized and tested pre-clinically and clinically. In our laboratory, various camptothecin were synthesized esters for biological evaluation (7-10). Crystalline camptothecin-20-O-propionate hydrate (CZ48) was prepared using an acid-catalyzed method with a high reaction yield (11). The structure of this agent is shown in Fig. 1. This compound has been tested against 29 human tumor xenografts grown in our nude mouse system thus far. Initially, in a previous study, we reported the results against the first 19 of these xenografts (12). However, further study of these xenografts has yielded 29 tumors in total. Besides one kidney tumor (28/29) with a 97% response rate, CZ48 has shown promising anticancer activity against almost every type of human tumor tested in our nude mouse system. The toxicity of this agent in mice has been found to be minimal.

From the above investigations, the effective dose (ED) with which tumor growth is inhibited >50% was found to vary widely from tumor to tumor. For instance, human breast xenograft (CLO) was treated with only 100 mg/kg/day to achieve complete growth inhibition in nude mice, whereas the human colon McCN requires a dose as high as 1000 kg/mg/day to achieve an 88.2% inhibition (12). Sensitivity of the tumors to treatment by CZ48 were relatively different. The difference in dose from the most to the least sensitive tumor for the 29 tumors tested thus far is 20-fold. CZ48 itself is not active. An enzymatic cleavage of the side ester chain of CZ48 to release the molecule of camptothecin activates the process of killing cancer cells (13,14). The esterase activity of each tumor is the determining factor for the local concentration of the active metabolite camptothecin.

The doses of a chemotherapeutic agent required for a particular treatment are calculated based on the pharmacological and toxicological data of the agent in patients obtained from clinical trials. The theoretical formula guiding the dosing of an anticancer agent is not currently available, and at present the correlation between the sensitivity of an agent to treatment and the type of tumor remains to be determined. To understand why the sensitivity of treatment with CZ48 varies widely from one tumor type to another, we used nude mice carrying human tumors as the animal model in order to determine the local concentrations of the parental CZ48 and its active metabolite

Correspondence to: Dr Zhisong Cao, Christus Stehlin Foundation for Cancer Research, 10301 Stella Link, Houston, TX 77025, USA
E-mail: zcao@stehlin.org

Key words: biodistribution, crystalline camptothecin-20-O-propionate hydrate, camptothecin, anticancer activity, metabolite, tumor tissues

corresponding metabolite camptothecin (CPT) in the tumors and major organ tissues. The assay used in this study was duplicable and consistent for the measurements. The aim of this study was to determine whether the data obtained in this study may be used for ongoing and future clinical studies.

Materials and methods

Chemicals. High-performance liquid chromatography (HPLC)-grade acetic acid, dimethyl sulfoxide (DMSO), acetonitrile, dichloromethane and diethyl ether were obtained from Sigma-Aldrich (St. Louis, MO, USA). Chromatographic-grade water was produced by a Millipore Milli-Q system (Billerica, MA, USA). CZ48 and CZ44 (internal standard for HPLC quantification) were synthesized in-house as previously described (7). Camptothecin, with a purity of 99%, was purchased from China and used according to the manufacturer's instructions.

Growth of human tumors in mice as xenografts. A tumor xenograft growing in a nude mouse (size, ~1 cm^3) was surgically removed under sterile conditions, finely minced with iridectomy scissors and suspended in a cell culture medium at a ratio of 1:10, v/v. One-tenth to one quarter of 1 ml of this suspension, containing ~50 mg of wet-weight tumor mince, was subcutaneously inoculated into the upper half of the dorsal thorax of the mouse. Groups of female, non-inbred Swiss nude mice,(17 groups, 4 animals in each group) with a weight of ~25 g, were used.

Tissue sample preparation. Human tumors PC3, MIA, SW48, CLO, DOY, MUR, HT29, SQU and SU86 were individually grown in each group of mice as xenografts. Tumors growing in animals were monitored daily and measured using a caliper two times per week. Treatment with 1000 mg/kg/day CZ48 was initiated when the tumor reached a volume of ~80 mm^3, i.e., well-vascularized and measurable. CZ48 was finely suspended in cottonseed oil. Each group of animals was treated once a day by gavage with the above dose. The animals were sacrificed at the pre-determined time points, and tumor and major organ tissue samples were then collected surgically. Following a series of processing, the tissue samples were extracted a number of times and the extracts obtained from each animal were combined and frozen until analysis.

Measurement of CZ48 and CPT. Animal extracts were analyzed using HPLC. The detailed procedure for analyzing the concentrations of CZ48 and CPT was described in a previous study (15). Experiments were performed by following the protocol approved by the Institutional Animal Care and Use Committee.

Results and Discussion

To obtain the time point when the steady-state concentrations (or saturation) of CZ48 and CPT in tumor tissues was reached, 3 groups of nude mice carrying DOY lung carcinoma xenografts were treated with 1000 mg/kg/day of CZ48 by gavage, for 2, 4 and 6 days, respectively. At each time point, a group of animals was sacrificed and tumor tissues were collected and processed for analysis (Table I).

Table I. Accumulation of CPT and CZ48 in tumor tissues of nude mice lung (DOY) human xenografts after daily oral treatment with 1000 mg/kg CZ48 for 2, 4 and 6 days.

Day	CZ48 (ng/mg tissue) ± SD	CPT (ng/mg tissue) ± SD
2	218.15±77.90	35.97±4.10
4	218.17±73.06	31.31±4.12
6	191.66±60.76	27.86±3.61

CPT, corresponding metabolite camptothecin; CZ48, crystalline camptothecin-20-O-propionate hydrate; SD, standard deviation.

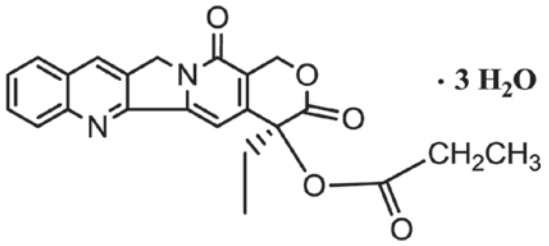

Figure 1. Molecular structure of crystalline camptothecin-20-O-propionate hydrate (CZ48).

To evaluate the sensitivity of tumors to CZ48 treatment, 9 groups of the tumor-carrying mice were treated with 1000 mg/kg of CZ48 daily for 5 days. The animals were then sacrificed and their tumor tissues were collected and extracted. The local concentration of CZ48 and its active metabolite CPT in tumors were determined using an established HPLC procedure (Table II). Measurement of the biodistributions of CZ48 in major organs of mice was also performed. The local concentrations of CZ48 and its metabolite CPT in the brain, heart, kidney, liver, lung and spleen, are shown in Table III.

To compare the concentrations of CZ48 and CPT in tumor tissues with those in plasma, a group of mice carrying LIE pancreatic carcinoma was treated with 100 mg/kg/day for two weeks and then sacrificed for analysis (Table IV).

Previously, we reported the results obtained against the first panel of 19 xenografts (12). ED is commonly defined as the amount of drug required to completely inhibit the growth of tumors in animals. The EDs required for these 9 human xenografts grown in nude mice in this study ranged from 100 to 1000 mg/kg/day. The correlation between the tumor lines and the EDs is shown in Table V. The sensitivity of a particular tumor type to chemotherapy was also assessed by the amount of agent used; thus, the lower the dose of an agent required to inhibi tumor growth, the more sensitive the tumor is to the therapy. Different types of tumors may have different sensitivities to the same agent. The quantitative correlation of the relationship between the types of tumors and their required EDs for a particular agent is difficult to achieve due to the various number of factors involved. However, the reason for which EDs of CZ48 required for these 9 human xenografts varied from 100 to 1000 mg/kg/day may

Table II. Accumulation of CPT and CZ48 in tumor tissues of nude mice carrying human xenografts following oral treatment with 1000 mg/kg/day for 5 days.

Tumor line	CPT (ng/mg tissue) ± SD	CZ48 (ng/mg tissue) ± SD	Ratio of CPT/CZ48
Breast-CLO	12.60±3.87	122.51±7.92	0.10
Breast-MUR	14.32±2.49	78.16±16.84	0.18
Colon-HT29	18.30±5.80	45.44±3.36	0.40
Colon-SQU	20.16±1.78	50.51±9.26	0.40
Colon-SW48	10.30±2.92	82.85±6.37	0.12
Lung-DOY	29.58±7.47	121.16±10.73	0.24
Pancreatic-MIA	13.35±1.46	72.66±3.54	0.18
Pancreatic-SU86	9.53±3.96	126.14±5.15	0.08
Prostate-PC3	24.17±6.82	34.01±6.20	0.71

CPT, corresponding metabolite camptothecin; CZ48, crystalline camptothecin-20-O-propionate hydrate; SD, standard deviation.

Table III. Biodistribution of CZ48 and its metabolite CPT in major organs.

Organ	CPT (ng/mg tissue) ± SD	CZ48 (ng/mg tissue) ± SD	Ratio of CPT/CZ48
Brain	15.98±17.17	92.91±56.90	0.17
Heart	19.37±6.41	141.32±50.67	0.14
Kidney	193.70±131.62	337.13±11.63	0.57
Liver	129.52±38.62	486.31±142.57	0.27
Lung	32.27±16.01	87.65±25.02	0.37
Spleen	37.29±1.61	178.69±54.13	0.21

CZ48, crystalline camptothecin-20-O-propionate hydrate; CPT, corresponding metabolite camptothecin; SD, standard deviation.

Table IV. Comparison of concentration levels of CZ48 and CPT between plasma and tumor tissues of mice following treatment with effective doses of CZ48.

Group	Organ	CPT (ng/mg plasma) ± SD	CZ48 (ng/mg tissue) ± SD	CPT/CZ48
Pancrea-LIE	Plasma	4.49±1.59	61.77±26.94	0.07
	Tumor	15.29±6.27	119.75±38.26	0.13

CZ48, crystalline camptothecin-20-O-propionate hydrate; CPT, corresponding metabolite camptothecin; SD, standard deviation.

Table V. The EDs required for 9 human xenografts and the calculated local CPT and CZ48 concentrations according to the conversion ratios listed in Table II.

Lines	CLO	MUR	HT29	SQU	SW48	DOY	MIA	PC3	SU86
EDs (mg/kg) (ref. 12)	100	300	300	300	200	200	1000	1000	1000
CZ48 (ng/mg tissue)[a]	12.25	23.45	13.63	15.15	16.57	24.23	72.66	34.01	126.14
CPT (ng/mg tissue)[b]	2.20	4.22	5.45	6.06	1.99	5.82	13.08	24.15	10.08

[a]Calculated CZ48 concentration = ED x concentration of measured CZ48 (listed in Table II)/1000. [b]Calculated CPT concentration = Calculated CZ48 x ratio of CPT/CZ48 (listed in Table II). EDs, effective doses; CPT, corresponding metabolite camptothecin; CZ48, crystalline camptothecin-20-O-propionate hydrate.

be understood better by studying the biodistribution of this agent in tumor tissues and major organs. Therefore, 9 tumor lines, breast-CLO, breast-MUR, colon-HT29, colon-SQU, colon-SW48, lung-DOY, pancreatic-MIA, prostate-PC3 and pancreatic-SU86, were selected for the biodistribution investigations in this study.

To establish the time point of drug saturation for the biodistribution studies, the tumor tissues of groups of animals were collected at 3 time points of 2, 4 and 6 days, respectively. No time point was selected prior to day 2 considering that any shorter duration was probably insufficient for CZ48 to saturate the mouse body. The results showed that the concentration of CZ48 in tumor tissues of the mice carrying DOY reached the steady state (i.e., saturation) after 2 days of treatment and thus, any time points after 2 days were acceptable for the biodistribution studies (Table I). We chose 5 days for the duration of treatment, ensuring sufficient drug-saturation and a convenient work schedule. A total of 9 human tumors were xenotransplanted into 9 groups of nude mice, with 4 mice per tumor group. After reaching 80 mm^3 in tumor size, treatment with 1000 mg/kg/day of CZ48 for each group was initiated and continued for 5 days. The animals were sacrificed 1 h following the fifth administration and their tumor tissues were collected and processed for analysis.

The data in Table II were obtained by performing the HPLC procedure on tissue samples, and the results clearly demonstrate that the local concentrations of CZ48 in tissues of breast-CLO, lung-DOY and pancreatic-SU86 are much higher compared to the remaining tumor lines. The concentrations of CPT, the active metabolite, were in the range of 10-20 ng/mg tissue, considering the standard deviations. The data in Table II also suggest that the esterase activity varies depending on the tumors. Prostate-PC3 was found to have the highest esterase activity in this panel of tumors due to its exhibiting the highest CPT/CZ48 ratio, while the pancreatic tumor line SU86 showed the least esterase activity with a CPT/CZ48 ratio of only 0.08. The difference in esterase activity in the two breast tumor lines, CLO and MUR, is small (0.10 vs. 0.18). Two colon lines, HT29 and SQU, revealed an identical esterase activity level. Uptake of CZ48 by Br-CLO, lung-DOY and pan-SU86 were better compared to other lines in the panel (Table II). Prostate-PC3 had the least uptake and the highest cleavage. The EDs of CZ48 required for the 9 tumor lines listed in Table V range from 100 to 1000 mg/kg/day. The calculated local concentrations of CPT relative to EDs range from 1.99 to 24.15 ng/mg tissue. These results clearly demonstrate that breast-CLO and colon-SW48 tumor lines are the most sensitive to CZ48 treatment, requiring only 2 ng/mg tissue of the active metabolite to achieve complete inhibition. Prostate-PC3 and the two pancreatic lines MIA and SU86 were found to be the least sensitive group in this panel, ~5- to 10-fold less sensitive compared to the group of CLO and SW48, requiring ≥10 ng/mg tissue of the active CPT for the same level of inhibition. The four lines, breast-MUR, colon-HT29 and SQU and lung-DOY, were similar in the level of sensitivity to the treatment, requiring 4-6 ng/mg tissue of the active CPT to inhibit tumor growth.

The biodistribution of CZ48 among major organs including brain, heart, kidney, liver, lung and spleen were also measured. Liver tissues contained the highest local concentrations of CZ48 (Table III). The kidney tissue was also detected to have a high concentration of CZ48. Kidney esterase(s) showed great ability of transferring CZ48 into CPT (CPT/CZ48 ratio, 0.57). Lung tissue also demonstrated good esterase activity with a CPT/CZ48 ratio of 0.37. Thus, physicians would need to pay attention to potential renal toxicity induced by high kidney conversion of CZ48 into CPT. Of note, 2 patients, enrolled in an early phase 1 trial (unpublished data), were found to have light grades of bladder toxicity. Increased hydration in these patients solved the problem.

The comparison of concentrations of CZ48 and CPT in plasma and tumor tissues was performed using the pancreatic-LIE line. The results indicate that concentrations of CZ48 and CPT for this tumor line were much higher in tumor tissues compared to those in plasma (Table IV). CZ48 was stable in plasma with an extremely low CPT/CZ48 ratio (0.07).

In conclusion, the EDs of CZ48 required to achieve complete inhibition of tumor growth are influenced by the properties of each tumor: the sensitivity to CZ48 and the esterase activity (i.e., cleavability). The ratios of CPT/CZ48 in tumor tissues range from 0.08 to 0.71, almost 9 times, for the panel of 9 assayed tumor tissues, suggesting that the activity of esterase varies widely from tumor to tumor. The most sensitive tumors only need ~2 ng/mg tissue of the active metabolite to kill cancer cells, while one prostate and two colon lines required at least 10 ng/mg tissue of the active CPT to achieve the cell-killing. Major organs, such as liver and kidney, contain high local concentrations of CZ48. The kidney esterase(s) has the highest cleavability of converting CZ48 into CPT. Hydration with water or juices is highly recommended for patients enrolled in the clinical trials to avoid urinary tract toxicity when treated with CZ48.

Acknowledgements

The authors would like to thank the Christus Stehlin Foundation for Cancer Research and the Friends of the Stehlin Foundation for Cancer Research for their financial support.

References

1. ten Bokkel Huinink W, Gore M, Carmichael J, Gordon A, Malfetano J, Hudson I, Broom C, Scarabelli C, Davidson N, Spanczynski M, Bolis G, Maimstrom H, Coleman R, Fields C and Heron F: Topotecan versus paclitaxel for the treatment of recurrent epithelial ovarian cancer. J Clin Oncol 15: 2183-2193, 1997.
2. Schiller JH, Adak S, Cella D, DeVore RF III and Johnson DH: Topotecan versus observation after cisplatin plus etoposide in extensive-stage small-cell lung cancer: E7593-a phase III trial of the Eastern Cooperative Oncology Group. J Clin Oncol 19: 2114-2122, 2001.
3. von Pawel J, Schiller JH, Shepherd FA, Fields SZ, Kleisbauer JP, Chrysson NG, Stewart DJ, Clark PI, Palmer MC, Depierre A, Carmichael J, Krebs JB, Ross G, Lane SR and Gralla R: Topotecan versus cyclophosphamide, doxorubicin, and vincristine for the treatment of recurrent small-cell lung cancer. J Clin Oncol 17: 658-667, 1999.
4. Pizzolato JF and Saltz LB: The camptothecins. Lancet 361: 2235-2242, 2003.
5. Saltz LB, Cox JV, Blanke C, Rosen LS, Fehrenbacher L, Moore MJ, Maroun JA, Ackland SP, Locker PK, Pirotta N, Elfring GL and Miller LL: Irinotecan plus fluorouracil and leucovorin for metastatic colorectal cancer. N Engl J Med 343: 905-914, 2002.

6. Douillard JY, Cunningham D, Roth AD, Navarro M, James RD, Karasek P, Jandik P, Iveson T, Carmichael J, Alakl M, Gruia G, Awad L and Rougier P: Irinotecan combined with fluorouracil compared with fluorouracil alone as first-line treatment for metastatic colorectal cancer: a multicenter randomized trial. Lancet 355: 1041-1047, 2000.
7. Cao Z, Harris N, Kozielski A, Vardeman D, Stehlin J and Giovanella B: Alkyl esters of camptothecin and 9-nitrocamptothecin: Synthesis, in vitro pharmacokinetics, toxicity, and antitumor activity. J Med Chem 41: 31-37, 1998.
8. Cao ZS, Mendoza J, Dejesus A and Giovanella B: Synthesis and anti-tumor activity of alkenyl camptothecin esters. Acta Pharmacol Sin 26: 235-241, 2005.
9. Cao Z, Mendoza J, Dejesus A, Vardeman D and Giovanella B: Synthesis and antitumor activity of aromatic camptothecin esters. Int J Mol Med 21: 477-487, 2008.
10. Cao Z, Mendoza J, Kozielski A, Liu X, Dejesus A, Wang Y, Zhan CG, Vardeman D and Giovanella B: Anticancer activity of new haloalkyl camptothecin esters against human cancer cell lines and human tumor xenografts grown in nude mice. Anticancer Agents Med Chem 12: 818-828, 2012.
11. Cao Z, Kozielski A, Harris N, Vardeman D and Giovanella D: Sulfuric acid catalyzed preparation of alkyl and alkenl camptothecin ester derivatives and antitumor activity against human xenografts grown in nude mice. Open J Med Chem 2: 10-14, 2012.
12. Cao Z, Kozielski A, Liu X, Wang Y, Vardeman D and Giovanella B: Crystalline camptothecin-20(S)-O-propionate hydrate: a novel anticancer agent with strong activity against 19 human tumor xenografts. Cancer Res 69: 4742-4749, 2009.
13. Cao ZS, Pantazis P, Mendoza J, Early J, Kozielski A, Harris N and Giovanella B: Structure-activity relationship of alkyl 9-nitrocamptothecin esters. Acta Pharmacol Sin 24: 109-119, 2003.
14. Cao Z, Pantazis P, Mendoza J, Early J, Kozieslki A, Harris N, Vardeman D, Liehr J, Stehlin J and Giovanella B: Structure-activity relationship of alkyl camptothecin esters. Annu N Y Acad Sci 992: 122-135, 2000.
15. Liu X, Wang Y, Vardeman D, Cao Z and Giovanella B: Development and validation of a reverse-phase HPLC with fluorescence detector method for simultaneous determination of CZ48 and its active metabolite camptothecin in mouse plasma. J Chromatogr B Analyt Technol Biomed Life Sci 867: 84-89, 2008.