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ORIGINAL ARTICLE

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Assessment of normal and tumor tissue uptake of MAG-CPT, a polymer-bound prodrug of camptothecin, in patients undergoing elective surgery for colorectal carcinoma

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Abstract *Purpose*: MAG-camptothecin (MAG-CPT) is the lead compound of a novel drug delivery system in which an active cytotoxic moiety, camptothecin (CPT), is covalently linked to a soluble polymeric carrier (MAG) to form an inactive prodrug. The mechanism of action of CPT remains unaltered, but the delivery system is thought to allow the carrier-bound drug to accumulate in tumor tissues and release the active CPT locally. This proof-of-concept clinical study was designed to determine whether MAG-CPT was preferentially delivered to or retained in tumor tissue compared to adjacent normal tissue or plasma, and to estimate the degree of intratissue release of CPT. *Methods*: This was an open, non-randomized study in ten adult patients scheduled for elective surgery for colorectal cancer. Patients received a single dose of 60 mg/m^2 (CPT equivalent) of MAG-CPT 24 h, 3 days or 7 days prior to surgery. Plasma, tumor, and adjacent normal tissue samples were collected simultaneously at the time of surgery and analyzed for MAG-bound and released CPT concentrations. *Results*: MAG-bound and free CPT concentrations in plasma, tumor, and normal tissue achieved equilibrium by 24 h after dosing, declining in parallel up to 7 days after dosing. MAG-bound CPT was delivered to similar levels to tumor and normal tissue. At 24 h after dosing, the mean ± SD MAG-bound CPT concentrations were 861 ± 216 ng/g in tumor and 751 ± 215 ng/g in adjacent normal tissue, and free CPT concentrations were lower in tumor than in normal tissue (12.2 ± 4.7 ng/g and 21.9 ± 6.7 ng/g, respectively). At 24 h after dosing, mean ± SD ratios of MAG-bound and free CPT in tumor and plasma were 0.13 ± 0.03 and 0.22 ± 0.09, respectively, and the ratios did not change for up to 7 days after dosing, indicating a lack of preferential retention of MAG-bound CPT or release of free CPT in tumor. These results are in marked contrast to previous data from animal tumor xenograft studies, where MAG-CPT levels were higher in tissue than in plasma at 3 and 7 days after a single i.v. dose. *Conclusions*: Delivery of CPT to the target tumor tissue is achievable by means of the MAG-CPT polymer-bound delivery system, with the equilibrium between plasma and tumor tissue concentrations of released CPT being established within 24 h after dosing. However, preferential retention of MAG-bound or released CPT in the tumor relative to normal tissue or plasma was not detected during the 7 days after dosing. The methods

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employed in our study could be of use in making "go/no-go" decisions on further development of anticancer drugs.

Keywords Camptothecin · Polymer-bound · Prodrug · Pharmacokinetics · Tissue concentrations · MAG-CPT

Introduction

Administration of maximum tolerated doses (MTD) of cytotoxic drugs, aimed at maximizing cytoreduction and minimizing drug resistance, results in considerable toxicity to the patient [27]. Thus, there is the need to better target drugs to the site of action, in order to increase antitumor efficacy while minimizing toxicity to normal tissues and increasing antitumor efficacy.

MAG-CPT (methacryloyl-glycyl-aminohexanoyl-glycyl-camptothecin) is a prodrug formulation developed to more effectively deliver the cytotoxic agent, camptothecin (CPT), to solid tumors [5]. The molecule consists of CPT (approximately 10% w/w) covalently bound through a glycyl-aminohexanoyl-glycyl spacer to a soluble polymer (*N*-[2-hydroxypropyl]-methacrylamide, HPMA) to form an inactive macromolecular system with a mean molecular weight of 20 kDa and a polydispersivity of 1.5 [23]. MAG-CPT was designed to release active CPT at the tumor site by esterolytic and proteolytic cleavage.

The design of this polymeric prodrug was based on previous experimental evidence that large molecules such as antibodies, polymers, and polymer-drug conjugates accumulate in tumors [15, 33]. In addition, solid tumors have an atypical vascular system with a discontinuous endothelium that facilitates macromolecular extravasation, and reduced lymphatic drainage, which prevents clearance of penetrated macromolecules [2, 14, 30]. Both these effects were postulated to contribute to the preferential accumulation and retention of macromolecular drugs in tumors compared to normal tissues, thus increasing the local antitumor effect while reducing the systemic toxicity of the free drug [22].

Although the chemotherapeutic mechanism of action of MAG-CPT is considered equivalent to that of CPT (i.e., inhibition of topoisomerase I), the compound differs profoundly from the original CPT in terms of pharmacologic, toxicologic, and pharmacokinetic behavior. CPT exhibits notable toxicity, characterized by hemorrhagic cystitis, myelosuppression, and gastrointestinal toxicity [6, 11, 24, 25]. Most of the toxicity is caused or exacerbated by the nonspecific systemic effects of CPT, which shows no selective uptake or retention in tumor. In contrast, the MAG-CPT delivery system is thought to allow the carrier-bound drug to accumulate in tumor tissues and release the active CPT locally. Preclinical studies with MAG-CPT in xenografted human colon, mammary, ovarian, melanoma, and non-small-cell lung cancer tumors in mice have indicated that the drug accumulates preferentially in tumor, especially at its more vascularized areas, and that CPT is released from carrier-bound drug inside the tumor mass (data on file, Pharmacia). MAG-CPT at doses of 15 to 22.5 mg/kg (CPT equivalent) demonstrated strong cytotoxic activity against these tumors, with up to 100% of tumor growth inhibition, cure, inhibition of distant metastases, and decreased toxicity, compared to CPT, which did not produce any cure and was toxic to animals at doses of 12.5 mg/kg [34]. However, three phase I clinical trials of MAG-CPT in a total of 50 patients with advanced cancer have provided no conclusive indication of antitumor activity of the drug despite dosing up to levels of dose-limiting hematological and gastrointestinal toxicity (240 mg/m^2 CPT equivalent) [7].

Consequently, this study in patients with colorectal carcinoma undergoing elective surgery was designed to compare the delivery of MAG-CPT and the release of active moiety in tumor, adjacent normal tissue, and plasma by simultaneous measurement of MAG-bound and released CPT concentrations in these tissues at different time-points after a single intravenous (i.v.) dose of the drug, administered at various times before surgery. A MAG-CPT dose of 60 mg/m^2 CPT equivalent (25% of MTD) was selected for the study based on its favorable safety profile in phase I trials in patients with refractory solid cancer [7].

Patients and methods

Study design

This open, non-randomized study was conducted at two clinical sites in Sweden and Germany in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice. The population consisted of adult patients scheduled for elective surgery for primary or locally recurrent colorectal cancer (confirmed histologically or by double-contrast barium enema scan). Patients had to have adequate bone marrow, liver, and renal function at screening to be eligible for participation in the study: neutrophil count ≥2000/mm^3, platelet count ≥100,000/mm^3, hemoglobin concentration ≥11 g/l, normal serum bilirubin, serum SGOT and SGPT not more than three times the upper limit of the normal range, and serum creatinine < 1.5 mg/dl (< 133 μmol/l). Patients could not receive chemotherapy or radiotherapy within 4 weeks of dosing in this study. Prior to enrollment, all patients provided written informed consent, approved by the independent local ethics committees.

Eligible patients were enrolled into three treatment groups according to the scheduled time of surgery and received MAG-CPT 24 h, 3 days, or 7 days prior to elective surgery for colorectal cancer. MAG-CPT was administered as a single dose of 60 mg/m^2 CPT equivalent given as a 30-min i.v. infusion. Patients were monitored after surgery on an inpatient and ambulatory basis. During surgery, the colorectal cancer was extirpated in a block of tissue that included adjacent normal colon tissue.

Safety evaluations at screening and during the study included a physical examination, blood pressure and heart rate, 12-lead electrocardiograms, clinical laboratory tests (hematology, biochemistry, and Dipstick urinalysis), and adverse event monitoring throughout the study.

Tissue sampling and assessments

Blood samples for concentrations of MAG-bound and free CPT in plasma were taken at the end of the MAG-CPT i.v. infusion and within 15 min of extirpation of the tumor.

Immediately after surgical removal, tissue residual blood and intestinal contents were washed, and at least 1 cm^3 of solid non-necrotic tumor tissue and a sample of normal gut wall (at least 2 cm^2 in size and at least 2 cm away from the tumor) were removed by a pathologist. The samples were obtained within 30 min after surgical excision. Tissue samples were frozen at −80°C immediately upon dissection to minimize the ex-vivo release of free CPT from the polymer prior to assay. At the time of assay, samples of tumor and normal tissue were homogenized and extracts were analyzed for the determination of MAG-bound and free CPT levels.

The proportion of necrosis in the tumor tissue and the malignant cellularity were assessed by the pathologist. The extent of tumor necrosis was expressed as a percentage of tissue involved and determined from the proportion of necrotic cells in ten microscopic visual fields of the tumor tissue (×10 magnification). The malignant cellularity was estimated from the proportion of malignant cells in ten microscopic visual fields of the tumor tissue (×10 magnification). Tumor tissue was classified as having low (less than one-third of cells), medium (between one-third and two-thirds of cells), or high (more than two-thirds cells) malignant cellularity.

Bioanalytical methods

Plasma CPT

Fully validated HPLC methods with fluorescence detection were used for measurement of MAG-bound and free CPT in plasma [9, 29]. Concentrations of free (released) CPT were determined directly in the stabilized plasma samples and included both the fractions bound and unbound to plasma proteins. The concentrations of total (free plus MAG-bound) CPT were determined following alkaline hydrolysis of MAG-CPT. MAG-bound CPT concentrations were calculated by subtracting the free from the total CPT levels. The assay was linear over the ranges of approximately 1–2500 ng/ml for free CPT and 120–480,000 ng/ml for total CPT. Within- and between-day variation was 4–13% for free CPT and 11.5–15.7% for total CPT.

Tissue CPT

Following homogenization, concentrations of total and free CPT in tissue and tumor samples were determined using similar HPLC methods to those utilized for plasma. The assays were linear over the ranges of approximately 2–2000 ng/g for free CPT and 100–200,000 ng/g for total CPT, and analyte recovery, which was assessed in animal tissues due to difficulty in obtaining control human tissue, was good (> 70%).

Statistical methods

MAG-bound and free CPT data are presented using descriptive statistics. By-subject ratios (tumor versus normal tissue, tumor versus plasma, and normal tissue versus plasma) were calculated for MAG-bound and free CPT.

Results

Patient and disease characteristics

Of the ten patients enrolled (seven males and three females, mean age 64.3 years, mean body weight 83.8 kg), two had rectal adenocarcinoma, seven had adenocarcinoma of the colon (five colon ascendens, two sigmoid colon) and one had colorectal cancer with location not detailed (Table 1). None of the patients had a history of prior surgery, radiotherapy, or chemotherapy for cancer.

Tumor homogeneity was mixed in the 24-h group (0, < 5, 20, 20 and 30% necrotic tumor cells; one patient with less than one-third of cells malignant, three patients with one-third to two-thirds malignant, and one patient with more than two-thirds malignant), while the tumors in the 3- and 7-day groups were more homogeneous (all with 10% necrotic cells; one patient with less than one-third and one patient with more than two-thirds malignant cells in the 3-day group; one patient

Table 1 Patient characteristics

Characteristic	Treatment group			
	24 h ($n=5$)	3 days ($n=2$)	7 days ($n=3$)	Total ($n=10$)
Age (years)				
Mean	66.0	70.5	57.3	64.3
Range	59–74	66–75	54–61	54–75
Race (n)				
White	5	2	3	10
Gender				
Female (n)	2	0	1	3
Male (n)	3	2	2	7
Weight (kg)				
Mean	78.6	92.0	87.0	83.8
Range	67.5–104.1	82–102	75–104	67.5–104.1
Tumor location (n)				
Ascending colon	2	1	2	5
Sigmoid colon	0	1	1	2
Rectum	2	0	0	2
Unknown	1	0	0	1

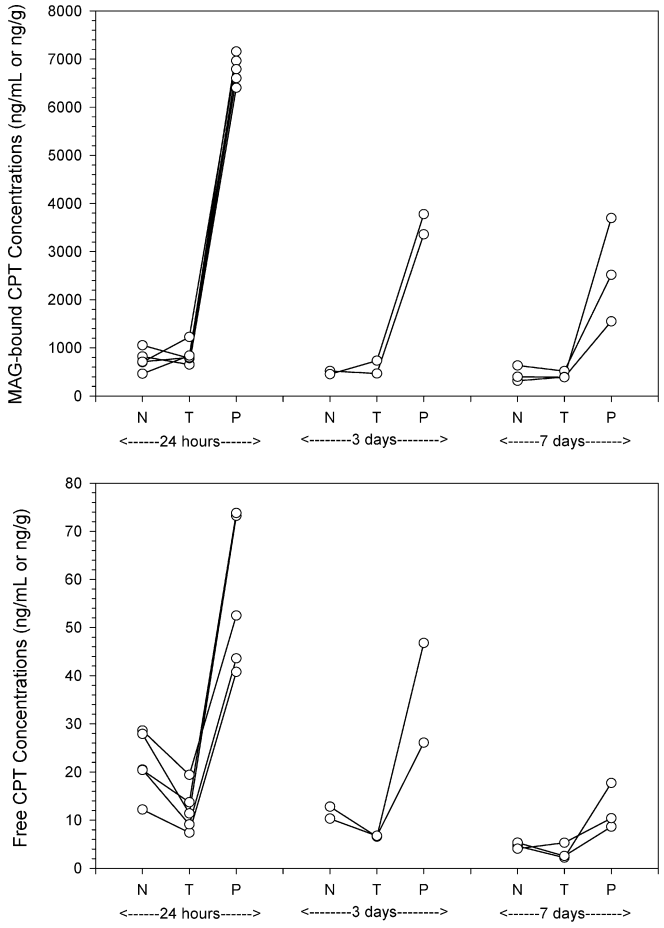

Fig. 1a, b Concentrations of MAG-bound CPT (**a**) and free CPT (**b**) for individual patients in normal tissue (*N*), tumor (*T*), and plasma (*P*), by treatment group. *Solid lines* connect data from individual patients

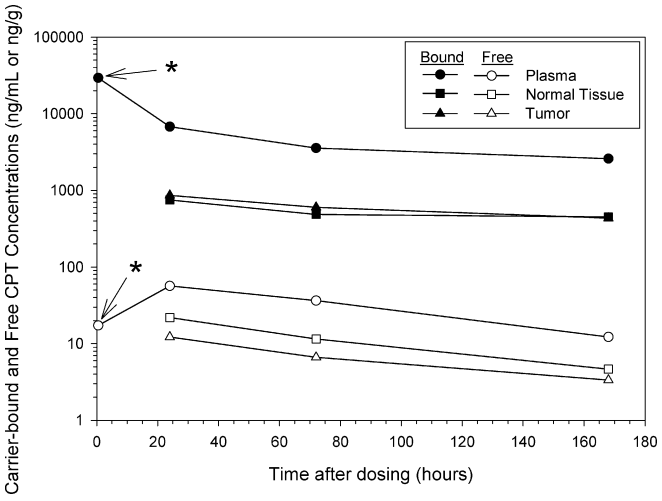

Fig. 2 Mean concentrations of MAG-bound and free CPT as a function of time after dosing (*concentrations measured at the end of infusion)

with one-third to two-thirds and two patients with more than two-thirds of malignant cells in the 7-day group).

Concentrations of MAG-bound and free CPT in plasma and tissues

MAG-bound CPT was successfully delivered to the tumor tissue, albeit to levels similar to those in normal tissue (Fig. 1 and Table 2). At 24 h after dosing, the mean ± SD MAG-bound CPT concentrations were 861 ± 216 ng/g in tumor and 751 ± 215 ng/g in normal tissue. However, the concentrations of free CPT were lower in tumor than in normal tissue at all time-points after dosing (24 h to 7 days). Tumor to normal tissue concentration ratios of both MAG-bound and free CPT remained similar from 24 h up to 7 days after dosing (Table 3).

The concentrations of both MAG-bound and free CPT were much lower in tumor and normal tissue than in plasma at all time-points after dosing (24 h to 7 days; Fig. 1 and Table 3).

In a subgroup analysis, tumor to normal tissue ratios of MAG-bound and free CPT were compared between patients with low tumor necrosis and high malignant cellularity (0–10% necrosis, more than two-thirds of cells malignant; $n = 4$) versus patients with low to medium necrosis and low to medium cellularity (< 5–30% necrosis, less than one-third to two-thirds of cells malignant; $n = 6$), regardless of their treatment group. The ratios for MAG-bound CPT were similar between the two subgroups (mean ± SD ratio of 1.15 ± 0.38 for the low necrosis/high cellularity group and 1.20 ± 0.47 for the low–medium necrosis/low–medium cellularity

Table 2 Mean (range) concentrations of MAG-bound and free CPT in plasma, tumor, and normal tissue (*N/A* not applicable)

		End of infusion ($n = 9$)	Treatment group		
			24 h ($n = 5$)	3 days ($n = 2$)	7 days ($n = 3$)
Plasma	MAG-bound CPT (ng/ml)	29,378 (22,022–36,088)	6783 (6403–7157)	3568 (3777–3358)	2588 (1550–3697)
	Free CPT (ng/ml)	17.3 (9.36–30.1)	56.8 (40.8–73.8)	36.5 (26.1–46.8)	12.2 (8.63–17.7)
	Ratio (bound/free)	2027 (732–2986)	127 (90–164)	108 (72–145)	240 (142–428)
Normal tissue	MAG-bound CPT (ng/g)	N/A	751 (462–1054)	486 (454–519)	451 (317–635)
	Free CPT (ng/g)	N/A	21.9 (12.2–28.6)	11.6 (10.3–12.8)	4.66 (4.06–5.31)
	Ratio (bound/free)	N/A	37 (23–67)	42 (41–44)	99 (60–138)
Tumor	MAG-bound CPT (ng/g)	N/A	861 (653–1226)	602 (467–736)	434 (389–517)
	Free CPT (ng/g)	N/A	12.2 (7.39–19.4)	6.64 (6.54–6.74)	3.35 (2.21–5.29)
	Ratio (bound/free)	N/A	74 (61–88)	90 (71–109)	154 (74–234)

Table 3 Descriptive statistics for MAG-bound and free CPT concentration ratios

		Treatment group					
		24 h ($n=5$)		3 days ($n=2$)		7 days ($n=3$)	
		Mean	Range	Mean	Range	Mean	Range
Normal tissue/plasma[a]	MAG-bound CPT	0.11	0.07–0.16	0.14	0.12–0.15	0.20	0.09–0.26
	Free CPT	0.39	0.28–0.54	0.33	0.27–0.39	0.42	0.26–0.62
Tumor/plasma[a]	MAG-bound CPT	0.13	0.10–0.18	0.17	0.14–0.19	0.19	0.11–0.25
	Free CPT	0.22	0.16–0.37	0.20	0.14–0.26	0.31	0.12–0.51
Tumor/normal tissue	MAG-bound CPT	1.25	0.74–1.82	1.26	0.90–1.62	1.01	0.81–1.25
	Free CPT	0.56	0.41–0.68	0.58	0.51–0.65	0.76	0.48–1.30

[a]Plasma concentrations at time of tissue removal

group). For free CPT, the tumor to normal tissue ratios were slightly higher (0.71 ± 0.41) for the low necrosis/high cellularity group than for the low–medium necrosis/low–medium cellularity group (0.57 ± 0.10).

The concentrations of both MAG-bound and free CPT in tissues and plasma declined slowly in a parallel fashion from 24 h after dosing (Fig. 2).

Safety results

A single i.v. dose of 60 mg/m^2 of MAG-CPT was safe and well tolerated when administered between 1 to 7 days prior to major elective surgery. All patients enrolled in the study recovered from surgery within a normal timeframe and without any complications related to participation in this study. There were no deaths, serious adverse events, or withdrawals due to adverse events. The majority of clinical adverse events reported were of mild or moderate severity and most were considered by the investigators not to be related to MAG-CPT. There were no clinically significant changes in laboratory results, vital signs, or electrocardiograms.

Discussion

The focus of medical cancer treatment has shifted towards designing rational mechanism-based strategies, including optimal biologically effective dosing, drug targeting, and individually tailored therapy to achieve therapeutic activity with lower systemic toxicity [3, 28]. Better therapeutic activity and reduced toxicity of anticancer drugs may be achieved by selectively delivering the drug to the tumor tissue, such that tumor exposure to drug is increased but systemic exposure of normal tissue is reduced. Improved targeting is being attempted by physical or chemical modifications of the drug molecule or formulation, such as development of alternative formulations (e.g., liposomes or polymeric delivery systems, improvement of stability, solubility, or drug release characteristics) [1, 4, 12], chemical derivatives and analogs based on structure-activity relationships (to improve activity and receptor binding) [17], and drug carriers such as antibody-directed or polymer-bound complexes (such as MAG-CPT), with the aim of preferentially delivering the macromolecule to the tumor [17, 19, 21, 26].

In this study, we utilized a polymer-bound, macromolecular prodrug system in an attempt to selectively target CPT to colorectal tumor tissue. Our study showed that MAG-bound CPT was indeed delivered to the target tumor tissue after i.v. administration. Tumor tissue concentrations of MAG-bound and free CPT were quantifiable at 24 h and remained so until 7 days after dosing. However, there was no evidence of selective delivery or retention of MAG-bound CPT or preferential release of free CPT in tumor versus normal tissue for up to 7 days after dosing. On the contrary, exposure to MAG-bound CPT was similar in normal and tumor tissue and exposure to free CPT was lower in tumor than in normal tissue. Additionally, the degree of necrosis and malignancy did not affect the level of tumor retention as demonstrated by the similarity of tumor to normal tissue ratios for MAG-bound and free CPT in patients with low necrosis/high malignant cellularity versus low–medium necrosis/low–medium malignant cellularity.

The levels of both MAG-bound and free CPT declined in parallel in tissues and plasma, indicating that equilibrium between these compartments was achieved by 24 h after dosing. Plasma levels of MAG-bound and free CPT were comparable to those observed in previous phase I studies [7]. The plasma concentrations of both analytes never decreased below those of the tumor tissue even up to 7 days after dosing, demonstrating that MAG-bound and free CPT are not selectively retained in tumor tissue. These results are in marked contrast to those from animal studies with MAG-CPT, where after a single i.v. administration of 20 mg/kg CPT equivalent to HT-29 tumor-bearing male nude mice, the plasma levels of MAG-CPT had decreased below the tumor levels 3 days after dosing, and at 1 week the plasma levels were less than 20% of tumor tissue concentrations [34].

Tumors have been shown to exhibit high interstitial pressure which can retard the extravasation of macromolecules and their penetration into tissue [16, 31]. In this study, however, concentrations of MAG-CPT were comparable in tumor and normal tissue, arguing against the importance of high interstitial pressures in the penetration of this macromolecule into colorectal tumor tissue. However, it is possible that other factors such as increased vascular permeability, high interstitial diffusivity, or lack of efficient lymphatic drainage, which are also characteristics of many tumor tissues [31], may have

compensated for any decreases in tumor penetrability due to increased interstitial pressure.

While concentrations of MAG-bound CPT were similar, concentrations of free CPT were slightly lower in tumor than in normal tissue. This may be attributable to impaired cleavage of the ester bond in tumor tissue, since the tumor mass is hypothesized to be generally acidic in nature [10, 13, 16], while MAG-CPT is more readily cleaved under alkaline conditions. It should also be noted that, while the study did measure free CPT in tumor and normal tissues, it did not establish that free CPT was necessarily generated from MAG-bound CPT within these tissues. Thus, it is possible that the levels in these tissues could have resulted from the equilibration of free CPT from plasma distributing into normal tissue and tumor.

With the paradigm shift towards more rationally designed and targeted anticancer therapies, there has been a need for modified clinical trial designs [8, 18, 20, 32] to allow easier, quicker, and more accurate proof-of-concept of the biological activity of novel anticancer drugs. The strengths of the current study design should be viewed in this context. The plasma and tissue sampling times for this study were selected based on the results of compartmental pharmacokinetic simulations using data from the phase I trials with MAG-CPT, and were aimed at evaluating the disposition of carrier-bound and free CPT over an adequate timeframe to allow completion of distribution and equilibration of the drug across the tissues of interest.

The limitations of this study design include the fact that surgical extirpation of tumor tissue can only be done once per individual (thus precluding the collection of temporal intrasubject data), and only the administration of a single dose of a cytotoxic drug would usually be permissible prior to surgery. Additionally, it can be difficult to recruit patients scheduled for elective surgery since no direct therapeutic benefit is offered. With drugs with a long half-life such as MAG-CPT (approximately 200 h), dosing long before surgery and prolonged blood sampling after surgery would be challenging to implement. Handling and processing of tissue samples is also associated with practical difficulties, such as dissection of the solid tumor to obtain only the homogeneous tissue and the need for rapid freezing of tissue samples. There are also a number of analytical considerations in the performance of such a study, especially in the case of MAG-CPT where the levels of free CPT are generally much lower than those of the bound species, and thus any ex-vivo release during processing (which would have a dramatic effect on the relative ratios) needs to be minimized. Another challenge for such an early-phase study is the proper definition of the study dose, since dose selection must be done within the framework of selecting a dose low enough to ensure patient safety, without compromising the study results because of undetectable drug levels in the tissues of interest.

In conclusion, the delivery of CPT to the target tumor tissue is achievable by means of the macromolecular polymer-bound delivery system, MAG-CPT, with the equilibrium between plasma and tumor tissue concentrations of free CPT being established within 24 h after dosing. A preferential retention of MAG-CPT in the tumor relative to normal tissue or plasma was not detected during the 7 days after dosing. While the clinical study did not reflect the findings of the preclinical studies, a longer period between dosing and surgery may be required to determine whether retention within the tumor is feasible. However, ethical and practical considerations constrained treating patients beyond the 1-week period used in this study.

Coupled with the lack of efficacy in clinical trials where MAG-CPT was administered in repeated cycles and in different dosing regimens up to the MTD, the results of this study indicated that MAG-CPT offers no advantage as a treatment for colorectal cancer, leading to the termination of the development program. However, this study showed that a well-designed proof-of-concept study conducted in a homogeneous target population can provide useful information about the delivery, retention, release, and pharmacokinetics of an anticancer drug in the target cancer tissue. Thus, the methods employed in our study were proved to be potentially useful in making "go/no-go" decisions on further development of anticancer drugs.

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