Evaluation of antimetastatic activity and systemic toxicity of camptothecin-loaded microspheres in mice injected with B16-F10 melanoma cells

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ABSTRACT Purpose: The aim of this work was to evaluate the pulmonary antimetastatic activity and the systemic toxicity of camptothecin-loaded microspheres. **Methods**: PCL microspheres containing camptothecin (CPT) were prepared by the emulsion solvent/evaporation method and characterized according to their encapsulation efficiency, particle size, morphology, and drug release. The ability of CPT to inhibit the lung metastasis was verified using an experimental mouse model intravenously injected with metastatic B16-F10 melanoma cells. The microspheres and the free drug were given intraperitoneally at a dose of 7 mg/kg at intervals of three or five days for 24 days. The systemic toxicity of CPT was evaluated by weight measurements, survival and hemograms of the animals. **Results:** The encapsulation efficiency was nearly 80%. The drug release was complete after 72 hours, but the burst effect increased from 7% to 35% with the increase in CPT content in the particles. It was observed during the *in vivo* essays that all groups treated with CPT had a decrease of nearly 70% in the number of lung metastases. However, systemic toxicity was verified in animals that received the free drug. **Conclusion**: Camptothecin-loaded microspheres demonstrated similar therapeutic efficacy when compared to those of the free drug, but the toxicity was significantly reduced.

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INTRODUCTION

Camptothecin is the alkaloid obtained from *Camptotheca acuminata* presenting a considerable anticancer activity, in which the mechanism involves the inhibition of topoisomerase I, an enzyme which is highly expressed in tumors. This protein reduces the torsion stress of supercoiled DNA to facilitate the replication, recombination and transcription processes. Camptothecin stabilizes the normally transient DNA topoisomerase complex, leading to the cleavage of doubled-strand DNA, and consequently, to cellular killing (1, 2). This drug is widely distributed in the body, including the central nervous system, lungs, liver and bowels (3). However, the use of camptothecin has shown some drawbacks, which, in turn, have limited its application in therapeutics. This drug encloses in its structure a highly conjugated pentacyclic ring with an α-hydroxylactone portion at carbon 12 which is essential for its *in vitro* and *in vivo* antitumor activity (4). At physiological pH, the lactone ring undergoes a rapid pH-dependent non-enzymatic hydrolysis to form a less active and more toxic carboxylate form (5). Stability studies in phosphate buffer pH 7.4 have demonstrated that camptothecin's half-life is about 10 minutes, and that only 13% of the drug is found in lactone form at equilibrium. Therefore, the inactivation of this drug occurs quickly a few minutes after intravenous administration. The inactivation in plasma is further increased by preferential binding of the carboxylated form to albumin that is about 200-fold over the lactone form (6). These drawbacks, together with the poor water solubility conferred by the unusually weak basic feature of its quinolone nitrogen atom, prevent the use of camptothecin by the intravenous route (7). Several hydrophilic derivatives have been developed in order to bypass the low aqueous solubility of camptothecin. In spite of the water solubility improvements provided by chemical modification, camptothecin derivatives approved for human use, i.e. topotecan and irinotecan, are also susceptible to inactivation in a physiological medium (7, 8, 9). Nevertheless, clinical trials have been carried out with these camptothecin derivatives against a wide variety of tumors to optimize administration schedules. So far, these studies have demonstrated that large doses of camptothecins given intermittently are not effective. Camptothecins require prolonged administration given continuously

at low doses, or frequently fractionated to produce a more effective antitumor activity (10). This can be explained by the fact that topoisomerase I inhibitors exert their activity in the S phase of the cell cycle. Hence, once the cytotoxic threshold is achieved, the exposure time rather than the dose becomes the parameter which determines antitumor activity. In addition, diarrhea and myelosuppression have been reported as the most important dose-limiting toxicities of camptothecins (7, 11). The severity of the undesirable effects of camptothecin is also dependent on the administration schedule.

In view of the issues concerning low water solubility, poor stability and the need to maintain the CPT concentration in therapeutic levels at the tumor for prolonged time, there has been a considerable interest in the development of formulations that allow continuous delivery and the protection of camptothecin from inactivation in a physiological environment. Microspheres exhibiting prolonged release of a CPT derivative, the irinotecan (CPT-11), were firstly prepared by Machida et al. (1998) using poly(D,L-lactide) or poly(D,L-lactide-co-glycolide) (PLGA) as matrix. The CPT-11-loaded microspheres displayed marked antitumor activity against P388 ascitic tumor via intraperitonial administration; however, they were not significantly effective against Sarcoma 180 solid tumor implanted subcutaneously. Thus, the prolonged release of CPT-11 has shown to be effective in the i.p.-i.p. system but not in the i.p.-s.c. system (12). Furthermore, the *in vivo* studies have shown that therapeutic efficacy was better when the *in vitro* release rate from microspheres was higher (12). The PLGA microspheres were also proposed as a vehicle to stabilize the camptothecins inside the particles (13, 14). In fact, studies have shown that the acidic microclimate created from the hydrolysis of the PLGA into acidic oligomers and monomers favors the stabilization of CPT within the delivery device. The potential of local delivery of 10-hydroxycamptothecin-loaded PLGA micropheres in providing effective inductive chemotherapy was evaluated using a murine human oral squamous cell carcinoma regression model. In this study, PLGA microspheres showed significantly higher intratumor-drug concentrations relative to local bolus and i.p. administration (approximately 10 and 100 folder higher, respectively) leading to significant reduction of the tumor weight (15). Finally, *in vitro* cytotoxicity studies have demonstrated that CPT-loaded microspheres are more effective than free camptothecin against human derived RPMI-8402 lymphoid and THP-1 myeloid leukemia cell lines (16). More recently, *in vitro* cytotoxicity studies revealed that camptothecin encapsulated in PLGA microspheres retains its antitumor potency against B16 cells, being quickly uptaken by these cells (17). Even though many studies have been carried out involving the antitumor activity of microspheres incorporated with camptothecin, the improvement of the drug efficacy after systemic administration of the microspheres remains to be verified. In this study, then, we are interested in evaluating whether or not camptothecin microspheres are able to inhibit the growth and the lung metastatic spread in the mice intravenously injected with B16-F10 melanoma cells. These cells were obtained by the Fidler method which results in a cell line with increased pulmonary metastatic capacity (18). In our case, CPT microspheres were prepared and characterized using poly-ε-caprolactone (PCL) as the polymer matrix former. Toxicity studies on microspheres and on the free drug were carried out after hemogram analysis and weight measurements of the mice. Since antimetastatic activity of camptothecin-loaded microspheres has not yet been described in detail in the literature, the present study attempts to shed light on this matter.

MATERIAL AND METHODS

Materials

Camptothecin and poly-ε-caprolactone (MW 65000 Da) were obtained from Sigma-Aldrich (USA). Hydroxypropylmethylcellulose (Methocel E4M Premium CR) was purchased from Colorcon (USA). Except for methanol and acetonitrile used in HPLC analysis (Tedia, USA), all other reagents and solvents were of analytical grade and were used as received.

Cells

A highly metastatic B16-F10 mouse epithelial-like melanoma cell line was donated from Bio-Rio (Rio de Janeiro, Brazil). The cells were cultured in a RPMI-1640 medium, buffered with 2g/L of HEPES and sodium bicarbonate, and supplemented with dextrose, penicillin, streptomycin and 10% of fetal bovine serum. The cells were cultured in a humidified atmosphere containing 5% CO_2 at 37^{o}C, were harvested with trypsin:EDTA (0.05:0.03 w/v) solution, and were washed and inoculated in recipient animals in PBS pH 7.4.

Preparation of the microspheres

Camptothecin-loaded poly-ε-caprolactone microspheres were prepared by an oil-in-water solvent emulsion/extraction technique. PCL (1g) was dissolved in 10 mL of methylene chloride solution containing 15 or 30 mg of camptothecin. This solution was gradually poured in 100 mL of aqueous phase containing hydroxypropylmethylcellulose 0.25% (w/v), which was previously saturated with the organic solvent. After the emulsion had been formed, 5 mL of ethanol were added and the mixture was kept under stirring at 500 rpm for five hours at room temperature. After the evaporation of the organic solvent, the hardened microspheres were centrifuged at 4000 rpm, washed three times with water, and freeze-dried. The microspheres prepared with initial amounts of 15 and 30 mg of camptothecin were denominated MC15 and MC30, respectively.

Determination of encapsulation efficiency and drug content

The camptothecin content in the microspheres was determined by a reversed-phase HPLC method. The analysis was carried out using a Supelcosil LC-18 column (15 cm x 4.6 mm ID, 5 μm; Supelco). The mobile phase was composed of methanol: KH2PO4 10mM (50:50, v/v) adjusted at pH 2.8 with phosphoric acid, and it was delivered at flow rate of 1.0 mL/min. The CPT was detected by UV absorption at 254 nm. Exactly weighed microspheres were dissolved in a 1:1 methylene chloride:dimethyl sulfoxide mixture and the resulting solutions were properly diluted with the mobile phase prior to HPLC analysis. The samples were injected in triplicate and the camptothecin concentration was determined by comparing the peak area corresponding to the drug with that obtained with a standard camptothecin solution. The encapsulation efficiency (%) was estimated as being the percentage of camptothecin incorporated into the microspheres in relation to the amount of drug initially added to the internal phase of the formulations. The drug content was expressed as milligrams of camptothecin per 100 mg of microspheres.

Morphological examination and particle size determination

The morphological examination of the microspheres was carried out using a Philips XL30 scanning electron microscope (SEM) after coating the samples with gold under vacuum. After their dispersion in water, camptothecin-loaded microspheres were analyzed for their average size and size distribution using a laser diffraction analyzer (CILAS 1064, France) and were plotted for size distribution using the software supplied by the manufacturer.

In vitro drug release studies

In the release studies, an amount of microspheres corresponding to 250 μg of camptothecin was exactly weighed and placed in 50 mL of a PBS pH 7.4 containing 2% (w/v) of Tween 80 in order to obtain *sink* conditions. Samples were maintained under stirring at 37ºC and at time intervals of 0, 1, 2, 4, 6, 10, 24, 48, 72 hours the microspheres were centrifuged and the supernatant was withdrawn and frozen for further analysis. Camptothecin concentration in the release media was determined by spectrofluorimetry. The solutions were excited at 374 nm and the sample spectra were recorded in the wavelength region of 390 and 550 nm. The samples' emission spectrum areas were compared with those obtained with a standard solution of camptothecin analyzed under the same conditions. The analyses were carried out in triplicate and the camptothecin release (%) versus time (hours) profiles were then plotted.

Evaluation of the antimetastatic activity of camptothecin-loaded microspheres

In the assays, 60-day-old Swiss male mice were used. The animals were maintained in a room (23 ± 2oC and 60 ± 10% humidity) under a 12-hour light/dark cycle. Food and water were given *ad libitum*. The *in vivo* assays were previously approved by our University's Ethics Committee for Animal Use based on the Principles of Animal Care.

Eight mice groups, each containing eight animals were employed in the evaluation of the antimetastatic activity of the microspheres. The mice were injected with 5 x 10^4 B16-F10 cells in 100 μL of PBS pH 7.4 via the intraorbital vein. Camptothecin-loaded microspheres, unloaded microspheres or the free drug were suspended in PBS pH 7.4 containing 0.3% (w/v) sodium carboxymethylcellulose and 0.2% (w/v) Tween 80, in order to improve the dispersion of the particles in the vehicle. On the second day after B16-F10 cells had been injected, the resulting suspensions were administrated intraperitoneally to the mice at a concentration of 7 mg/kg at intervals of three or five days, according to the experimental schedule tested.

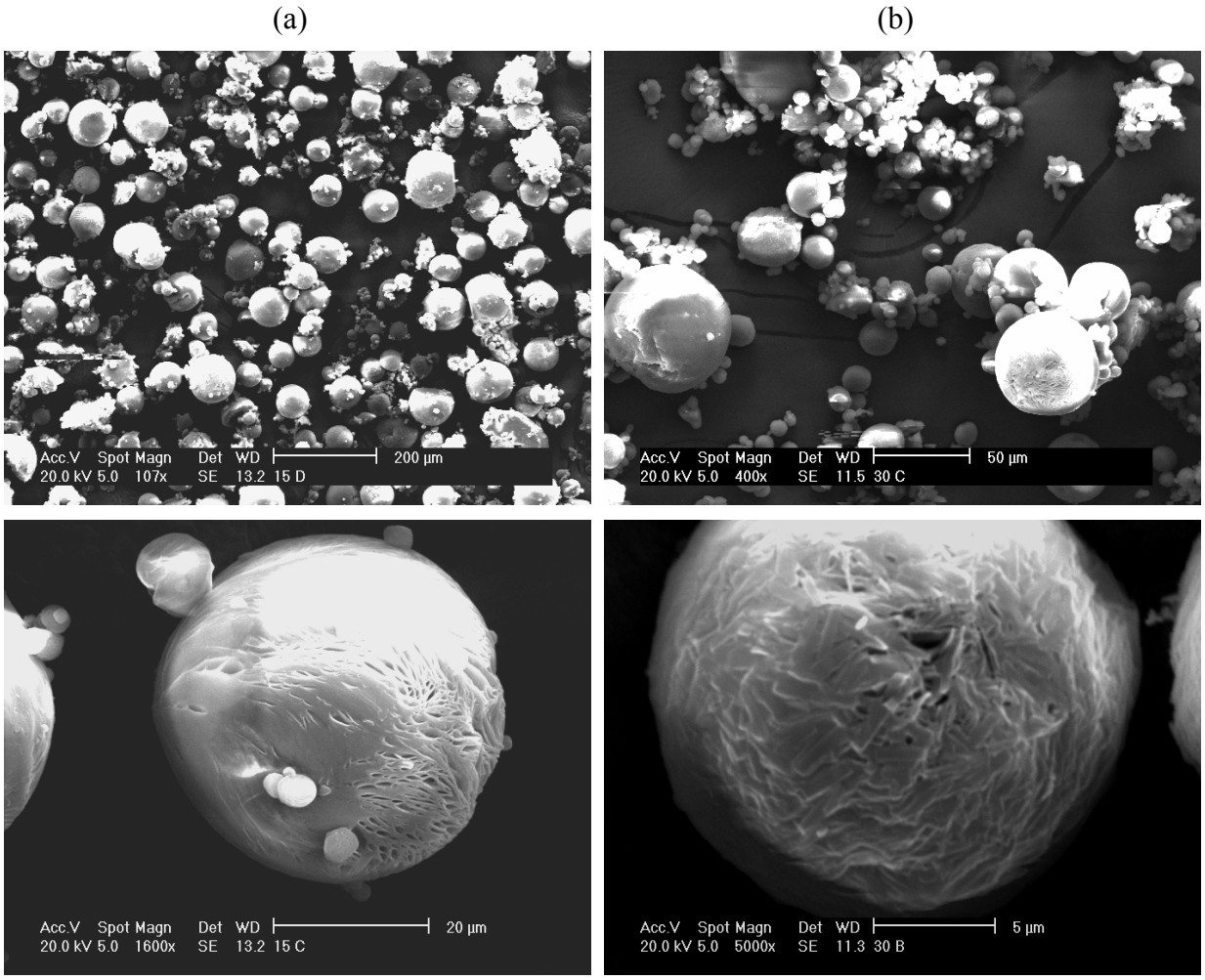


Figure 1: Scanning electron micrographs of CPT-loaded microspheres: (a) MC15, (b) MC30.

Negative control only received the vehicle, while the positive control received both the cells and the vehicle. The animals were sacrificed in a CO_2 chamber after 24 days. The lungs were excised and fixed with a 10% formaldehyde solution and the metastatic colonies were counted using a dissection microscope. The number of pulmonary metastasis observed in the mice treated with the free drug, vehicle, unloaded microspheres and camptothecin-loaded microspheres were compared. The statistical analysis was performed using analysis of variance followed by the Bonferoni's post-hoc, using the Graph-Pad Prism (San Diego, CA, USA) software. The difference was considered significant at $p<0.001$.

Toxicity studies

The hematological toxicity of the free drug and of the camptothecin microspheres was evaluated using blood collected by cardiac puncturing in heparinized propylene tubes. Hemograms were obtained by the flow cytometry technique using a hematological Serono Baker System 9000 counter coupled with a Hematology Analyzer. The animals were weighted throughout the experiment and the dead ones were recorded; these data were also used as indicators of systemic toxicity.

RESULTS AND DISCUSSION

Microsphere characterization

In order to obtain microspheres with high drug loading, formulations containing two initial amounts of camptothecin were prepared. The results displayed in Table 1 indicated that the encapsulation efficiency of camptothecin was around 81% for both formulations.

Table 1: Camptothecin encapsulation values obtained for MC15 and MC30 (n = 3).

	Initial amount of CPT (mg)	Encapsulation Efficiency (%)[a]	Drug content (%, w/w)[b]
MC15	15	81.49 ± 8.95	1.17 ± 0.15
MC30	30	81.66 ± 3.94	2.37 ± 0.08

[a] *weight of the encapsulated drug/ weight of total drug used in preparation.*
[b] *weight of encapsulated drug/ weight of microspheres.*

However, the drug content increased from 1.17% to 2.37% (w/w) with the increase in the amount of camptothecin initially added to the formulations. Several factors may affect the encapsulation efficiency of the drug in the microparticles. In general, high encapsulation values are observed when the partitioning of the drug tends toward the internal phase of the emulsion, while the fraction not encapsulated is eliminated in the filtration and washing procedures (19). In view of this, the high values of encapsulation efficiency obtained for camptothecin could be related to a higher affinity of this hydrophobic drug for the internal phase of the emulsion. Similar results were obtained when camptothecin was encapsulated in poly(D,L-lactic-co-glycolic acid) microspheres, using the same drug to polymer ratio (14).

The morphology of microspheres was investigated by SEM. As can be observed in the micrographs (Figure 1), the microencapsulation method employed led to the formation of spherical particles with a rough surface.

This surface characteristic may be related to rapid methylene chloride removal resulting from the addition of ethanol to the external phase during the evaporation process (20). The average diameter of camptothecin-loaded microspheres was 32.64 and 40.49 μm for MC15 and MC30, respectively, and the size distribution ranged from 0.4 and 120 μm for both formulations.

In vitro camptothecin release

The release profile of camptothecin from both MC15 and MC30 was evaluated after suspension of the microspheres in PBS pH 7.4, containing 2% (w/v) Tween 80. The solubility of the drug in the release medium was previously evaluated and it was found to be 79 μg/mL. Since the total amount of camptothecin in the microspheres corresponded to 6.33% of its saturated concentration, the perfect *sink* conditions were reached. Spectrofluorimetry was used to determine the drug concentration in the release medium because it is a very simple and rapid method. As can be observed in Figure 2, nearly 100% of the drug was released after 72 hours for both MC15 and MC30.

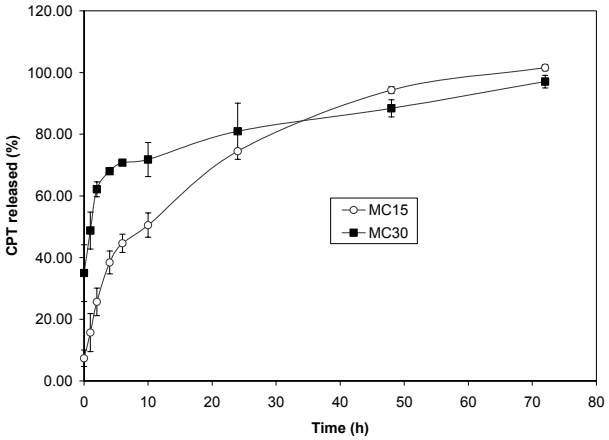


Figure 2: *In vitro* CPT release from PCL microspheres in phosphate buffer solution pH 7.4 containing 2% (w/v) Tween 80 at 37° C.

However, the release profile was affected by the microsphere drug content. The initial drug burst increased from 7 to 35% with the increment in drug loading from 1.17 to 2.37 % for MC15 and MC30, respectively. Since all formulation parameters remained constant, the higher burst effect obtained for MC30 could be attributed to the presence of the fraction of the associated drug at the particle surface (21). The burst effect concomitantly increasing with camptothecin loading has been reported when PLGA was used to prepare the microspheres, which is in agreement with the release of lipophilic drugs from matrix systems (14).

Evaluation of the antimetastatic activity of camptothecin-loaded microspheres

The ability of malignant neoplasms to produce secondary growths (metastases) in organs distant from the primary tumors is the lethal event in the clinical course of most neoplasic diseases. While primary cancers can be surgically resected or locally irradiated, it is usually difficult to use these therapeutic modalities against disseminated disease (18). The lungs and liver are the earliest sites colonized by most metastatic tumors. Delivery of drugs is considered valuable not only for lung treatment, but also for metastasis prevention even though the lung is not the metastatic target organ (22). An important result of experimental metastasis models employing direct injection of cells into the circulation has been the development of clonally related variants that differ in metastatic potential.

Table 2: Effect of free drug and CPT-loaded microspheres on pulmonary spontaneous metastasis in mice injected with $5x10^4$ B16-F10 melanoma cells with different administration schedules (n = 8).

	Dose and schedule	Total dose (mg/Kg)	Number of pulmonary metastasis (M ± σ)	Number of deaths[(a)]
Control (-)	-	-	0	0
Control (+)	-	-	22.38 ± 4.34	0
Unloaded microspheres	With intervals of three days	-	20.35 ± 4.30	0
Unloaded microspheres	With intervals of five days	-	18.63 ± 3.50	0
Free drug	7 mg/kg with intervals of three days	42	Nd[(b)]	8
Free drug	7 mg/kg with intervals of five days	28	7.50 ± 1.69***	0
MC30	7 mg/kg with intervals of three days	42	6.37 ± 1.77***	0
MC30	7 mg/kg with intervals of five days	28	6.50 ± 3.12***	0

*Statistical significance was evaluated by analysis of variance followed the Bonferoni's post-hoc: *** $p < 0.001$ vs. positive and negative control*

[(a)] Total number of deaths after 24 days

[(b)] Nd – Not determined

A B16 melanoma cell line that is characterized by progressively higher metastatic potential was developed by Fidler *et al* (23). While B16F1 parental cells are capable of forming experimental metastases at a rate of ~1,3 × 10^{-5} per cell per generation, the B16F10 cells generated by successive tail vein metastasis had an effective metastasis rate of 5 x 10^{-5} per cells per generation. This experimental metastasis model provides several advantages including the rapid time course for model maturity, the reproduction and consistence of the biology of metastasis, and the control of the number of cells that are introduced in the circulation. Some of the disadvantages have been attributed to most of the experimental metastasis models are chiefly related to the fact that the early steps in the metastatic cascade are eliminated and the compressed time course of metastasis can preclude their use in defining active agents against established metastatic cancers (24). In spite of these disadvantages, several studies have emphasized the interest in spontaneous lung metastasis model to evaluate the antitumor and antimetastatic activity of drugs and drug delivery systems (22,25,26). Shao *et al.* (22), for instance, demonstrated that a cell-based drug delivery system containing doxorubicin was more effective than the drug solution for both the early treatment of metastasis and the eradication of established metastasis in the lungs.

In this study, the effect of camptothecin-loaded microspheres on spontaneous lung metastasis was verified in mice inoculated via the retrorbital vein with B16-F10 cells. In this case, the therapeutic efficacy of the microspheres was evaluated for their ability to inhibit the number of lung colonies produced by the B16-F10 cells injected by the intravenous route. MC30 was selected for the *in vivo* studies due to its higher drug content. Since the antitumor effectiveness of camptothecin is highly dependent on the administration schedule (10), the microspheres and the free drug were given intraperitoneally in the dose of 7 mg/kg, at intervals of either three or five days for 24 days. The number of pulmonary metastasis and the number of deaths for each treated group are demonstrated in Table 2.

The lungs with metastatic melanoma nodules produced after B16-F10 cell inoculation can be seen in Figure 3. We observed an increase in the melanoma nodules in positive controls as well as in the unloaded microparticles, whereas for all camptothecin schedules the amount of lesions was greatly diminished. As can be seen in Table 2, the number of lung metastases was significantly reduced after administration of the free drug at intervals of five days (± 64.5%), and after administration of MC30 at intervals of three (± 71.5%) or five days (± 71.0%), when compared to the number of metastases produced in animals used as positive controls ($p < 0.001$). All animals of the groups treated with camptothecin at intervals of 3 days died after 18 days, indicating the high drug toxicity with this administration schedule. In addition, the antimetastatic activity was similar in the groups treated with free camptothecin and microspheres.

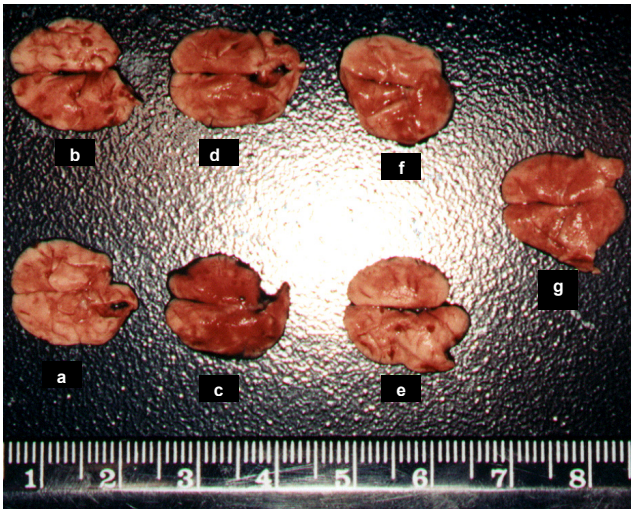


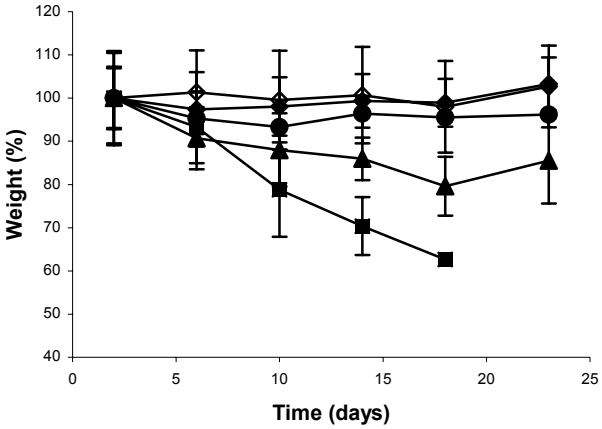
Figure 3: Appearance of the lungs from mice injected intravenously with highly metastatic B16-F10 melanoma cells ($5x10^4$) after treatment with (a) negative control; (b) positive control; (c) unloaded microspheres given at intervals of three days; (d) unloaded microspheres given at intervals of five days; (e) free camptotecin given at intervals of five days; (f) MC30 given at intervals of three days and (g) MC30 given at intervals of five days.

Toxicity studies

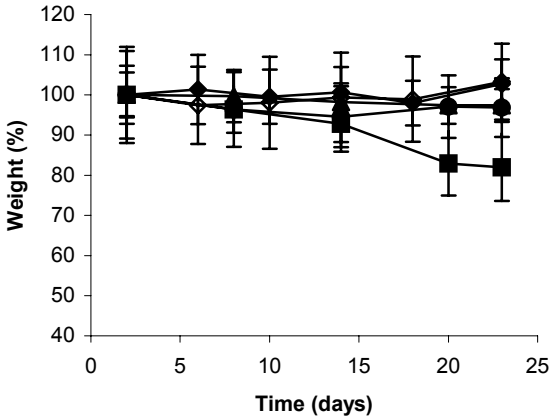
The administration of camptothecin in prolonged and continuous schedules has shown that the hematopoietic and mucosal progenitor cells with low topoisomerase I levels are somewhat spared, while, more importantly, antitumor effects are maintained. In these studies, myelosuppression caused mainly by severe neutropenia was found to be the dose-limiting toxic effect for most of the tested protocols, but many other disturbances, such as hemorrhagic cystitis, thrombocytopenia and diarrhea were also observed (27).

The weight measurements of the mice were performed during the experiments to evaluate the systemic toxicity of camptothecin. At the end of the experiments, significant weight loss of the animals was observed neither in the negative and positive control groups, nor in the group that received the unloaded and CPT-loaded microspheres at intervals of five days. However, when the free drug was administered at intervals of 5 days, and the MC30 was administered at intervals of three days, the weight of the animals was significantly reduced as observed in Figure 4 ($p < 0.001$).

Furthermore, the group that received free camptothecin at intervals of three days developed severe diarrhea and did not survive until the end of the experiments. Severe diarrhea have been demonstrated to occur after consecutive daily injections of free drug and it was considered to be an enterocolitis caused by the high level of CPTs retained for a long period in the intestine (28).



(a)



(b)

Figure 4: Percentage of weight loss of animals injected intravenously with highly metastatic $5x10^4$ B16-F10 melanoma cells and treated in administration schedules with intervals of (a) three days and (b) five days over a 24-day period. (♦) negative control, (◊) positive control, (●) Unloaded microspheres, (■) Free camptotecin, (▲) MC30.

In order to compare the toxicity produced by the administration of camptothecin in the free and encapsulated forms, the hematological parameters of the animals were determined on the twenty-fourth day, and were then compared with the respective values obtained for normal control mice. The results of the hemograms of the animals are shown in Table 3.

Table 3: Effect of MC30 and free camptothecin (CPT) on hematological parameters for each animal group after 24 days of treatment (n = 5).

	Reference values	Control (-)	Control (+)	MB[(a)]	MB[(b)]	CPT[(b)]	MC30[(a)]	MC30[(b)]
Red Blood Cells								
Total RBC Count (x 10^6/mm^3)	7.0 -12.5	8.12 ± 0.53	8.21 ± 0.76	8.74 ± 0.30	8.51 ± 0.28	7.15 ± 1.03	7.25 ± 0.34	6.96 ± 0.44
Hemoglobin (g/dL)	10.2 -16.6	13.73 ± 0.66	14.07 ± 0.79	14.46 ± 0.73	14.50 ± 0.66	11.13 ± 1.20	12.87± 0.57	10.45 ± 2.04
Hematocrit (%)	39 - 49	40.96 ± 3.36	41.22 ± 3.80	42.73 ± 1.55	42.80 ± 2.80	34.73 ± 4.62	36.45 ± 1.80	31.82 ± 4.28
White Blood cells								
Total WBC Count (mm^3)	6000 - 15000	10133.33 ± 1556.70	9100 ± 4573.11	9733.33 ± 2968.3	9675± 3525.50	8266.67± 3074.63	10825± 5483.54	7112.50± 2172.7
Neutrophil (%)	10 -40	17.00± 7.07	15.75± 6.30	24.66± 13.58	25.50± 15.02	2.33± 2.52	24.33± 10.41	13.25 ± 7.80
Eosinophil (%)	0 - 4	0	1	0	0	0	0	0
Lymphocyte (%)	55 - 95	85.66 ± 8.02	82.25 ± 7.09	74.6 ± 14.15	73.75± 14.93	97.33± 2.08	79.75± 12.58	86.50 ± 8.22
Monocyte (%)	0.1 - 3.5	1.5 ± 0.7	2.33 ± 1.15	2	1	1	1	1
Platelet count (x10^6)	0.8 - 1.1	1.01 ± 0,07	1.11 ± 0.13	1.49 ± 0.20	1.35± 0.22	1.82± 0.23	1.7± 0.04	1.8 ± 0.16

[(a)] Given at intervals of three days (total dose = 42 mg/Kg).

[(b)] Given at intervals of five days (total dose = 28 mg/Kg).

The count of red blood cells, hemoglobin content, and hematocrit values were slightly lower in the groups that received the free drug and the MC30 microspheres. These results can be related to some toxicity induced by the camptothecin and not by the tumor, since the positive control group did not show a reduction of these elements. Surprisingly, after the administration of camptothecin there was an increase in the count of platelets (thrombocytosis), rather than thrombocytopenia. Thrombocytosis was, nevertheless, also verified after the administration of the unloaded microspheres, suggesting that this may be a consequence of an inflammatory processes caused by the intraperitoneal administration of microparticles in suspension.

On the other hand, neutropenia was found in the group of mice treated with free camptothecin at intervals of 5 days. In groups that received the MC30 microspheres, at intervals of 3 or 5 days, this effect was not observed, which, in turn, indicates a decrease in hematological toxicity when the encapsulated drug is administered.

The pharmacokinetics of camptothecin has been described elsewhere. Under physiological conditions, camptothecin exists in equilibrium between its lactone (CPT) and carboxylate forms (CPT-Na), this equilibrium favoring the carboxylate form. The activity of CPT was found to be approximately 10-fold greater than Na-CPT and this was attributed to a small amount of *in vivo* conversion from carboxylate to lactone (29). Furthermore, the distribution clearance for lactone was greater than the carboxylate form, indicating that the lactone is quickly distributed into the tissue compartment (30). In comparative pharmacokinetics studies performed in rats, the plasma concentration of irinotecan, a water soluble camptothecin derivative, was gradually increased when microspheres were administrated by i.p route, while a quick decreasing on the plasma concentration of the drug was observed after the administration of the drug solution (31). In our case, since the same dose was given when either microspheres or CPT dispersion were administered, the reduction in the systemic toxicity can be associated with both the protection of the drug inside of the particles, avoiding its conversion in the carboxylate form, and the slower delivery of lactone form towards the blood stream. Therefore, it is

possible that the administration of microspheres provides lower CPT plasma concentration in relation to the drug dispersion, contributing to the toxicity reduction but maintaining the antimetastatic effect.

CONCLUSIONS

Camptothecin-loaded microspheres displayed similar antimetastatic activity as compared to the free drug, but their systemic toxicity was lower as evidenced by the evaluation of the weight loss and survival of the animals, and blood neutrophil count. The decrease of toxicity may be related to the maintenance of the camptothecin lactone ring in the microspheres, which leads the drug to achieve systemic circulation in lower concentrations than when it is administrated in its free form. The hematological toxicity reduction was observed for both administration schedules when the drug was encapsulates in PCL microspheres, indicating the microencapsulation of CPTs can be advantageous to minimize the inconvenient of the systemic administration of these drugs.

ACKNOWLEDGEMENTS

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