Cancer Chemother Pharmacol (1999) 44: 187–192

ORIGINAL ARTICLE

Nadezhda V. Koshkina · Brian E. Gilbert
J. Clifford Waldrep · Alexander Seryshev
Vernon Knight

Distribution of camptothecin after delivery as a liposome aerosol or following intramuscular injection in mice

Received: 23 September 1998 / Accepted: 11 January 1999

Abstract *Purpose*: The plant alkaloid camptothecin (CPT) has shown significant antitumor activity against a wide variety of human tumors xenografted in nude mice. In previous studies we have found that administration of dilauroylphosphatidylcholine (DLPC) liposome aerosols containing 9-nitrocamptothecin (9-NC) inhibits the growth of human breast, colon and lung cancer xenografts. The purpose of this study was to analyze the pharmacokinetics and tissue distribution of inhaled CPT formulated in DLPC liposomes. *Methods*: C57BL/6 mice with subcutaneous Lewis lung carcinoma, Swiss nu/nu mice with human lung carcinoma xenografts and BALB/c mice without tumors were used for pharmacokinetic studies of CPT administered as a liposome aerosol and BALB/c mice were given CPT intramuscularly. *Results*: After 30 min inhalation of CPT liposome aerosol, drug was deposited in the lungs (310 ng/g) and was followed promptly by the appearance of high concentrations in the liver (192 ng/g) and with lesser amounts appearing in other organs. Drug concentration in the brain was 61 ng/g. After intramuscular injection of CPT dissolved in DMSO, drug was released from the site of injection very slowly and accumulated mainly in the liver (136 ng/g). Only trace amounts appeared in the lungs (2–4 ng/g). These results demonstrate a prompt pulmonary and later systemic distribution of CPT following liposome aerosol administration. *Conclusions*: The substantial concentrations of CPT in lungs and other organs following inhalation of liposome aerosol suggest the possible benefit of it and of its more active derivative, 9-NC, in the treatment of lung, liver, kidney and brain cancer in humans.

Key words Aerosol · Liposome · Cancer · Pharmacokinetics · Camptothecin · Mice

N.V. Koshkina (✉) · J.C. Waldrep · A. Seryshev · V. Knight
Department of Molecular Physiology and Biophysics,
Baylor College of Medicine,
One Baylor Plaza, Houston,
TX 77030, USA
Tel. +1-713-798-5607; Fax +1-713-798-3125

B.E. Gilbert
Department of Microbiology and Immunology,
Baylor College of Medicine,
Houston, TX, USA

Introduction

Camptothecin (CPT), a plant alkaloid, exhibits significant in vivo activity against a broad spectrum of human tumor xenografts in athymic mice, including cell lines resistant to many clinically used chemical agents [8, 11, 13, 19]. The intracellular target of camptothecin and its derivatives is topoisomerase I [14], which is active during the S-phase of cell replication.

Along with their anticancer activity, these drugs possess significant toxic effects, particularly myelosuppression [17, 18]. We attempted to circumvent this problem by incorporating CPT and its 9-nitro derivative (9-NC) into liposomes and administering them in small-particle aerosol. In other studies it has been shown that liposomes can be effectively used as carriers for CPT and its derivatives [3, 6, 7, 9, 22]. Liposomes could also potentially protect the drugs from binding by albumin which, in humans, has high affinity for the carboxyl form of the drug, thus leading to the depletion of the active lactone forms [4, 5]. Moreover, by deposition within the lungs where there is little albumin [20], and with rapid transit to sites of cancer, more of the lactone-mediated effect could be preserved.

A previous report has described treatment of human breast, colon and lung cancer xenografts in nude mice principally with the 9-NC derivative delivered by liposome aerosol [15, 16]. The results were very favorable with significant tumor regression in these studies. The daily calculated doses associated with suppression of the tumor growth ranged from 0.008 to 0.153 mg/kg, administered 5 days weekly for several weeks. In one study, 0.306 mg/kg per day was given for a short period. These doses were significantly lower than the generally used

effective dose in mice of 1.0 mg/kg per day, 5 days per week for several weeks given by direct injection of an oily suspension of the drug into the stomach of nude mice with human cancer xenografts [12].

In the present study the pharmacokinetics of CPT in mice following inhalation of a small-particle aerosol containing the drug-liposomal formulation were examined. CPT was chosen for these pharmacokinetic studies because it is a highly fluorescent compound and can be detected in nanogram amounts in tissues and body fluids. While the 9-NC derivative has more potent anticancer activity [12, 15, 16], the limit of sensitivity of the assay precludes its use for pharmacokinetic studies at the doses delivered by aerosol.

Materials and methods

Chemicals

20-(*S*)-CPT was purchased from Sigma (St. Louis, MO). 9-NC was a gift from Dr. B. Giovanella of the Stehlin Institute for Cancer Research, Houston, Tx, and was >99% pure as determined by HPLC analysis. Dilauroylphosphatidylcholine (DLPC) was purchased from Avanti Polar Lipids (Alabaster, Ala.). Tertiary butanol (*t*-butanol) and acetonitrile were obtained from Fisher Scientific. Sterile, pyrogen-free water for irrigation came from Baxter Healthcare Corporation (Deerfield, Ill). Dimethylsulfoxide was purchased from Sigma.

Mice

Nu/nu Swiss immunodeficient nude mice of the NIH-1 high-fertility strain were obtained from the Stehlin Foundation for Research, Houston, Tx. These mice had been implanted with human lung adenocarcinoma cells (SPA) over the thorax and had served as chemotherapy controls for several weeks. Female BALB/c and C57BL/6 mice were purchased from Harlan Sprague Dawley, Indianapolis, Ind. Food was removed from the animals approximately 18 h before the start of the experiment.

Murine Lewis lung carcinoma

Murine Lewis lung carcinoma cells (LLC1) were obtained from the American Type Culture Collection (Rockville, Md.). LLC1 cells in the pharmacokinetic studies were obtained from subcutaneous tumors after homogenization with scissors and filtration through a sieve. Cell suspensions were washed with DMEM supplemented with 10% fetal calf serum with L-glutamine and 1% antibiotic-antimycotic mixture. Medium supplements were purchased from Gibco (Grand Island, N.Y.). C57BL/6 mice were injected subcutaneously (s.c.) over the thorax with 10^6 cells suspended in saline and were used for study 10–14 days after injection.

Drug administration

Aerosol was administered to groups of mice in sealed plastic cages or from a nose-only exposure chamber (Small Animal Exposure Chamber System, In-Tox Products, Albuquerque, N.M.). Aerosol was generated from an Aerotech II nebulizer flowing at 10 l/min (CIS USA, Bedford, Mass.) connected by accordion tubing (1 cm inside diameter) to the aerosol entry orifice of the plastic cage or the nose-only exposure system. Effluent aerosol was discharged from an opening at the end of the cage or at the top of the nose-only exposure chamber. The aerosol particles had a mass median aerodynamic diameter (MMAD) of 1.6 μm and a geometric standard deviation of 2.1 as determined using an Andersen cascade impactor. The concentration of CPT in the aerosol was 9.0 μg/l. Aerosol exposure was begun at zero time and at 15 min three mice were sacrificed. The remaining mice were continued on treatment until 30 min had elapsed, when aerosol treatment was stopped. At this time, three additional mice were sacrificed and then three each at various times after cessation of aerosol exposure.

In all aerosol studies the initial drug concentration in the nebulizer was 0.5 mg CPT and 25 mg DLPC per 1 ml water. The inhaled aerosol dosage of CPT, was 40.5 and 80.9 μg/kg, for 15- and 30-min exposures, respectively. The dosage (D) was calculated from the following formula:

$$D = C \times V \times DI \times T$$

where C is the concentration of the drug in aerosol measured in the Andersen cascade impactor [2] (in the present study it was 9.0 μg/l CPT), V is the volume of air inspired by the animal (1 ml/min per g body weight [21]), DI is the estimated deposition index (fraction of inhaled dose deposited in the respiratory tract; for mice DI = 0.3 [10]), and T is the duration of treatment (min). Values given in Tables 1–5 have been corrected for concentrations measured in untreated mice.

Table 1 Tissue distribution of CPT in BALB/c mice during and after a 30-min inhalation of CPT-DLPC aerosol in a sealed plastic cage. During this treatment (at 15 min) three mice were removed from the cage and sacrificed. The drug dose for the 30-min exposure was 80.9 μg/kg. When the treatment was stopped, three animals were sacrificed immediately and then three each at 40, 60 and 90 min counting from the beginning of treatment. Values are mean CPT concentration ± SD (ng/g tissue)

Organ	15 min	30 min	40 min	60 min	90 min
Lungs	181 ± 153	179 ± 127	45 ± 23	9 ± 13	1 ± 2
Liver	29 ± 14	132 ± 38	166 ± 34	124 ± 177	64 ± 24
Spleen	5 ± 5	16 ± 11	30 ± 26	11 ± 11	4 ± 8
Kidney	28 ± 15	52 ± 13	33 ± 9	11 ± 10	7 ± 6
Blood	8 ± 8	12 ± 4	24 ± 15	18 ± 19	2 ± 3

Table 2 Tissue distribution of CPT in Swiss nude mice following 30 min inhalation of CPT-DLPC aerosol in a nose-only exposure chamber. The drug dose was 80.9 μg/kg. When the treatment was stopped, three animals were sacrificed immediately (30 min) and then three more were sacrificed 15 min later (45 min). Values are mean CPT concentration ± SD (ng/g tissue)

Organ	30 min	45 min
Lungs	638 ± 580	36 ± 11
Liver	90 ± 36	213 ± 101
Spleen	26 ± 13	50 ± 17
Kidney	146 ± 127	123 ± 27
Blood	39 ± 2	50 ± 20
Tumor	14 ± 8	19 ± 4

Table 3 Distribution of CPT in C57B/6 mice with subcutaneous Lewis lung carcinoma during and after 30 min of inhalation of CPT-DLPC in a nose-only exposure chamber. Except for the "nose-only" treatment, the procedure was as described for Table 1(n = 3 for each time-point)

Organ	15 min	30 min	40 min	60 min	90 min
Lungs	84 ± 39	113 ± 87	182 ± 185	66 ± 60	41 ± 24
Liver	48 ± 44	86 ± 26	198 ± 148	47 ± 44	11 ± 10
Kidney	43 ± 12	88 ± 34	59 ± 24	23 ± 13	8 ± 3
Spleen	42 ± 41	38 ± 7	15 ± 6	15 ± 6	7 ± 5
Blood	18 ± 6	26 ± 6	16 ± 6	15 ± 2	5 ± 2
Tumor	12 ± 0.4	39 ± 14	32 ± 30	16 ± 3	7 ± 3
Brain	6 ± 6	61 ± 80	15 ± 4	11 ± 5	9 ± 1

Table 4 Tissue distribution in BALB/c mice at various times up to 120 min after a single i.m. injection of CPT in DMSO at a dose of 0.233 mg/kg (7 μg). Values are mean CPT concentration ±SD (ng/g tissue) (n = 3 for each time-point)

Organ	1–3 min	30 min	60 min	120 min
Lungs	2 ± 2	4 ± 2	3 ± 3	4 ± 3
Liver	3 ± 2	87 ± 74	136 ± 107	126 ± 116
Kidney	2 ± 0	40 ± 14	26 ± 7	15 ± 5
Spleen	2 ± 2	18 ± 9	11 ± 5	5 ± 2
Blood	2 ± 2	12 ± 5	8 ± 1	4 ± 2
Injection site	6918 ± 260	4309 ± 1548	4609 ± 1412	1544 ± 751

Table 5 Concentration of CPT in five organs and tumor(s) of mice treated for 30 min with CPT liposome aerosol at a dose of 0.081 mg/kg and in five organs and tumor(s) of mice treated by the oral, intravenous and intramuscular routes with tritiated CPT in a lipid dispersion at a dose of 4 mg/kg. The data for the aerosol administration are pooled from the three mouse strains used (see Fig. 1). The data for the other routes of administration are from reference 1 for which the conversion of 1 nCi = 13.3 ng of CPT was used. Values are ng CPT/g tissue. The mean concentrations of CPT in five organs and tumor(s) of mice in the aerosol group were compared using Student's t-test, two-tailed, with each of the three non-aerosol treatment groups at both time-points and within the non-aerosol group when indicated. At 2 h, mice treated intramuscularly had significantly higher CPT concentrations than the aerosol-treated mice (P = 0.004) and, in addition, at 2 h mice treated intravenously had significantly lower concentrations than mice treated intramuscularly (P = 0.049)

Organ	Aerosol		Oral		Intravenous		Intramuscular	
	30 min	90 min	30 min	120 min	30 min	120 min	30 min	120 min
Lungs	310	17	43	5.3	32	36	44	170
Tumor	26	6.8	31	6.0	36	56	42	57
Brain	61	9.0	37	2.7	12	4	27	74
Blood	26	3.2	39	5.3	23	19	60	144
Liver	103	42.9	137	21.3	50	54	105	118
Kidney	95	7.2	243	210	109	146	176	294
Mean	104	14.4	88	42	44	53	76	143
SD	106	14.7	86	83	35	50	56	85

Intramuscular (i.m.) injections were made with a 26-gauge needle into the thigh muscles of the mice. Initial CPT stock in DMSO (10 mg/ml) was diluted with saline up to 0.14 mg/ml CPT and 50 μl was injected into each animal (approximately 233 μg/kg).

Extraction procedure for CPT from tissues

After sacrifice, organs were resected and specified amounts of 9-NC in DMSO were added as an internal standard to estimate the extraction efficiency. Organs were frozen in liquid nitrogen and kept at −20 °C. Before extraction, samples were melted and 1.0 ml of 0.1% acetic acid in water extraction solution (pH 4.5) was added to each sample. Organs were homogenized on a mini-beadbeater (Wig-L-Bug, Model # 3110B, Crescent Dental MFR. Co., Lyons, Ill.) for 1 min and homogenates were centrifuged at 16 000 rpm for 5 min. Supernatant fractions were collected and reextraction was performed with 8 ml methylene chloride. The organic fraction was separated and dried by air flow at room temperature. The dried extracts were reconstituted in 0.1 ml acetonitrile and analyzed by HPLC.

HPLC analysis

A Waters 710B WISP automatic injector and a Waters Nova-Pak C18 column (3.9 × 150 mm; Waters, Milford, Mass.) at room temperature were used to quantitate CPT and 9-NC. The mobile phase was composed of 30% acetonitrile and 70% water containing 0.1% glacial acetic acid (pH 3.5). The flow rate was 1.2 ml/min. CPT was detected using a Waters 470 scanning fluorescent detector set to excitation and emission wavelengths of 370 and 440 nm, respectively. 9-NC was detected using a Waters 440 UV absorbance detector monitoring at 254 nm. The data were analyzed with Waters Millenium software. This extraction procedure converts all of the drug to the lactone form.

The extraction efficiency was different for the different organs: the highest extraction values were obtained for blood (41 ± 13%), spleen (39 ± 7%), lungs (34 ± 6%) and kidneys (32 ± 5%).

Statistics

For comparisons of CPT concentrations in the same tissues at the same times in the three different mouse strains the unpaired t-test, two tailed (Microsoft Excel software) was used. For comparisons of drug concentrations in different tissues at the same sampling time the unpaired t-test was also used. For comparisons of concentrations in the same organ or tissue at different times of sampling the Mann-Whitney Rank Sum test (Primer of Biostatistics software) was used.

Results

Aerosol treatment

Table 1 shows the mean concentrations of CPT in organs of BALB/c mice during and after a 30-min inhalation of CPT-DLPC liposome aerosol. The highest concentrations of the drug were detected in the lungs. The levels were 181 ± 153 ng/g after 15 min and 179 ± 127 ng/g after 30 min. Substantial concentrations were first detected in the liver at 30 min (132 ± 38 ng/g). Other organs had lower mean concentrations of CPT, but similar clearance patterns.

Two other animal models were used to detect CPT amounts in the tumor besides the other main organs.

Table 2 represents tissue distribution of CPT in Swiss nu/nu mice with s.c.-implanted tumor. Although we found higher mean concentrations of drug in the lungs and kidneys of these animals after 30 min of aerosol treatment, these values were not significantly different from those of the other strains of mice ($P > 0.05$, Student t-test, two-tailed). CPT concentrations in the tumor reached 14 ± 8 ng/g at the end of 30 min aerosol treatment and were still at the same level 10 min after the end of treatment (19 ± 4 ng/g).

In the other experiment with C57BL/6, mice with s.c. Lewis lung carcinoma (Table 3) the highest mean concentration in the tumor was detected at the end of 30-min aerosol treatment and reached 30 ± 14 ng/g. In this experiment we studied CPT deposition in the brain, and 61 ± 80 ng/g was detected in the brain also at the end of aerosol treatment.

Pooling the data from all three strains of mice (Fig. 1) showed that more than 90% of sets of the 48 values at the same sampling times were not significantly different ($P > 0.05$, Student t-test, two tailed). Statistical analysis by the Mann-Whitney Rank Sum test showed that peak values for lungs, liver and kidney were significantly higher than the values obtained at later time-points, whereas for the spleen, blood, tumor and brain they were not significantly different (data not shown). It was also shown ($P > 0.05$, Student t-test, two tailed) that concentrations in livers and lungs did not differ from each other at the 30- and 40-min time-points.

Analysis of the area under the organ concentration-time curve (AUC) over the 90-min period (Fig. 1) using the trapezoidal rule gave the following AUC values for lungs, liver, kidneys, spleen, blood and tumor: 8713, 7551, 3433, 1514, 1444, and 1392 ng CPT · min/g of tissue, respectively.

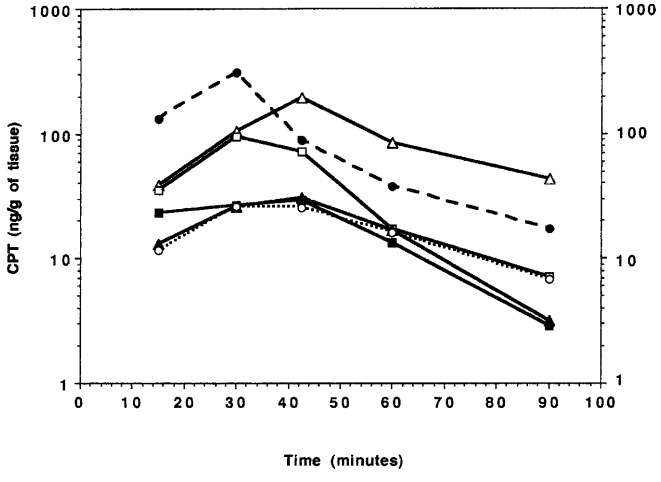

Fig. 1 Pooled results of CPT tissue distribution in the three strains of mice following a 30-min liposome aerosol treatment. Data for 40 and 45 min were combined and are plotted as 42.5 min (● lungs, △ liver, □ kidney, ■ spleen, ▲ blood, ○ tumor)

Intramuscular injections

Table 4 shows the distribution of drug in BALB/c mice following an approximate i.m. dose of 233 μg/kg dissolved in DMSO. This dose was higher than the estimated aerosol dose in order to achieve the detectable amounts of drug in the organs. Mice were sacrificed at the times indicated in Table 4. After i.m. injection, CPT was released from the site of injection very slowly. About 60% of the drug was still present in the muscle tissue 1 h after injection and 20% after 2 h. A small amount of drug reached the liver, with some additional deposition in the kidney. Only trace amounts of CPT were found in the lungs.

Comparison of aerosol dosage with other routes of administration of CPT

Table 5 shows a quantitative comparison of aerosol dosing (present report) with previously reported oral, intravenous and intramuscular dosing of CPT in mice [1]. The aerosol consisted of CPT in liposomes, while for the other routes of administration CPT was dispersed as a fine emulsion in Intralipid 20. The comparison of efficiency of drug penetration was based on CPT recovered from five organs and tumor(s) of aerosol-treated mice at 30 min and 1.5 h after the start of a 30 min aerosol treatment, and similarly from mice given CPT in lipid dispersion by oral, intravenous and intramuscular routes when examined for tritiated label at 30 min and 2 h after dosing. The dosage by aerosol was 81 μg/kg while the dose for the non-aerosol routes of administration was 4 mg/kg. The ratio of aerosol to non-aerosol dosage was, therefore, 1:50. Table 5 shows that the tissue concentrations of CPT were considerably greater after aerosol treatment at 30 min than after administration by the other routes. At 2 h, concentrations in mice treated by the non-aerosol routes were reversed, being three to ten times greater in the non-aerosol-treated animals. However, if consideration is given to the 50-fold greater dose to the non-aerosol treated groups, the aerosol provided quicker penetration and relatively larger concentrations in the five organs and tumor(s) of the mice that were examined.

Discussion

Aerosol drug delivery represents an effective method for targeting inhaled drugs to the pulmonary tissues. However, about 30% of inhaled drug in a particle range of 1–2 μm MMAD in mice will deposit in the respiratory tract [10]. Of the deposited amount more than 1one-half will remain in the nasopharynx while the remainder will reach the lungs. Of the inhaled particles, 70% will not deposit and will be exhaled. Particles deposited in the nose and head will be transported promptly by mucociliary action to the orifice of the esophagus and

swallowed, and some of the particles deposited in the central airways will also be carried into the esophagus. It is a reasonable approximation that about 12% of the inhaled particles will reach the lungs.

In previous studies [15, 16], we have shown that administration of equal amounts of 9-NC-DLPC (up to about 0.15 to 0.2 mg/kg per day) orally or via aerosol to nude mice bearing cancer xenografts leads to tumor regression only in aerosol-treated mice. Thus, the drug that reaches the stomach during aerosol treatment probably has very little antitumor effect, while the smaller fraction of drug deposited in the lungs provides the therapeutic effect. It follows from this hypothesis that a good portion of the drug found in organs of mice in the present study, except the gastrointestinal tract, must have come primarily from the fraction that was deposited in the lungs.

Within a few minutes after the end of aerosol treatment, in all three strains of mice, lung concentrations decreased slowly and liver concentrations increased to amounts almost equal to that in the lungs, with smaller amounts appearing in other organs. In the study of C57BL/6 mice, concentrations of CPT in the brain were measured and the mean concentrations were similar in trend, but lower, than kidney concentrations.

Prior to the present study, i.m. injections were shown to be the most effective route for CPT administration to immunodeficient mice bearing human cancers; oral administration was less effective and i.v. injection had little effect [11, 19]. The oral route of treatment was found to be effective in humans [17, 23]. Our studies of CPT deposition in tissues after i.m. injection of CPT dissolved in DMSO showed that 20–30% of the injected drug was retained at the site of injection for a long period, with gradual accumulation in the liver. No CPT was measurable in the lungs during the 2-h period of observation. However, Ahmed et al. have shown substantial absorption of CPT given i.m. in lipid suspension (Table 5). Smaller amounts appeared in the kidneys with still smaller amounts in the spleen and blood. We have previously reported [15, 16] that a 9-NC-DLPC i.m. dose comparable to the aerosol dose inhibits the growth of a human lung cancer xenograft in nude mice, but the effect is significantly less than that observed with 9-NC liposome aerosol treatment.

The foregoing findings suggest that aerosol has an advantage in treatment. This may be because the albumin concentration in tracheal and bronchial surface liquids is only 1–2% of the plasma concentration (0.5–0.7 mg/ml [20]) and because the drug probably reaches the tumor quickly by the arterial circulation, possibly preserving more of the lactone form of the drug.

Another basis for the better response is shown in Table 5: the CPT dosage in liposome aerosol in the present study was 1/50th of the dosage by oral, intravenous and intramuscular routes of a lipid-CPT formulation [1]. Yet, at 30 min following treatment concentrations of CPT in the organs of the aerosol-treated mice were 1.18 to 2.36 times greater than those in the non-aerosol-treated mice. At 1.5 to 2.0 h after the start of treatment, the situation was reversed with concentrations of CPT in non-aerosol-treated mice three to ten times greater than in the aerosol-treated animals. Despite these higher concentrations of CPT in organs of non-aerosol-treated mice, when adjusted for the 50-fold margin of difference in dosage, the aerosol route overall produced higher concentrations of CPT than the other treatments and with a shorter time to peak concentrations.

The formulations used and the methods of assay may also have had some role in these differences, but it is reasonable to suppose that the aerosol route of treatment with liposomal CPT is the major basis for the differences observed.

Acknowledgement This work was supported by the Clayton Foundation for Research, Houston, TX.

References

1. Ahmed EA, Jacob S, Giovanella BC, Kozielsky AJ, Stehlin JS, Liehr JG (1996) Influence of route of administration on [3H]-camptothecin distribution and tumor uptake in CASE-bearing nude mice: whole-body autoradiographic studies. Cancer Chemother Pharmacol 39: 122
2. Andersen AA (1958) New sampler for the collection, sizing and enumeration of viable airborne particles. J Bacteriol 76: 471
3. Burke TG, Gao X (1994) Stabilization of topotecan in low pH liposomes composed of distearoylphosphatidylcholine. J Pharm Sci 83: 967
4. Burke TG, Mi Z (1993) Preferential binding of the carboxylate form of camptothecin by human serum albumin. Anal Biochem 212: 285
5. Burke TG, Mi Z (1994) The structural basis of camptothecin interactions with human serum albumin: impact on drug stability. J Med Chem 37: 40
6. Burke TG, Staubus AE, Mishra AK (1992) Liposomal stabilization of camptothecin's lactone ring. J Am Chem Soc 114: 8318
7. Burke TG, Mishra AK, Wani MC, Wall ME (1993) Lipid bilayer partitioning and stability of camptothecin drugs. Biochemistry 32: 5352
8. Chen AY, Yu C, Potmesil M, Wall ME, Wani MC, Liu LF (1991) Camptothecin overcomes MDR1-mediated resistance in human KB carcinoma cells. Cancer Res 51: 6039
9. Daoud SS, Fetouh MI, Giovanella BC (1995) Antitumor effect of liposome-incorporated camptothecin in human malignant xenografts. Anticancer Drugs 6: 83
10. Fairchild GA (1972) Measurement of respiratory volume for virus retention studies in mice. Appl Microbiol 24: 812
11. Giovanella BC, Hinz HR, Kozielsky AJ, Stehlin JS, Silber JR, Potmesil M (1991) Complete growth inhibition of human cancer xenografts in nude mice by treatment with 20-(S)-camptothecin. Cancer Res 51: 3052
12. Giovanella BP, Natelson E, Harris N, Vardeman D, Stehlin JS (1996) Protocols for the treatment of human tumor xenografts with camptothecins. Ann NY Acad Sci 803: 181
13. Hendrics CB, Rowinsky EK, Grochow LB, Donchower RC, Kaufmann SH (1992) Effect of P-glycoprotein expression on the accumulation and cytotoxicity of topotecan (SK&F104864), a new camptothecin analogue. Cancer Res 52: 2268
14. Jaxel C, Kohn KW, Wani MC, Wall ME, Pommier Y (1989) Structure-activity study of the actions of camptothecin derivatives on mammalian topoisomeraze I: evidence for a specific receptor site and a relation to antitumor activity. Cancer Res 49: 1465

15. Knight V, Koshkina NV, Waldrep JC, Giovanella BC, Gilbert BE (1999) Anti-cancer effect of 9-nitro camptothecin liposome aerosol on human cancer xenografts in nude mice. Cancer Chemother Pharmacol 44: 177
16. Koshkina NV, Waldrep JC, Gilbert BE, Giovanella BC, Knight V (1998) Anticancer effect of 9-nitrocamptothecin aerosol delivery on human cancer xenograft models. Proc. of the 89th annual meeting of the Am Assoc Cancer Research 39: Abstract #1902, Cadmus Journal Services, Linthicum, Md., p 278
17. Natelson EA, Giovanella BC, Vershraegen CF, Fehir KM, De Ipolyi PD, Harris N, Stehlin JS (1996) Phase I clinical and pharmacological studies of 20-(S)-camptothecin and 20-(S)-9-nitrocamptothecin as anticancer agents. Ann NY Acad Sci 803: 224
18. Pantazis P (1995) Preclinical studies of water-insoluble CPT congeners: cytotoxicity, development of resistance and combination treatment. Clin Cancer Res 1: 1235
19. Pantazis P, Kozielsky AJ, Vardeman DM, Petry ER, Giovanella BC (1993) Efficacy of CPT congeners in the treatment of human breast carcinoma xenografts. Oncol Res 5: 273
20. Patton JC (1996) Mechanisms of macromolecule absorption by the lungs. Adv Drug Delivery Rev 19: 3
21. Phalen RF (1984) Inhalation studies; foundations and techniques. CRC Press, Boca Raton p 222
22. Sugarman SM, Zou Y, Wasan K, Kumi R, Reddy S, Perez-Soler R (1996) Lipid-complexed camptothecin: formulation and initial biodistribution and antitumor activity studies. Cancer Chemother Pharmacol 37: 531
23. Verschraegen CF, Natelson EA, Giovanella BC, Kavanagh JJ, Kudelka AP, Freedman RS, Edwards CL, Ende K, Stehlin JS (1998) A phase I clinical and pharmacological study of oral 9-nitrocamptothecin, a novel water-insoluble topoisomerase I inhibitor. Anticancer Drugs 9: 36