Spray-Dried Poly(D,L-Lactide) Microspheres Containing Carboplatin for Veterinary Use: In Vitro and In Vivo Studies

Elisabetla Gavini,[1] Lucia Manunta,[2] Stefano Giua,[2] Giannina Achenza,[3] and Paolo Giunchedi[1]

[1]Dipartimento di Scienze del Farmaco, University of Sassari, 07100 Sassari, Italy
[2]Istituto di Clinica Chirurgica Veterinaria, University of Sassari, 07100 Sassari, Italy
[3]Istituto Zooprofilattico Sperimentale della Sardegna, 07100 Sassari, Italy

ABSTRACT

The aim of this study was the development of a veterinary dosage form constituted by injectable biodegradable microspheres designed for the subcutaneous release of carboplatin, a chemotherapeutic drug. Poly(D,L-lactide) (PDLLA) microspheres were prepared by an emulsification/spray-drying method, using the drug-to-polymer weight ratios 1:9 and 1:5; blank microspheres (1% w/v) were prepared as a comparison. Microparticles were characterized in terms of morphology, encapsulation efficiency, and in vitro drug release behavior. In vivo tests were conducted on rats by subcutaneous injection of microsphere aqueous suspensions. Levels of carboplatin were evaluated both in the skin and in serum. The microparticles obtained had a spherical shape; particle size ranged from 5 to 7 μm, dependent on drug loading. Microspheres were able to control the in vitro release of the drug: approximately 90% to 100% of the carboplatin was released over 30 days. In vivo results showed that the microspheres were able to release high drug amounts locally, and sustained serum levels of drug were also achieved. Based on these results, carboplatin-loaded PDLLA microspheres may be useful for local delivery of the antineoplastic drug to the tumor, avoiding tumor recurrence in small animals, and may decrease the formation of distant metastases.

KEYWORDS: local chemotherapy, companion animals, poly(D,L-lactide), carboplatin, microspheres, spray-drying

INTRODUCTION

Over the past years neoplastic pathologies represent the most frequent cause of death of companion animals.

Surgical treatment is often combined with radiation therapy and/or systemic chemotherapy.[1,2] However, in animals (like in humans), antineoplastic agents cause a number of side effects concerning the bone marrow, the gastroenteric apparatus, and the skin.[3]

The toxicity of conventional systemic cancer chemotherapy has severely limited its safety and effectiveness. Safer and more aggressive (higher doses) administration of toxic chemotherapy directly to the tumor site (intratumoral therapy) is an attractive alternative to the systemic treatment.[4]

To minimize the side effects and to improve the efficacy of the therapy, different kinds of local chemotherapy have been developed (regional, arterial, and intralesional chemotherapy) using polymeric biodegradable devices. Biodegradable polymers for local drug delivery directly to the tumor have been studied in animal models of brain tumors and used more recently for the treatment of the human glioma.[5-8]

These systems allow the local delivery of chemotherapeutic agents after intratumoral implantation of a drug delivery system or injection: local therapy increases drug exposure and improves the therapeutic index targeting the drug directly into the tumoral mass.[9-11] With this goal, monoclonal antibodies and growth factors have been used, because they bind to the membrane receptors leading the drug inside the tumoral cell.

Théon et al[12-14] used sesame oil as carrier of cisplatinum in intralesional chemotherapy for the treatment of tumors in horses; the oily carrier permits the reduction of the systemic diffusion of the drug, minimizing the side effects of cisplatin and furnishing high drug concentrations at the tumoral tissue. However, this method can be used only for lipophilic drugs.

Liposomes represent a valid strategy either to overcome the chemoresistance or to act as a vehicle for hydrophilic drugs that can not penetrate through the cell membranes. They fuse with the membrane lipids and release the active agents directly into the cell.[15]

The regional advantages can be additionally increased by a sustained release of the drug. In fact, sustained release of anticancer drugs from polymers may also prevent the local recurrence of tumors.[9,16,17]

Over the past decade, microspheres prepared using biodegradable polymers, such as poly-lactide and poly-

Corresponding Author: Paolo Giunchedi, Dipartimento di Scienze del Farmaco, via Muroni 23/a, 07100 Sassari, Italy; Tel: +39-079-228754; Fax: +39-079-228733; E-mail: pgiunc@uniss.it

lactide-coglycolide, received much interest as carriers for chemotherapy.[18-21] They provide a useful and practical means of maximizing the efficacy of antineoplastic drugs by providing adequate concentrations of drug directly to the tumor; they have the potential to achieve a suitable duration of drug release for predefined periods of the time ranging from days to months, preventing the local recurrence of the tumor.[9,16]

The purpose of this work was the preparation of injectable biodegradable microspheres loaded with carboplatin (CP) for the subcutaneous treatment of animal skin cancer.

Poly(D,L-lactide [PDLLA]) was selected as a polymer because of its biocompatibility and biodegradability.[22]

CP, an antitumoral drug characterized by a relatively high solubility in water, has been already used successfully in cancer chemotherapy. It is an analog of cisplatin, with a similar spectrum of activity but with a short half-life (about 3 hours in the dog), because it binds to the plasmatic proteins.[23] CP is less nephrotoxic with respect to cisplatin, but it can cause thrombocytopenia.[24]

The incorporation of CP into PDLLA microspheres and then their direct intratumoral injection would achieve high and sustained drug concentrations selectively in the tumor, thus reducing the number of administrations.

Microspheres containing antitumoral agents have been already prepared,[19-21,25-27] but little work has been done on the encapsulation of anticancer drugs in polyesters by the spray-drying technique,[19,28] because solvent evaporation is the method commonly used.[29,30]

The spray-drying process received great attention for the preparation of microparticulate controlled release systems,[31,32] because it is a 1-step process and involves, simply, the preparation of a solution of drug and polymer. The emulsification/spray-drying method has been already proposed as an alternative to double emulsification for the preparation of poly-lactide-coglycolide microspheres containing water-soluble drugs,[33,34] and it is proposed here for the preparation of CP-loaded PDLLA microspheres.

Microspheres were characterized in terms of drug loading, morphology (scanning electron microscopy [SEM] and laser diffraction method), and in vitro drug release. Blank microparticles were prepared as a comparison.

In vivo studies were conducted on rats. Microparticles were injected as aqueous suspensions, via hypodermic, directly into the skin of the animals. The amount of drug released at the site of injection and diffused in the surrounding skin was evaluated within 21 days. The in vivo absorption of CP from microspheres was evaluated by the determination of pharmacokinetic parameters.

MATERIALS AND METHODS

Materials

CP 98% was purchased by Aldrich (Milan, Italy); PDLLA, Resomer R203 (inherent viscosity = 0.3, Mw [gel permeation chromatography] = 28,000) was supplied by Boehringer Ingelheim KG (Ingelheim am Rhein, Germany); sodium carboxymethylcellulose E 466 (low viscosity, 400 to 1,000 cPs) was purchased from Cruciani (Rome, Italy); Tween 80 was supplied by Sigma-Aldrich Chemie GmbH (Steinheim, Germany); and polytetrafluoroethylene syringe filters (13 mm, 0.45-μm porosity) were provided by Alltech Italia Srl, (Sedriano, Milan, Italy).

All of the other solvents and chemicals used were of reagent grade.

Preparation of the Spray-Dried Microspheres

Two batches of drug-loaded microspheres were prepared using different amounts of drug-to-polymer weight ratios: CP-MS10 constituted by 10% w/w of drug and 90% w/w of polymer and CP-MS17 constituted by 17% w/w of drug and 83% w/w of polymer.

A w/o emulsion was prepared by dissolving Poly(D, L-lactide); PDLLA polymer in dichloromethane and carboplati (CP) in the smallest volume of water possible (1% w/v total solid concentration in the emulsion). The amounts of drug and polymer were different depending on the composition of the microspheres prepared. The aqueous drug solution was added dropwise to the polymer solution under stirring at 9,000 rpm with a homogenizer (Ultra-Turrax T25 basic, IKA, Staufen, Germany). The temperature was maintained at about 5°C for the entire process. The drop size of the emulsion was analyzed by optical microscopy according to a method described.[35]

The microspheres were obtained by spraying the solutions through the nozzle of a spray-dryer (model Mini Spray HO Pabisch, W. Pabisch S.p.A., Milan, Italy), cocurrent flow type, equipped with standard 0.7-mm nozzle, using the following conditions: inlet air temperature, 70°C; outlet air temperature, 50°C; spray pressure, about 203 kPa; and spray-rate feed, about 15 mL/min.

Blank Poly(D,L-lactide); PDLLA polymer microspheres (1% w/v) were prepared in the conditions described above, as a comparison. The process conditions were, as follows: inlet air temperature, 60°C; outlet air temperature, 40°C; spray pressure, about 203 kPa; and spray-rate feed, about 13 mL/min.

The volume of feed solution sprayed for the preparation of each batch of both loaded and unloaded microspheres was 200 mL. The solid microparticles were collected and stored under vacuum at room temperature for 48 hours.

Each preparation was conducted in duplicate.

SEM

Shape and surface morphology of spray-dried microspheres were studied by SEM (Zeiss DSM 962, Zeiss, Oberkochen, Germany). The samples were mounted on double-sided tape, which had been secured previously on aluminum stubs and then analyzed at 20-kV acceleration voltage after gold sputtering under an argon atmosphere.

Particle-Size Analysis

Particle size and particle-size distributions were determined by laser diffractometry using a Coulter LS 100Q laser sizer (Beckman Coulter Particle Characterization, Miami, FL). Particle size analysis was performed both on empty and drug-loaded, spray-dried microspheres after their suspension in distilled water and sonication for 1 minute. The average particle size was expressed as volume-surface diameter (d_{vs}, micrometer).[36]

The results were expressed as mean of 3 determinations (SD within 0.3).

Drug Content Determination

Microspheres weighing about 4 mg were dissolved in 50 mL of Milli-Q water acidified with HNO_3 (1% v/v). The amount of CP entrapped into microspheres was determined by Inductively Coupled Plasma (ICP) mass spectrophotometer (Perkin Elmer Monza [Milan], Italy) in standard conditions. Calibration standards were prepared at 20 and 40 ppb concentration levels from platinum 1,000 ppm standard (Merck, Darmstadt, Germany) using 2-step dilution with 1% HNO_3. Platinum was quantified against the external calibration curve and calculated as a percentage of the total weight of microspheres.

In Vitro Release Studies

In vitro drug-release tests were conducted placing 125 mg of microspheres in 20 mL of phosphate buffered saline (pH 7.4), incubated at 37°C; 1 mL of supernatant was withdrawn after centrifugation (7,000 rpm, 15 minutes) at predetermined time intervals (1, 3, 5, 10, 15, and 30 days), and the same volume was replaced. The microparticles were resuspended after each drawing. The CP released was quantified by ICP-mass spectrometry using the same conditions reported for drug analysis.

The tests were conducted in triplicate.

In Vivo Studies

Animals

Twenty-four male Wistar Institute rats, weighing approximately 300 to 350 g each, were used. The rats were housed in standard cages in a temperature-controlled environment (22°C, humidity 60%) with alternating 12-hour cycles of light and dark. Rats were acclimated to the environment for 7 days before the study and received standard rodent feed and water ad libitum throughout the acclimation and study periods. The protocol was approved by the Ministry of Health, Rome.

CP Microsphere Administration

CP-MS10 microspheres were chosen as example for the in vivo studies.

Microspheres were administered as suspensions (40 μL; 10% w/v was the amount of microspheres in the medium) in an aqueous vehicle (3.0% sodium carboxymethylcellulose; 0.1% Tween 80; 0.9% NaCl) in the right abdominal subcutis of each rat with a dose of 0.45 mg of CP per cubic centimeter of tissue (16-gauge needle).

Single-dosing tests were conducted. CP-MS0 was administered as comparison and to verify any possible local irritation effect of the polymer. Pictures of the rat skin were taken immediately after injection and after 1 month.

Skin samples surrounded the injection site ("skin-in") were recovered after 2 hours, and 1, 7, and 21 days to evaluate the amount of CP diffused in the skin. In particular, at each time the animals were sacrificed by inspiration of diethyl ether, the right abdominal wall of each rat was harvested, and skin samples of the surrounded area of the injection site (skin-in) were excised to the evaluation of the CP concentration. The area considered as skin-in was a square of 1 cm for side and a thickness of about 3 to 4 mm. Before drug determination, the skin-in area was carefully cleaned from the microspheres.

Furthermore, blood samples were taken from the jugular vein after the same time points. The CP concentration in both the plasma and the skin was determined by ICP-mass spectrometry.

Skin samples were digested with concentrated HNO_3. The process was microwave assisted. The digested solution was diluted with Milli-Q water to 50 mL. The amount of CP recovered in skin-in was expressed as microgram per gram of skin.

Serum samples were diluted 1:20 with Milli-Q water and acidified with HNO_3 to 1% v/v. The amount of CP recovered in serum was expressed as microgram per milliliter of serum.

Pharmacokinetic parameters were determined by plotting the CP plasma concentrations versus time data. The maximum plasma concentration and the time to reach the maximum concentration were read directly from the curve obtained. The area under the curve from time zero to the

Table 1. Characterization of Spray-Dried Microspheres

Formulation	Encapsulation Efficiency (%)	d_{vs} (μm) $\pm$ SD	Mode (μm)
CP-MS0	–	5.14 ± 0.36	11.29
CP-MS10	99.21	5.77 ± 0.24	13.61
CP-MS17	96.85	7.3 ± 0.16	15.20

time of last measurable plasma concentration point was determined according to the trapezoidal rule.

RESULTS AND DISCUSSION

Preparation and Characterization of the Spray-Dried Microspheres

The emulsification/spray-drying method described here appeared to be a suitable and simple technique to prepare PDLLA microspheres loaded with a water-soluble drug. The method was rapid and involved the preparation of single w/o emulsions, with the polymer (PDLLA) dissolved in the organic phase and the hydrophilic drug (CP) in the aqueous phase. The spray-drying method, in the case of preparation of an injectable particulate system presents the additional advantage that it can be conducted in an aseptic condition, because atomization air can be filtered, and the apparatus could be easily cleaned and sterilized.

From microscopic observation, the dispersed phase of the feed emulsions (kept at about 5°C) was constituted by droplets of regular shape and uniform size (about 20 μm), and the droplets maintained their stability during all the entire spraying process. This stability permitted the avoidance of surfactants in the preparation. The spraying of the emulsions led to the drying of the solvents and to the formation of solid microparticles. As described previously,[33] the morphologic characteristics of particles are strictly dependent on the stability of the emulsion.

Production yields were found to be between 40% and 50% (SD $\pm$ 2.0). Drug contents were always close to the theoretical values; in fact, the microspheres were characterized by good encapsulation efficiencies of approximately 99%

and 97%, respectively, of the amount of CP entrapped in CP-MS10 and CP-MS17 (Table 1).

Particle size and particle-size distributions were determined by laser diffractometry. Mean diameter values were expressed as d_{vs} (Table 2). CP-MS17 microparticles, characterized by the highest CP content (about 17%), showed the higher d_{vs} (about 7 μm), whereas the other batches characterized by a lower drug loading (10%) or by the absence of drug (CP-MS10 and CP-MS0, respectively) both had a d_{vs} of about 5 μm. Mode values of particle size distribution were in a range between 11 and 15 μm.
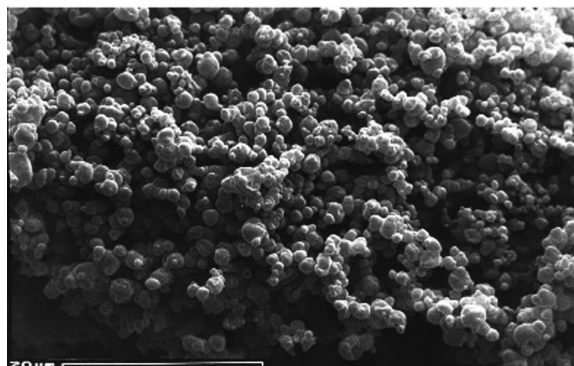
The SEM picture of CP-MS10, chosen as an example (Figure 1), showed particles with a spherical shape and a smooth surface that are partially melted and aggregated.

Analogous results were obtained from SEM pictures of the other microspheres (both drug loaded and blank). This indicated that the different compositions did not substantially influence the morphologic characteristics of the spray-dried particles.

In Vitro Release Studies

In vitro release tests were conducted in phosphate buffered saline (pH 7.4) within 30 days (Figure 2).

The 2 batches of microspheres had the following release behavior: CP-MS10 microspheres, with lower drug content (about 10%) and higher percentage of polymer (about 90%), were characterized by a slower release rate with respect to CP-MS17 microspheres (drug content of about 17%). At the end of the test (1 month), 100% of the

Table 2. Pharmacokinetic Parameters in Serum After In Vivo Administration in Rats of the Aqueous Suspension of CP-MS10*

Variable	Number
C_{max} (μg/mL)	24.7
T_{max} (days)	7.0
AUC (min * μg/mL)	257.7

*C_{max} indicates the maximum concentration; Tmax, time to reach the maximum concentration; and AUC, area under the curve from time zero to the time of last measurable plasma concentration point.

Figure 1. Scanning electron micrograph of CP-MS10 (magnification, ×2,000; acceleration voltage, 20 kV).
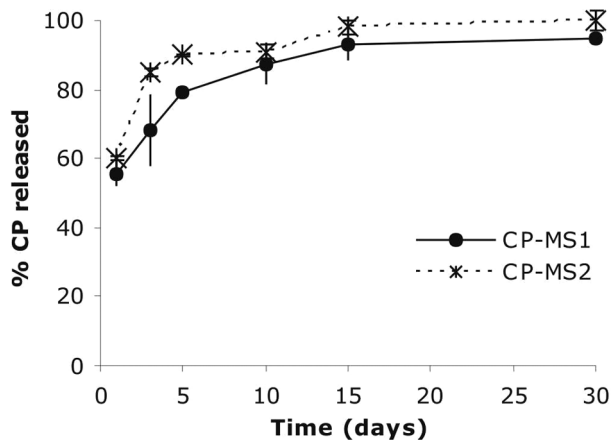
Figure 2. In vitro release profiles of drug-loaded PDLLA microspheres (n = 3; mean ± SD).

Figure 4. Amount of CP diffusion from the injection site toward the skin (μg/g) at different days after single administration (columns). Data are compared with the serum levels of drug (μg/mL; dots).

released drug was achieved from CP-MS17 microspheres, whereas about 92% was reached from CP-MS10. Additionally, CP-MS17 microspheres had a less uniform release rate, which was higher at the beginning (first 5 days).

In Vivo Studies

Preliminary in vivo tests were conducted on blank microspheres to evaluate the effect of the polymeric microparticles on the skin.

Immediately after administration of the blank microspheres formulated as aqueous suspension, a small skin swelling at the skin-in area was observed (Figure 3).

After 30 days, a subcutaneous nodule of about 1 to 2 mm was recovered and histologically analyzed. It was a lipidic

Figure 3. Photograph of rat skin immediately after administration of blank microspheres (top) and 30 days later (bottom).
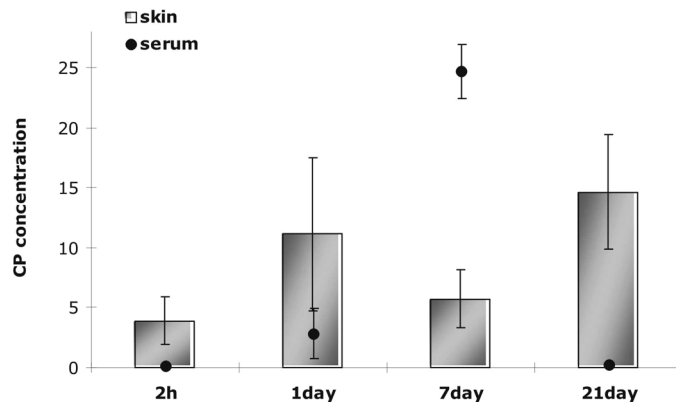
granuloma induced by the microsphere presence, and it appeared to be absorbed (Figure 3). In any case, no evidence of severe skin irritation and/or toxicity induced by PDLLA was found.

Based on the results of the in vitro tests, the drug-loaded batch CP-MS10 has been chosen for the in vivo study, because these microparticles were characterized by a more-sustained and uniform release with respect to CP-MS17 microspheres.

The aqueous suspension in which CP-MS10 microparticles were formulated was characterized by a good stability, because no sedimentation was observed during preparation and injection (2 to 5 minutes) and by a good syringability.

Results of the evaluation of the amount of CP (expressed as micrograms per gram) determined in the skin-in at different time points (2 hours and 1, 7, and 21 days) were illustrated in Figure 4.

These results showed that the drug loaded in the microparticles diffused quite quickly from the injection site to the skin, because 2 hours after the single administration, about 3.9 μg of CP can be detected.

The release of the drug into the skin at all times tested showed a bimodal behavior with 2 peaks: the first peak (corresponding to about 11.14 μg of CP) at the day 1 and the second peak (14.63 μg of CP) at the day 21.

Serum CP concentrations versus time profiles after subcutaneous administration of CP-MS10 formulation were shown in Figure 4. Small amounts of the drug were detected in serum at the first time point (2 hours). Drug levels increased within 7 days, reaching about 24 μg/mL. In the following 2 weeks the serum CP concentration gradually decreased.

The comparison between the drug amount in the skin and the drug level in the serum at the corresponding times

showed that after 7 days, the CP concentration in the skin decreased, whereas the serum level reached the highest value. This may indicate an initial release of drug from microspheres in the skin-in from where, through a process of absorption, the drug moved to the plasma.

At day 21, the highest drug concentration into the skin corresponding with the lowest concentration in the serum may be attributable to an additional release of the drug from the microspheres (biphasic release behavior).

These results showed that the delivery system that was prepared was able to sustain the release of CP giving effective skin and serum concentration over 14 days.

Based on these results, this delivery system can be useful for the local treatment of animal neoplasia. In fact, the deposition of the microspheres in the pathologic site after surgical resection of the tumor may release high drug concentrations locally, preventing tumor recurrence. Simultaneously, slow but sustained serum levels may decrease the formation of distant metastases.

Table 2 lists the pharmacokinetic parameters obtained directly from the CP serum concentration versus time curve. The maximum serum concentration was about 25 μg/mL at the day 7. These values should be considered preliminary and indicative because of the few points of the serum curve.

CONCLUSION

PDLLA-biodegradable microparticles containing the water-soluble drug CP can be easily prepared by the emulsification-spray drying technique. The microspheres obtained were an in vitro-effective controlled release delivery system of CP.

Drug-free microparticles showed skin biocompatibility; they did not induce any local toxic reaction. Based on these preliminary results, biodegradable microspheres may have an application as an efficient system for the local delivery of CP in supporting surgical resection of the tumor.

REFERENCES

1. Bergman PJ, MacEwen G, Kurzman ID, et al. Amputation and carboplatin for treatment of dogs with osteosarcoma: 48 cases (1991 to 1993). *J Vet Intern Med.* 1996;10:76-81.

2. Lana SE, Dernell WE, LaRue SM, et al. Slow release cisplatin combined with radiation for the treatment of canine nasal tumours. *Vet Radiol Ultrasound.* 1997;38:474-478.

3. Withrow SJ. Surgical oncology. In: Withrow SJ, MacEwen EG, eds. *Small animal clinical oncology*, 3rd ed. Philadelphia: WB Saunders, 2001;70-75.

4. Goldberg EP, Hadba AR, Almond BA, Marotta JS. Intratumoral cancer chemotherapy and immunotherapy:opportunities for nonsystemic preoperative drug delivery. *J Pharm Pharmacol.* 2002;54:159-180.

5. Utsuki T, Brem H, Pitha J, et al. Potentiation of anticancer effects of microencapsulated carboplatin by hydroxypropyl α-cyclodextrin. *J Control Release.* 1996;40:251-260.

6. Chen W, LU DR. Carboplatin-loaded PLGA microspheres for intracerebral injection: formulation and characterization. *J Microencapsul.* 1999;16:551-563.

7. Emerich DF, Winn SR, Hu Y, et al. Injectable chemotherapeutic microspheres and glioma I: Enhanced survival following implantation into the cavity wall of debulked tumours. *Pharm Res.* 2000;17:767-765.

8. Emerich DF, Winn SR, Hu Y, et al. Injectable chemotherapeutic microspheres and glioma II: Enhanced survival following implantation into deep inoperable tumours. *Pharm Res.* 2000;17:776-781.

9. Ehrhart N, Dernell WE, Ehrhart EJ, et al. Effects of a controlled-release cisplatin delivery system used after resection of mammary carcinoma in mice. *J Am Vet Med Assoc.* 1999;60:1347-1351.

10. Orenberg EK, Luck EE, Brown DM, Mitchell BE. Implant delivery system: intralesional delivery of chemotherapeutic agents for treatment of spontaneous skin tumors in veterinary patients. *Clin Dermatol.* 1991;9:561-568.

11. Orenberg EK, Miller BH, Greenway HT, et al. The effect of intralesional 5-fluorouracil therapeutic implant (MPI 5003) for treatment of basal cell carcinoma. *J Am Acad Dermatol.* 1992;27:723-728.

12. Théon AP, Pascoe JR, Carlson GP, Krag DN. Intratumoral chemotherapy with cisplatin in oily emulsion for treatment of tumours in horses. *J Am Vet Med Assoc.* 1993;202:261-267.

13. Théon AP, Pascoe JR, Galuppo LD, Fisher PE, Griffey SM, Madigan JE. Prospective randomised comparison of perioperative versus postoperative intratumoral administration of cisplatin for treatment of cutaneous of tumours in horses. *J Am Vet Med Assoc.* 1999;215:1655-60.

14. Théon AP, VanVechten MK, Madewell BR. Intratumoral administration of carboplatin for treatment of squamous cell carcinomas of the nasal plane in cats. *J Am Vet Med Assoc.* 1996;57:205-10.

15. Kubo T, Sugita T, Shimose S, Nitta Y, Ikuta Y, Murakami T. Targeted systemic chemotherapy using magnetic liposomes with incorporated adriamicin for osteosarcoma in hamsters. *Int J Oncol.* 2001;18:121-125.

16. Menei P, Venier MC, Gamelin E, et al. Local and sustained delivery of 5-fluorouracil from biodegradable microspheres for the radiosensitization of glioblastoma: a pilot study. *Cancer.* 1999;86:325-330.

17. Pohlen U, Berger G, Binnenhei M, Reszka R, Buhr HJ. Increased carboplatin concentration in liver tumors through temporary flow retardation with starch microspheres (Spherex) and gelatin powder (Gelfoam): An experimental study in liver tumor-bearing rabbits. *J Surg Res.* 2000;92:165-170.

18. Okada H, Toguchi H. Biodegradable microspheres in drug delivery. *Crit Rev Ther Drug Carrier Syst.* 1995;12:1-99.

19. Fu YJ, Mi FL, Wong TB, Shyu SS. Characteristic and controlled release of anticancer drug loaded poly(D,L-lactide) microparticles prepared by spray drying technique. *J Microencapsul.* 2001;18:733-747.

20. Shenoy DB, D'Souza RJ, Udupa N. Poly(DL-lactide-co-glycolide) microporous microsphere-based depot formulation of a peptide-like antineoplastic agent. *J Microencapsul.* 2002;19:523-535.

21. Zhu KJ, Zhang JX, Wang C, Yasuda H, Ichimaru A, Yamamoto K. Preparation and in vitro release behavior of 5-fluorouracil-loaded microspheres based on poly (L-lactide) and its carbonate copolymers. *J Microencapsul.* 2003;20:731-743.

22. Anderson JM, Matthew SS. Biodegradation and biocompatibility of PLA and PLGA microspheres. *Adv Drug Deliv Rev.* 1997;28:2-24.

23. Page RL, McEntee M, George SL, et al. Pharmacokinetic and phase 1 evaluation of carboplatin in dogs. *J Vet Intern Med.* 1993;7:235-240.

24. Moore MJ, Erlichman C. Pharmacology of anticancer drugs. In: Tannock IF, Hill RP, eds. *The Basic Science of Oncology*, 3rd ed. New York: McGraw-Hill Health Professions Division NY, 1998;370-391.

25. Ike O, Shimizu Y, Wada R, Hyon SH, Ikada Y. Controlled cisplatin delivery system using poly(D,L-lactic acid). *Biomaterials.* 1992;13:230-234.

26. Boisdron-Celle M, Menei P, Benoit JP. Preparation and characterization of 5-fluorouracil-loaded microparticles as biodegradable anticancer drug carriers. *J Pharm Pharmacol.* 1995;47:108-114.

27. Dubey RR, Parikh JR, Parikh RR. Effect of heating temperature and time on pharmaceutical characteristics of albumin microspheres containing 5-fluorouracil. *AAPS PharmSciTech.* 2003;4:E4.

28. Fu YJ, Shyu SS, Su FH, Yu PC. Development of biodegradable co-poly(D,L-lactic/glycolic acid) microspheres for the controlled release of 5-FU by the spray drying method colloids and surfaces. *B Biointerfaces.* 2002;25:269-279.

29. Ozalp Y, Özdemir N, Kocagöz S, Hasirci V. Controlled release of Vancomycin from biodegradable microcapsules. *J Microencapsul.* 2001;18:89-110.

30. Parikh RH, Parikh JR, Dubey RR, Soni HN, Kapadia KN. Poly(D,L-lactide-co-glycolide) microspheres containing 5-fluorouracil: optimization of process parameters. *AAPS PharmSciTech.* 2003;4:E13.

31. Giunchedi P, Conte U. Spray-drying as a preparation method of microparticulate drug delivery systems: an overview. *STP Pharma Sciences.* 1995;5:276-290.

32. Gavini E, Sanna V, Juliano C, Giunchedi P. Compressed biodegradable matrices of spray-dried PLGA microspheres for the modified release of Ketoprofen. *J Microencapsul.* 2003;20:193-201.

33. Giunchedi P, Gavini E, Bonacucina G, Palmieri GF. Tabletted polylactide microspheres prepared by a w/o emulsion-spray drying method. *J Microencapsul.* 2000;17:711-720.

34. Gavini E, Chetoni P, Cossu M, Alvarez MG, Saettone MF, Giunchedi P. PLGA microspheres for the ocular delivery of a peptide drug: vancomycin. Emulsification/spray-drying as preparation method; in vitro / in vivo studies. *Eur J Pharm Biopharm.* 2004;57:207-212.

35. Giunchedi P, Conti B, Puglisi G, Genta I, Conte U. Emulsion spray - drying for the preparation of albumin loaded PLGA microspheres. *Drug Dev Ind Pharm.* 2001;27:745-750.

36. Edmundson IC. Advances in Pharmaceutical Sciences. Vol 2. Bean HS, Carless JE, Beckett AH, eds. New York, NY: Academic Press; 1967.