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In Vitro and *In Vivo* Preparation Evaluations of Bleomycin Implants and Microspheres Prepared with DL-Poly (Lactide-Co-Glycolide)

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ABSTRACT In this investigation, poly(lactide-co-glycolide) (PLGA) gel implants and microspheric depot systems of bleomycin (BLM) were formulated and evaluated in vivo in mice bearing transplantable solid tumor (fibrosarcoma). The pharmacodynamic studies showed that both the formulations retarded tumor growth significantly ($p < 0.05$) when compared to the control animals (without any drug treatment). Preliminary pharmacokinetic studies illustrated controlled release of the drug into the systemic circulation to elicit the anti-neoplastic action. The gel implants showed better release characteristics and greater pharmacodynamic action when compared to the microspheres, thus demonstrating the feasibility of employing biodegradable depot polymer gel matrix for chronic cancer therapy.

KEYWORDS Poly(lactide-co-glycolide), Microspheres, In situ gel, Implants, Anticancer activity, Bleomycin

INTRODUCTION

Exploitation of polymeric drug delivery systems for parenteral controlled release of proteins, peptides, and poorly soluble compounds provides a number of advantages over daily injections with patient compliance being an important benefit. The conventional oral and intravenous routes of drug administration do not provide ideal pharmacokinetic profiles, especially for drugs which display high toxicity and/or a narrow therapeutic window. For such drugs, the ideal pharmacokinetic profile will be one wherein the drug concentration is maintained at therapeutic levels without exceeding the maximum tolerable dose and maintaining this dose continuously for prolonged periods until the therapeutic effect is reached. One of the ways in which such a profile can be achieved is to encapsulate the drug in a polymeric matrix. These new drug delivery systems are injected or implanted into the subcutaneous tissue and release the incorporated drug in a controlled manner, allowing the adjustment of release rates over extended periods of time ranging

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from several days to up to one year (Danckwerts & Fassihi, 1991).

Interest in the field of polymeric drug delivery has increased considerably especially after the commercial success of products such as Lupron Depot®, Zoladex®, Norplant®, and Gliadel®, all which use the principles of sustained and localized drug delivery. In case of localized drug delivery, one attempts to achieve high drug concentrations at the site of implantation without exposing the non-affected tissue. This aspect can be utilized for the effective delivery of antineoplastic agents, which have a considerable level of tissue toxicity in addition to a narrow therapeutic window.

Ideal cancer chemotherapy would involve a relatively constant drug concentration maintained between the minimum therapeutic level and the toxic level of the antineoplastic agent for an appreciably prolonged duration of time. However, conventional cancer therapy involves episodic or bolus administration of drugs which exposes the patient to peak drug concentrations as a result of which critical therapeutic levels is not always maintained. This leads to peak and trough fluctuations and toxic manifestations generally associated with conventional therapy. In addition to this, another major disadvantage is that the most potent drugs are also highly toxic to the normal tissue and are not well tolerated by the patients. Hence, the strategy of identifying new methods of delivery for existing drugs is more feasible and rewarding (Langer, 1981; Hnatyszyn et al., 1994; Singh et al., 2001). The design of an injectable system which will be biodegradable and which will release the drug at a rate affected by the erosion of the system seems to be a very promising way to overcome these problems nowadays. In this respect, the DL-polylactic/glycolic acid (PLGA) microspheres have had success because of the experience provided in terms of safety and biodegradability. Currently marketed products include microspheres, wafers, and polymer solutions that solidify at the injection site, while semi-solid materials and thermally gelling liquid formulations are currently under development (Zentner et al., 2001; Ravivarapu et al., 2000; Barr et al., 2002; Westphal et al., 2003; Persad, 2002). The examples of formulations of PLGA microspheres in the market are: Lupron Depot®, Enantone Depot®, Decapeptil®, and Pariodel LA®. In spite of the great potential of the biodegradable microspheres for the controlled release of macromolecules, there are important difficulties which explain, to a certain extent, the limited number of formulations on the market. The systemic bioavailability of peptide and protein drugs after oral administration, which is the most widely used route for drug administration, is primarily restricted by their extremely low absorptions for gastrointestinal tract (GIT) because of their low permeation through intestinal membrane and their high affinity for proteolytic enzymes. Toxicity forms a limiting factor in using these drugs as chemotherapeutic agents. In addition to the therapeutic aspects, factors such as the ease of synthesis, processing, sterilization, and formulation while maintaining low-cost product with a defined pathway have to be considered. However, the systemic bioavailability of the macromolecules can promisingly be enhanced by subcutaneous depot forming drug delivery systems.

With regard to these factors, the "in-situ gel forming" systems provide a viable alternative to the much researched microparticulate systems, which involve complex manufacturing procedures. These polymeric systems are a liquid at room temperature and a gel matrix similar to an implant forms immediately on contact with the body fluid (examples include OncoGel® and Atrigel®) and can be easily injected through a conventional 21–23-gauge needle (Chandrashekar & Udupa, 1996). Release of the entrapped drug occurs for prolonged periods depending on the degradation profile of the polymer, this property being exploited by numerous researchers (Jeong et al., 1997; Shah et al., 1993). The objective of this study was to develop and evaluate a thermally gelling liquid biodegradable formulation and a microparticulate system, which could deliver BLM, an anti-neoplastic glycopeptide, at controlled rate within the therapeutic window for prolonged periods of time.

MATERIALS AND METHODS

Materials

Bleomycin sulphate was purchased from M/s. Khandelwal Laboratories, Mumbai, India. PLGA (copolymer ratio 75:25; Mol. Wt. 90000 – 126000; intrinsic viscosity of 0.55 – 0.75 dL/g) was procured from M/s Birmingham Polymers, Inc., Birmingham, AL, USA. Triacetin was procured from Sigma Chemical Co., St. Louis, MO, USA. Poly vinyl alcohol (Mol. Wt. 78000; 98 mol% hydrolyzed) was purchased from Polysciences, Inc., Warrington, PA, USA. All others solvents and chemicals were of analytical grade and used as received as procured.

Animals

The animals (Swiss Albino mice; 6–8 weeks old), weighing between 25–30 g, were obtained from National Institute of Nutrition, Hyderabad, India. The Institutional Animal Ethical Committee of Kasturba Medical College, Manipal, approved the experimental protocol for all the in vivo studies. The animals were maintained under controlled conditions of temperature (25 ± 2°C), humidity (50 ± 5%), light and darkness (10 and 14 h, respectively), in polypropylene cages filled with sterile paddy husk as bedding material. They were fed a balanced diet (Lipton India Ltd.) and water ad libitum.

Preparation of Biodegradable PLGA Gel Implants

The method reported by Shah et al. (1993) was used. A solution of PLGA (75:25; 118 mg) was prepared by warming the polymer with 1 mL triacetin at a temperature of 65–70°C in vials and then cooling to room temperature to obtain a clear solution. Bleomycin (BLM) was dissolved in 100 µl of propylene glycol and added to the above cooled solution in portions and shaken gently until a clear solution was obtained. Propylene glycol was chosen, as it is miscible with the polymer, and was a good solvent to dissolve BLM.

Preparation of Biodegradable PLGA Microspheres

Bleomycin (BLM) loaded PLGA microspheres were prepared by emulsion–solvent evaporation method mentioned by Ogawa et al. (1998). Poly (lactide-co-glycolide) (PLGA) was dissolved in an appropriate volume of methylene chloride (MC). A primary emulsion (w/o) was prepared with an aqueous solution (0.1–0.5 mL) of the drug as internal phase and polymer solution (45–60%) as the external phase using a high-speed homogenizer (Remi, Mumbai, India). Once a stable emulsion was formed, it was poured into the bulk of the aqueous solution of poly vinyl alcohol (PVA) of suitable strength (0.1% w/v) and agitated using a mechanical stirrer for appropriate time period. The microspheres formed were then stirred using a magnetic stirrer for 4 h and were collected by centrifugation (3000 rpm for 15 min) followed by filtration using a Millipore® filtration assembly fitted with Sartorius filters (0.45 µm) and washed successively with distilled water. Finally, they were dried in a vacuum desiccator for suitable time and stored in airtight, amber colored containers in the refrigerator (2–8°C).

Determination of Size Distribution of Microspheres

The particle size distribution was determined using a particle size analyzer (Mastersizer 2000, Malvern Instruments Ltd., UK) by laser scattering technique. The obscuration level was maintained at 0–5% with an atomization pressure of 2 bar at 30% feed rate. The size range accepted by this system is 0.02 µm to 2000 µm.

Determination of Drug Encapsulation Efficiency of Microspheres

Twenty-five milligrams of drug-loaded microspheres were dissolved in 5 mL MC. The drug was back extracted into 10 mL phosphate buffered saline of pH7.4 (PBS) by agitation for 30 min. Aqueous layer was separated and was assayed for drug content after suitable dilution with PBS at 291 nm using Diode Array Spectrophotometer 8453 (HP, Palo Alto, CA, USA). From the data obtained, drug entrapment efficiency was computed. For UV spectrophotometric analysis of drug, a standard plot in PBS was prepared with drug concentrations ranging between 0.375–75.00 µg/mL (R^2 = 0.9987; Slope = 0.0100) where as for back extraction of drug from methylene chloride to PBS, a concentration range of 2.00–80.00 µg/mL was selected to prepare calibration curve (R^2 = 0.9955; slope = 0.0120).

In Vitro Drug Release Studies

Known quantity gel implant (about 0.44 mL) containing about 2 mg of BLM was injected into stoppered flasks containing PBS. In case of microspheres weighed amount (about 138 mg) containing known amount of drug (about 1 mg) were suspended in stoppered flasks containing PBS (10 mL). Both sets of flasks were placed in a horizontal shaker bath (Remi Thermostatic Shaking Water Bath, Mumbai, India) and maintained at a temperature of 37 ± 1°C at a speed setting

of 25 cycles/min. At predetermined time intervals, samples were collected from pre-labeled flasks and assayed for drug content as explained above. The experiment was performed in triplicate sets.

Pharmacodynamics Evaluation

Tumour Induction in Mice and Assessment

The mouse fibrosarcoma cell line was obtained from Department of Radiobiology, KMC, Manipal, India. For the experiments, 5×10^5 viable cells in 50 μl of Dulbecco's modified Eagle medium were injected intradermally into dorsal skin of Swiss mice. Once the tumor became palpable, diameters in three perpendicular planes were measured (Kamath et al., 1999). The tumor volume (V) was calculated using the formula:

$$V = \pi/6\ (D_1.D_2.D_3)$$

where $D_1.D_2.D_3$ are tumor diameters along three perpendicular planes. D1, D2, and D3 were determined using Slide Calipers (Mitutoyo, Tokyo, Japan).

For the purpose of evaluation of anticancer efficacy in mice bearing solid tumors the treatment modalities and other details followed are given in Table 1. The treatment was given in single dose modalities once the tumor size reached 100 ± 10 mm^3. The microspheres were suspended in a suitable vehicle (consisting of aqueous solution of 0.5% w/v sodium carboxymethyl cellulose of viscosity 9 cps, 0.9% w/v sodium chloride, and 0.1% w/v polysorbate 80). The bleomycin (BLM) control received a smaller dose because of the mortality obtained with the toxicity studies at higher dose levels. All the injections were given through 21-gauge needles subcutaneously on the back of mice.

The morphological tumor growth response was assessed on the basis of tumor regression, volume doubling time (VDT is the time taken for the tumor volume to reach double the treatment volume), growth delay (GD is the difference in the time between treated and untreated tumors to reach five-times the treatment volume), partial response (PR is the regression of 50% or more than the treatment volume), and no response (NR is less than 50% regression in the treatment volume) calculated from tumor volume measured every alternate day. Survival studies were not carried out due to ethical considerations and the animals were sacrificed once the tumor volume reached 1000 mm^3.

TABLE 1 **Treatment Schedules for BLM Delivery Systems in Mice Bearing Implantable Tumor (Fibrosarcoma)**

Formulation	Dose (mg/kg)	Route of administration	No. of animals per group
Control	—	—	6
BLM control	10 and 20	s.c.	12
BLM-microspheres	10 and 20	s.c.	12
BLM-gel implants	10 and 20	s.c.	12

Pharmacokinetic Evaluation

The pharmacokinetic profiles of BLM in the tumor-induced and healthy were studied by determining the plasma concentrations of BLM simultaneously in both the control and study groups. The treatment modality was similar to one followed for pharmacodynamic evaluation as in Table 1. For the purpose of evaluation, blood samples (about 0.8 to 1.0 mL) were withdrawn periodically by sino-arbital vein puncture using heparinized capillaries; plasma was separated by centrifugation and stored in vials under refrigeration (2–8°C) until further analysis. The blood samples were collected at periodic time intervals after the formulations were injected (0, 2, 4, 8, 12, 24, 48, 120, 240, and 360 h in case of tumor induced mice; 0, 2, 4, 8, 12, 24, 48, 120, 240, 360, 720, 1080, and 1440 h in case of healthy mice), the time being after the tumor volumes reached 100 ± 10 mm^3. The blood sampling was carried out periodically till the tumor volume reached 1000 mm^3 after which the animals were sacrificed for ethical considerations.

The plasma concentrations of BLM were determined by high performance liquid chromotography (HPLC) analysis by a method already established earlier (Shiu et al., 1979).

HPLC System

The system consisted of a delivery pump (Model 871, IRICA, Kyoto, Japan), a reversed phase analytical column (ODS; 5 μm; 150 × 4.6 mm ID; Zorbax, Tokyo, Japan) protected by a guard column (ODS; 5 μm; Zorbax, Tokyo, Japan), an integrator (Chromatocorder 11; System Instruments, Tokyo, Japan), a Rheodyne sample injector with a loop of 50 μL

(Model 7125, Rheodyne, USA), and a variable wavelength UV-visible detector (Model ERC 8710, IRICA, Kyoto, Japan).

Chromatographic Conditions

The mobile phase consisted of methanol:acetonitrile:0.0085M heptane sulfonic acid:acetic acid (30:10:59:1). The eluent was monitored with a UV-visible detector set at 254 nm and a flow rate of 1 mL per min was set up.

Extraction from Mouse Plasma

Mouse plasma (0.1 mL), obtained from healthy Swiss mice, was spiked with graded concentrations of BLM aqueous solution (0.1 mL each) in glass stoppered micro-centrifuge tubes. To these samples, 20 µL of 20% w/v trichloroacetic acid solution was added. The samples were gently vortexed and then centrifuged at 3000 rpm for 10 min. A 50 µL aliquot of clear supernatant was then injected onto the column. The drug concentration was estimated and a graph of plasma concentration of drug vs. time was plotted. The pharmacokinetic parameters were then calculated using Non-compartmental PK Solution software.

Statistical Analysis

The data was analyzed using student's *t*-test (Statistical Package for the Social Sciences (SPSS) software).

RESULTS AND DISCUSSION

Formulation Development

The gel implant was a solution of PLGA in triacetin containing BLM, which could be easily injected through a 22/24-gauge syringe needle. On injection of the formulation into PBS, a gel matrix was immediately formed in vitro. The consistency of the gel matrix formed depends on the concentration of the polymer in the vehicle (Shah et al., 1993).

The bleomycin-loaded microspheres were formulated using the water/oil/water method. The process followed was simple with minimum batch-to-batch (variations in drug content: ±3.50%; number of batches: 3) and intra-batch variations (variations in drug content: ±0.95%; number of samples: 3) with an entrapment efficiency of 59.10 ± 4.56% (Mean ± SD; n = 3) and practical yield of 76–78%. The size distribution of the particles was within the broad range of 15.136 µm (1.12 vol%) and 120.228 µm (3.11 vol%). The size of 90 vol% of the particles [d (0.9)] was 101.720 µm; whereas the size of 10 vol% of the particles [d (0.1)] and 50 vol% of the particles [d (0.5)] was 23.050 µm and 54.090 µm, respectively. Lower size ranges could be obtained if more powerful methods of dispersion are employed. Similar microspheres have been studied and discussed in-depth in our previous study (Shenoy et al., 2002).

In Vitro Drug Release Studies

The profile of in vitro release from BLM loaded gel implants and microspheres is given in Fig. 1. In the case of the gel implants, an initial rapid release (21.45 ± 2.52% in the first 96 h) is seen which could be due to the drug on the surface of the implants. On injection of the polymer containing drug into the aqueous environment, the biocompatible solvent diffuses out into the aqueous surroundings leaving behind the solid gel matrix of the polymer. This process is not immediate and takes some time after the administration of the polymer (approximately 1 min), during which time some of the drug on the surface diffuses into the surrounding area thereby accounting for the initial rapid release. The degradation process and thereby the release processes of all lactide-based polymers are a function of the crystallinity and hydrophilicity of their chemical composition. Bleomycin (BLM) although being a water-soluble drug, shows a slow release pattern during the later part of the study since it does not easily diffuse out of the polymer matrix. This is due to the high molecular weight of BLM (molecular weight: 1516.62), which does not permit direct diffusion through a non-biodegradable polymer matrix (Langer R. Folkman, 1976). For release to occur, the drug has to partition from its hydrophilic environment to a less hydrophilic environment of the polymer matrix and diffuse through a network into the aqueous medium. Due to the lower partition of drug into the polymer, the release has to take place mainly through the water filled channels that are formed as a result of hydration of the polymer. As a result of this, slow release of drug occurs and this is evident from the 62.32% release achieved in a period of 30 days (Fig. 1). The release pattern for microspheres shows a minimal burst effect due to removal

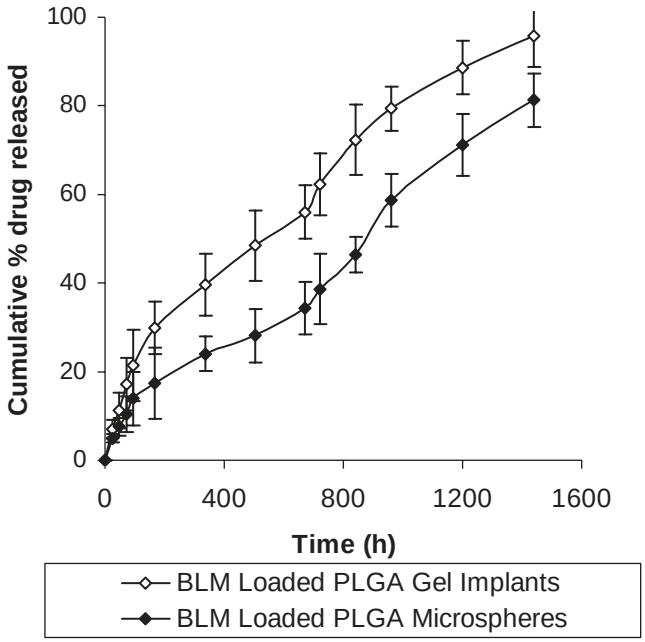

FIGURE 1 **In Vitro Release of BLM from Biodegradable Gel Implants and Microspheres. All Values are Presented as Mean ± SD; *n* = 3.**

of the surface drug during the washing process and, hence, release of drug takes place only once the process of degradation begins. Diffusion through pre-existing pore network in the formulated microspheres and subsequent enhanced diffusion via erosion-induced pore enlargement and evolution has been regarded as a pre-dominant drug-release mechanism (Shah et al., 1992). As a result, a release of 38.65% was observed in 30 days (Fig. 1). The degree of crystallinity of PLGA polymer directly influences the mechanical strength, swelling behavior, capacity to undergo hydrolysis, and subsequently the degradation rate (Jain et al., 1998). The resultant crystallinity of the PLGA copolymer is dependent on the type and the molar ratio of the individual monomer components (lactide and glycolide) in the copolymer chain. With both gel implants and microspheres, the drug release at later stages of the study (during 30 to 60 days) was again fast. At the end of 60 days, the gel implant and microspheres released 95.68% and 81.25%.

Pharmacodynamic Evaluation

These studies were carried out in order to assess the efficacy of the formulation and to determine if the biological activity is retained after formulating as a controlled release system. Since the aim of the formulation as a controlled release system is to alter the biodistribution of the drug, it is necessary to determine if the pharmacodynamics of the drug has been altered when compared to the conventional dosage forms at comparable dose levels. We chose solid tumor as a model for evaluation since the majority of the complex problems encountered in cancer chemotherapy is in treating solid tumors and BLM exhibits potent anticancer activity against solid tumors. In addition to this, the tumor model chosen exhibits well-defined growth properties and, hence, assessment of the pharmacodynamic activity of the drug in the delivery system becomes more meaningful. No spontaneous regression of the tumor was observed in the mouse colony and all the mice injected with the tumor cells developed a solid tumor. The results obtained with a single dose single modality treatment are shown in Table 2. Both the formulations showed a significant inhibition in tumor growth (VDT 9.76 for gel implants and 8.82 for microspheres as against 4.3 days for BLM control) at a dose level of 20 mg/kg. Further, the depot formulations exhibited significant increase in GD values (8.34 days for gel implants and 7.94 days for microspheres) when compared to the BLM control group. The pharmacodynamic behavior of formulations against fibrosarcoma can be explained on the basis of altered biodistribution of BLM. On administration of the formulations, not all of the injected drug is available for pharmacodynamic action but forms a depot at the site of administration. Only a small fraction of the drug is available for immediate action and further doses are slowly released depending on the release characteristics of the formulation, which form the maintenance dose. The main factor that contributes towards drug release is the hydrolytic cleavage of the polymer chains, which occurs after administration of the formulations. Water has to penetrate into the bulk and cause biodegradation through cleavage of the backbone ester linkages of the polymer. This bulk degradation occurs at a uniform rate throughout the PLGA matrix. As a result of these factors, it is the extent of biodegradation of the polymer matrix which plays a major role in deciding the drug fraction available for antitumor action. Another factor that could be responsible for drug release is the diffusion of the drug through the polymer matrix. Due to the high aqueous solubility of the drug, a rapid concentration gradient might have been established between the exterior and the core due to the drug which is transferred into a rapidly drug depleting zone in the vicinity

TABLE 2 **Antitumor Efficacy of BLM-loaded Biodegradable Systems in Swiss Mice Bearing Transplantable Tumour (Fibrosarcoma)**

Treatment	VDT (days)	GD (days)	Partial response (%)	No response (%)	Complete response (%)
Control	3.53 ± 0.68	—	—	100	—
BLM control					
10 mg/kg	4.3 ± 0.86	3.88 ± 1.58	—	100	—
PLGA- BLM Microspheres					
10 mg/kg	7.91 ± 1.29*†	6.98 ± 1.87	10	90	—
20 mg/kg	8.82 ± 1.12*†	7.94 ± 0.91†	30	70	—
PLGA-BLM gel implant					
10 mg/kg	8.68 ± 1.34*†	7.18 ± 1.78	20	80	—
20 mg/kg	9.76 ± 0.98**†	8.34 ± 2.41†	30	70	—

Control: no treatment.
BLM control: free bleomycin injection in sterile water for injection.
Partial response: regression ≥50% from the treatment volume.
No response: regression of <50% from the treatment volume.
Complete response: regression of the tumor.
Results are mean ± SD.
Number of animals per groups is 12.
*P < 0.05 compared with control.
**P < 0.01 compared with control.
†P < 0.05 compared with BLM control.

of the surface. As a result of these factors, there is a slow continuous supply of drug into the biological system at any given time, which leads to greater cytotoxic action when compared to the plain drug.

If we compare the two formulations for their cytotoxic action, the gel implants show greater pharmacodynamic action compared to the microspheres. This can be attributed to the greater amount of free drug that is available for cytotoxic effect when compared to the microspheres coupled with a faster release pattern. On administration of the implants, the biocompatible solvent diffuses out into the aqueous surroundings leaving behind the solid gel matrix of the polymer during which the free drug is available for rapid absorption. This free drug could have resulted in greater cytotoxic action when compared to the microspheres. On the other hand, in the case of the microspheres, the free drug available at the surface might be very small as a result of removal during the washing process and the rest of the drug is in the encapsulated form. The entrapped drug could not directly diffuse out of the polymer coat, and as a result, the microspheres are able to act only after an initial lag time whereas in the gel implants, the free drug compensates for the lag time.

Bleomycin (BLM) acts through producing single and double strands breaks in DNA by forming free radicals such as superoxide and hydroxyl radicals. This was effective in causing damage to tumor cells in the initial stages of their multiplication cycles and since a steady concentration of drug was maintained as a result of continuous supply of drug further inhibition of growth was possible. This was evident by the ($p < 0.05$) increase in GD values at 20 mg/kg body weight for both the systems. None of the animals exhibited complete regression, as the total quantity of BLM released might not have been sufficient to completely inhibit growth kinetics of tumor cells.

Pharmacokinetic Evaluation

The results of pharmacokinetic studies of BLM formulations in tumor bearing mice are shown in Fig. 2. In case of free BLM, the plasma concentrations were 8.25 μg/mL and 10.54 μg/mL (C_{max}) after 2 h (T_{max}) and 0.84 μg/mL and 1.03 μg/mL after 2 days at 10 and 20 mg/kg body weight, respectively. The elimination half-life, AUC (0-t) and elimination rate constant were 25.31 h, 107.4 μg/h/mL and 0.027 h^{-1} (for 10 mg/kg) and 23.34 h, 137.4 μg/h/mL and 0.030 h^{-1} (for 20 mg/kg), respectively. As free BLM is rapidly absorbed it attains its peak concentrations after parenteral administration and would be rapidly excreted out of the system. As a result of rapid peak blood concentration there is an initial increase in VDT but this effect is not sustained for prolonged periods as evident by GD values.

The results obtained are the initial trends of drug release in the period of 15 days and the trends could change upon longer periods of study. In the case of both the formulations, the maximal plasma concentrations could not be obtained due to their slow release rates and only the AUC has been calculated. As a result of variation in degradation rates, an AUC of 1235.63 μg/h/mL and 1982.02 μg/h/mL was obtained for the gel implants and plasma concentration of 4.89 μg/mL and 9.92 μg/mL was obtained after 15 days at 10 and 20 mg/kg body weight, respectively. For the microspheres, the AUC was calculated to be 1015.62 μg/h/mL and 1899.57 μg/h/mL with plasma concentrations of 4.02 μg/mL 9.44 μg/mL at the same dose. The drug release rate was just picking up to the time period studied and elimination had not commenced. When the pharmacokinetic data is correlated with the pharmacodynamic data a good correlation can be established with antitumor activity as VDT and GD are significantly different from those of BLM control group (Table 2).

Considering the gel implants, BLM being freely water soluble (solubility: 1 in 1 part), the absorption of the initial load is quite rapid after administration as a result of the presence of the vehicle used for the administration which facilitates drug absorption. This accounts for the higher plasma drug concentration in the initial period thereby giving an added advantage to the gel implants as far as the cytotoxic action is concerned. After the vehicle has been absorbed the, vehicle flow to the circulation stops and further absorption is dependent on the flow of tissue fluids at the implantation site and the normal diffusion processes and this release process is similar for both the gel implants and the microspheres. Due to this factor, the release of drug from the microspheres is quite low in the initial stages and, thus, the amount of drug reaching the systemic circulation in the initial stages of tumor growth is less. However, once the release of drug from the system accelerates then the drug is able to exert its cytotoxic action as evident from the significant ($p < 0.05$) effect on VDT and increase in GD values compared to the control groups. The presence of release-inducing material at the site of injection originating from serum filtrate, cell turnover, or injection trauma could be contributed towards more drug release and better in vivo performance.

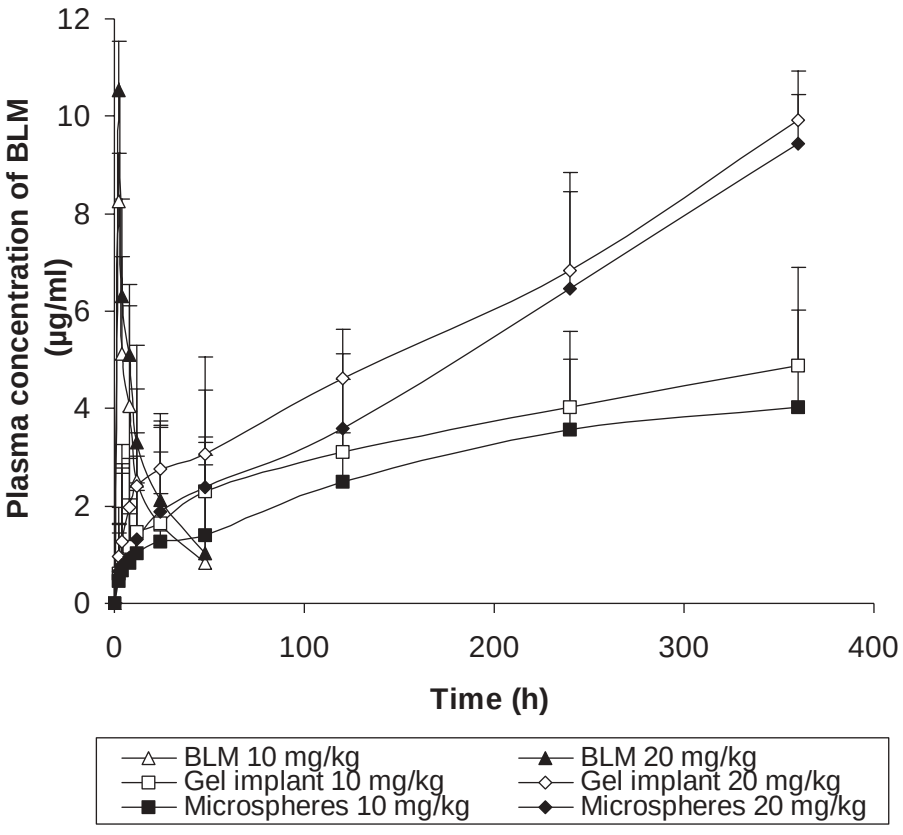

FIGURE 2 **Plasma-concentration Time Curve for BLM Loaded PLGA Gel Implants and Microspheres in Tumor Bearing Mice. All Values are Presented as Mean ± SD; *n* = 6-12.**

As the tumor bearing mice did not give us a complete picture of the pharmacokinetic profile of BLM in these controlled release systems, a separate study was carried out in healthy Swiss mice (Fig. 3). The animals were treated with a single dose of the formulation and the plasma samples were collected for a period of 2 months. It is observed from this figure that the pattern of drug release in vivo in healthy mice did not vary significantly over those bearing tumor. The parameters obtained with pharmacokinetic study in healthy mice are shown in Table 3. The results show that microspheres degrade at a much slower rate when compared to both the gel

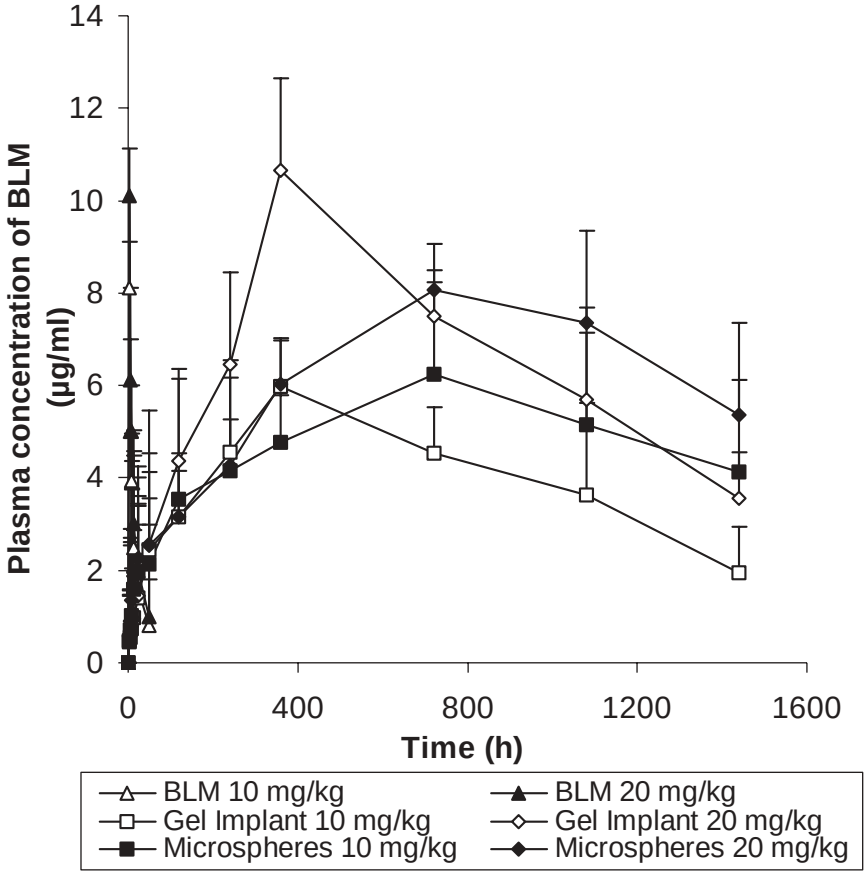

FIGURE 3 **Plasma BLM Concentration–Time Curve for PLGA Gel Implants and Microspheres in Healthy Swiss Mice. All Values are Presented as Mean ± SD; *n* = 6-12.**

TABLE 3 **Pharmacokinetic Parameters of BLM-loaded Biodegradable Systems in Healthy Swiss Mice**

Formulation	C_{max} (µg/mL)	T_{max} (h)	$[AUC]_0^t$ (µg/h/mL)	$t_{1/2}$ (h)	K_e (h^{-1})
BLM Control 10 mg/kg	8.12	2	105.40	23.99	0.02900
BLM Control 20 mg/kg	10.12	2	130.80	23.95	0.02800
Gel implant 10 mg/kg	5.98	360	5733.99	752.27	0.00092
Gel implant 20 mg/kg	10.65	360	9297.86	745.16	0.00093
Microspheres 10 mg/kg	5.19	720	6982.39	1504.55	0.00046
Microspheres 20 mg/kg	8.06	720	8963.49	1474.46	0.00047

implants. This process takes a longer time which is evident by the slower release pattern observed in vitro. In the case of the microspheres, the maximum plasma concentration of 5.19 μg/mL and 8.06 μg/mL was reached in 30 days with an AUC of 6982.39 μg/h/mL and 8963.49 μg/h/mL at a dose of 10 and 20 mg/kg.

As seen in Table 3, the PLGA 75:25 gel implants reached the maximum plasma concentration within 15 days. Similarly, the microspheres reached the maximum plasma concentration in 30 days. One of the drawbacks here is the large gap in the sampling time and so the C_{max} obtained and the time taken to reach the same may not be really accurate. A more definite picture can be obtained by taking lesser time intervals between the sampling times. The $t_{1/2}$ for free BLM given as i.v. bolus injection is reported to be 3.65 hours and K_{el} of 0.19 $hour^{-1}$ respectively (Naresh et al., 1996). It is clearly evident from this observation that formulation of implants and microspheres of BLM has significantly altered the biodistribution of the drug in vivo. The AUC values of the formulations are significantly higher than that given as i.v. injection indicate higher degree of bioavailability of the drug in vivo. This is because a constant fraction of the drug was released over a long period from the injection site into systemic circulation. This resulted in significant elevation in the plasma elimination half-life and rate of elimination. The implanted drug delivery system releases the drug into systemic circulation at a rate comparable to continuous i.v. infusion over a period ranging from 2 months to may be about 6 months depending on the polymer employed.

From the results obtained it is quite evident that these slow releasing systems of BLM are able to slow down the tumor growth. Between the two formulations studied the gel implants showed greater cytotoxic activity, although not statistically significant, compared to the microspheres. Further, the expensive and complex manufacturing process and inability to retrieve the microcapsules in the case of adverse drug reaction are perceived as limitations with these systems. On the other hand, a polymeric gel system does not have these limitations and can be equally efficient in a variety of drug delivery applications as shown in this work. Hence, in situ a gel could be retrieved better than microspheres. The research envisaged in this project would be more meaningful if we are able to manipulate the systems so as to deliver a greater load of the drug in the initial phase of tumor growth.

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