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Research Article

Formulation and evaluation of bendamustine hydrochloride poly (L-lactide) (PLA) microspheres

Gollapally Madhu*[1], Shobhan Babu T[1], Mallikharjun K[1], Lokesh T[1], Venugopal Pyata[2]

[1]Formulation Research & Development Celon Laboratories Limited, Hyderabad, Andhra Pradesh, India
[2]Analytical Research & Development, Celon Laboratories Limited, Hyderabad, Andhra Pradesh, India

ABSTRACT

The present investigation was aimed at developing Bendamustine-loaded poly (L-lactide) (PLA) based biodegradable microspheres by a double emulsion solvent evaporation technique which would have sustained release of the drug. The poly (L-lactide) (PLA) microspheres containing Bendamustine as a drug and evaluate the various physicochemical characteristics of the formulations, namely morphology, particle size, FTIR, Bendamustine encapsulation efficiency and *In vitro* Bendamustine release profile. Bendamustine-loaded microspheres were prepared by double emulsion solvent evaporation method with different Bendamustine, PLA ratios and at different speeds of homogenization keeping the amount of Bendamustine constant in all the formulations and different amount of salt (NaCl) concentrations Accelerated stability testing was performed with the optimized formulations for a period of two months. The mean particle size and encapsulation efficiency of the microspheres were found to decrease as the speed of homogenization increased and the encapsulation efficiency was increased with increase in salt (NaCl) concentration. The in vitro release study showed a slow and steady release pattern of Bendamustine. Thus a sustained release formulation of Bendamustine loaded PLA microspheres were developed.

Keywords: Double emulsion; Encapsulation efficiency; Homogenization; Poly (L-lactide); Salt concentrations

INTRODUCTION

Microspheres are one of the multiparticulate drug delivery systems and are prepared to obtain prolonged (or) controlled drug delivery, to improve bioavailability or stability and to target drug to specific sites. Microspheres can be defined as solid, approximately spherical particles ranging from 1 to 1000µm, containing dispersed drug in either solution (or) microcrystalline form (Remington, The Science and Practice of Pharmacy, 21st edition, 2006) (Vyaas SP & Khur RK, Targeted and controlled drug delivery- Novel carrier systems, 1st edition, 2006).

Chemically, the Bendamustine molecule is gamma-[1-methyl-5-bis (β-chloroethyl)-amino-benzimidazolyl-2]-butyric acid hydrochloride (Ozegowski W, Krebs D, 1971). The molecule has three structural elements: a mechlorethamine (nitrogen mustard) group, a benzimidazole ring and a butyric acid side chain. The nitrogen mustard group is structurally similar to cyclophosphamide and chlorambucil and gives the drug its alkylating properties, while the butyric acid group confers water solubility (Gandhi V, 2002).

Bendamustine is used in the treatment of leukemia and certain lymphomas. However, this compound has limited chemical stability in plasma, thereby requiring high or repeated doses in order to achieve a therapeutic effect. Thus there is a need for formulations of this drug which will exhibit increased stability. Attempts have been made to increase the stability of Bendamustine by complexing such molecule with polymeric materials. However, the approaches taken have only achieved marginal success (Valery Alakhov, *et al*., 2012).

To overcome these problems, an alternative approach is needed. In the present study Bendamustine HCl microspheres are formulated using bio degradable polymers to check effect of drug loading and particle size. Cancer chemotherapy is not always effective. Difficulties in drug delivery to the tumor, drug toxicity to normal tissues, and drug stability in the body contribute to this problem. Polymeric materials provide an alternate means for delivering chemotherapeutic agents. When anticancer drugs are encapsulated in polymers, they can be protected from degradation (Amass W *et al*., 1998).

The main objective of this work was to investigate the possibility of obtaining a sustained release formulation of Bendamustine HCl microspheres by using poly (L-lactide) (PLA) in various drugs, polymer ratios (1:6, 1:8, 1:16). The various physicochemical characteristics and

* Corresponding Author
Email: gollapally.madhu@gmail.com
Contact: +91-9160680048
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Table 1: Commercial biodegradable drug products

Product	Drug	Company	Delivery technology	Polymeric carrier
Decapeptyl SR	Triptorelin	Ipsen	Microparticles	PLGA
Nutropin Depot	Somatropin	Genetech	Microparticles	PLGA
Risperdal Consta	Risperidone	Janssen	Microparticles	PLGA
Sandostatin LAR	Octreotide	Novaris	Microparticles	PLGA
Trelstar Depot	Triptorelin	Watson Pharma	Microparticles	PLGA
Trelstar LA	Triptorelin	Watson Pharma	Microparticles	PLGA
Vivitrol	Naltrexone	Cephalon	Microparticles	PLGA

Table 2: Composition of Bendamustine microspheres

Composition	Formulation					
	F1	F2	F3	F4	F5	F6
Drug (Bendamustine), mg	50	50	50	50	50	50
Water, ml	2	2	2	2	2	2
Polymer (poly lactic acid), mg	300	400	300	400	800	800
Dichloromethane (DCM), ml	20	20	20	20	20	20
Polyvinyl alcohol (PVA) 0.5%, ml	100	100	100	100	100	100
Sodium chloride (NaCl), %	-	-	2.5	2.5	2.5	5
Homogenization speed (rpm)						
Primary (5min)	10000	10000	10000	10000	10000	10000
Secondary (3min)	8000	8000	8000	8000	8000	8000

Table 3: Composition of Bendamustine microspheres

Composition	Formulation					
	F7	F8	F9	F10	F11	F12
Drug (Bendamustine), mg	50	50	50	50	50	50
Water, ml	2	2	2	2	2	2
Polymer (poly lactic acid), mg	800	800	800	800	800	800
Dichloromethane (DCM), ml	20	20	20	20	20	20
Polyvinyl alcohol (PVA) 0.5%, ml	100	100	100	100	100	100
Sodium chloride (NaCl), %	7.5	10	10	10	12.5	15
Homogenization speed (rpm)						
Primary (5min)	10000	10000	15000	5000	10000	10000
Secondary (3min)	8000	8000	8000	8000	8000	8000

the in vitro release rates from these microspheres were thus examined.

Bendamustine is used in the treatment Chronic Lymphocytic Leukemia, Non-Hodgkin Lymphomas, and Multiple Myeloma (Kath R *et al*., 2011) and it is also being studied for the treatment of sarcoma (Bagchi S, 2007). A number of drug products based upon PLA and PLGA delivery systems have been launched into the global market (Table 1).

MATERIALS AND METHODS

Materials

Bendamustine was obtained from Emcure-Pharmaceuticals-Ltd Pune, Poly lactic acid obtained from Evonik roehmgmbh (Germany), Poly vinyl alcohol obtained from S.D. Fine chemicals (Mumbai). All solvents were HPLC grade and were obtained from Merck chemicals, Mumbai.

Preparation of Bendamustine Microspheres

This method for preparation of microsphere was reported to overcome the problem of low encapsulation efficiency of water soluble drug prepared by conventional double emulsion solvent evaporation method. Polymer [Poly (L-lactic acid) (PLA)] is dissolved in organic phase DCM (Dichloro methane). In this organic phase, aqueous drug solution is emulsified using high speed homogenizer operating around 10000 rpm for about 5 minutes to prepare water /oil (w/o) Primary emulsion. This primary emulsion is added to external aqueous phase containing surfactant (poly vinyl alcohol is used to prepare w/o/w emulsion) at homogenizer speed around 8000 rpm for 3 minutes and then reduce the stirring speed to 1000 rpm and stir for 1 hour at 2-8°C and continued stirring for 2 hours at room temperature to permit evaporation of DCM. The microspheres obtained is collected by centrifugation, filtration and then dried. Composition of Bendamustine microshperes showed in Table 2 & 3.

Evaluation of Microspheres

Percentage yield

Table 4: Percentage yield and entrapment efficiency of various formulations

S. No.	Batches	Percentage yield	Entrapment efficiency
1	F1	34.9%	6.2%
2	F2	36.5%	8.3%
3	F3	45.3%	16.7%
4	F4	59.6%	21.3%
5	F5	69.2%	43.6%
6	F6	75.1%	74.7%
7	F7	77.6%	84.2%
8	F8	84.2%	91.3%
9	F9	83.8%	79.4%
10	F10	86.9%	81.1%
11	F11	82.1%	85.6%
12	F12	67.3%	32.5%

The prepared microspheres were collected and weighted. The actual weight of obtained microspheres divided by the total amount of all material that was used for the preparation of the microspheres multiplied by 100 gives the % yield of microspheres (equation) (Chourasia MK and Jain SK, 2004).

Drug entrapment efficiency: The amount of drug entrapped was estimated by dissolving the 100 mg of microspheres in DCM and water in 3:1 ratio ,under vigorous shaking for 1hr, the resultant solution is centrifuged, both layers were separated, Bendamustine was soluble in water but not in DCM. The drug content in aqueous solution was analyzed spectrophotometrically by using UV-Vis spectrophotometer at 268.8 nm with further dilutions against appropriate blank. The amount of the drug entrapped in the microspheres was calculated using the formula (Nappinnai M. and Kishore VS, 2007).

Scanning electron microscopy

Microspheres were observed and photographed with scanning electron microscopy (SEM) (Using Hitachi-S-3700N). Scanning electron microscopy was carried out to study the morphological characteristics of Bendamustine PLA microspheres. The samples for the SEM analysis were prepared by sprinkling the microspheres on one side of adhesive stub. Then the microspheres were coated with gold (100A°) before microscopy. Finally the morphology of the microspheres was observed with the scanning electron microscopy (Amol Paharia *et al*., 2007).

Particle size analysis

Determination of average particle size of Bendamustine microspheres was very important character. It was carried out by using malvern instruments, startech labs pvt.ltd.

***In vitro* drug release**

An in vitro release method using a regenerated cellulose membrane dialysis apparatus (Float-a-Lyzer) was suitable for studying in vitro release of Bendamustine-loaded biodegradable microspheres. Microspheres suspension containing known amount of drug was placed in Float-a-Lyzer. The Float-a-Lyzer was placed in beaker containing 50 ml of Phosphate Buffer Solution (PBS pH 7.4), maintained at 37°C and stirred with the help of a magnetic stirrer. Aliquots (2ml) of release medium were withdrawn at different time intervals and the sample was replaced with fresh PBS pH 7.4 to maintain constant volume and sink conditions. The samples analyzed for drug content by UV-vis spectrophotometer at 268.8 nm. After every one week the complete medium was withdrawn and replaced by fresh medium to avoid saturation of the medium.

***In vitro* drug release kinetic study**

In order to describe the kinetics of the release process of drug in the different formulations, zero order (Qt = Q0+K0t), First order (lnQt = lnQ0+K1t), Higuchi KHt1/2) and Korsemeyer- Peppas (Qt/Q8= Ktn) models were fitted to the dissolution data of all formulations using linear regression analysis. A value of n=0.5indicates case-I (Fickian) diffusion or square root of time kinetics, 0.5<n<1anomalous (non-Fickian) diffusion, n=1 Case-II transport and n>1 Super Case-II transport 12 (Raslan HK and Maswadeh H, 2006).

Stability studies

To assess the physical and chemical stability of the microspheres, stability studies were conducted for 2 months under various storage conditions mentioned in ICH guidelines. The sample containing optimized formulation were placed in vials and stored at 40±2°C/75±5% RH. After 60 days the formulations were checked for physical appearance and drug content.

RESULTS AND DISCUSSION

Formulation optimization

The Microspheres were prepared by double emulsion technique using homogenizer (IKA). Formulations was optimized for in vitro release profile, particle size and entrapment efficiency. The drug polymer ratio was 1:16 for optimized formulation, PVA concentration was 0.5%, sodium chloride 10%, aqueous phase volume

was 2ml and DCM volume was 20ml. The formulation containing Bendamustine kept at constant strength was prepared with different excipients DCM, poly l-lactic acid, PVA, NaCl and all other parameters like temperature and rpm were optimized.

Evaluation of Microspheres

Percentage yield and Entrapment efficiency

The percentage yield and encapsulation efficiency were determined for all the formulations from F1to F12 it was in the ranges from, percentage yield (34.9% - 84.2%) and encapsulation efficiency (6.2% - 91.3%). Among those compositions 6 Formulations are selected as optimized batches for further evaluation based on *In vitro* dissolution profile and entrapment efficiency (Table 4).

Scanning Electron microscopy

SEM micrographs and typical surface morphology of the microspheres are given in Fig. 1 for F7, F8, F11 formulations. It was observed that microspheres were spherical with smooth surface. Fig. 1(d).

Particle size of Bendamustine microspheres

The particle size distribution was analyzed for F7, F8 and F11, formulations of Bendamustine **by** wet method. The particle size was optimum in F8 Formulation, when compared to F7 and F11. The results were denoted in Table 5 and Figure 2.

The release kinetics of F7, F8, F11 formulations was studied. All formulations follow Zero order release kinetics and follow Non-Fickian diffusion when it applied to the Korsemeyer-Peppa's Model for mechanism of drug release.

***In vitro* cumulative % drug release profile**

The *in vitro* dissolution profile of prepared formulations was determined by membrane diffusion method. The dissolution was carried out for a period of 30 days in 7.4 pH phosphate buffer. The cumulative percent release of F6, F7, F8, F9, F10 and F11 formulations at various time intervals was calculated and tabulated in Table No: 5 For F8 formulation 93.3% drug release was achieved on 30^{th} day. Drug release profile increases with increase in drug to polymer ratio.

***In vitro* release kinetics**

The release kinetics of F7, F8, F11 formulations was studied.

Stability studies

Accelerated stability studies of Bendamustine microspheres (F8) at temperature 40°C/75%RH as per ICH guidelines were studied for 60 days. The physical appearance of the formulation was a White to off-white and it was observed that there was no color change indicating physical stability. The drug content was analyzed and data is presented in table No7. From the data, it is observed that there was negligible change in the drug content indicating chemical stability.

CONCLUSION

From the executed experimental results, it could be concluded that the poly lactic acid and sodium chlorides were suitable for preparation of Bendamustine microspheres. Though the preliminary data based on *In vitro* dissolution profile, release kinetics and stability studies proved that the suitability of such formulations F8 formulation showed best particle size, better drug entrapment efficiency, and better sustained release profile for 30 days.

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