CODEN (USA): IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.344031

Available online at: http://www.iajps.com *Research Article*

PHARMACOKINETIC EVALUATION OF PLGA & PLA BASED LONG ACTING RELEASE (LAR) MICROSPHERE FORMULATIONS OF CETRORELIX IN RATS

Harish Kaushik Kotakonda[1, 4], Malothu Nagulu[2], Narsimha Reddy Yellu[3*]

[1]Dept of Pharmacy, Institute of Science and Technology, Jawaharlal Nehru Technological University, Hyderabad, India.

[2]Dept of Pharmacology, Swami Ramananda Tirtha Institute of Pharmaceutical Sciences, Nalgonda, India

[3]Dept of Pharmacology, University College of Pharmaceutical Sciences, Kakatiya University, Warangal, India.

[4]Dept of Drug Metabolism and Pharmacokinetics, JVR Biosciences, Hyderabad, India.

Abstract:

Cetrorelix is one of the potent luteinizing hormone–releasing hormone (LH-RH) antagonist which is indicated in the treatment of preventing premature ovulation in IVF (in vitro fertilization) procedures. The objective of the study was to prepare different poly-lactic-coglycolic acid (PLGA) and Polylactic acid (PLA) based long acting release (LAR) microsphere formulations and to further evaluate the pharmacokinetics, invitro and invivo release of cetrorelix from these formulations. Microsphere 1 (MS1) and Microsphere 2 (MS2) formulations prepared with PLGA 50:50 and PLGA75:25 copolymers could sustain the release of drug for 14-28 days, whereas microsphere 3 (MS3) and microsphere 4 (MS4) formulations prepared with PLGA 85:15 copolymer and PLA could extend the release for 120days. On comparing the invitro and invivo release of the microsphere formulations it was observed that release under invivo conditions release was faster than under invitro conditions. It was observed that increase in the drug loading lead to higher rise in the initial cetrorelix plasma levels and further lead to shorter duration of release. On SC injection of MS1, MS2, MS3 & MS4 microspheres formulation to adult male Sprague Dawley rats at 0.25mg/kg lead to an initial rise in plasma cetrorelix levels which is of 23.812 ± 8.554 ng/mL, 150.701 ± 51.772 ng/mL, 57.581 ± 19.781 ng/mL and 164.466 ± 56.512 ng/mL at the median T_{max} of 6hr for MS1-4 formulations respectively, where as a single subcutaneous (SC) administration of cetrorelix injection to rats at 0.25 mg/kg provided peak maximum concentration (C_{max}) of 79.274 ± 17.734 ng/mL at 1.5 hr (T_{max}). The exposure (AUC_{0-t}) achieved by the microsphere formulations are of the order 628.059±127.706, 4814.86 ± 1729.582, 8324.062 ± 2859.633, 10414.69 ± 3577.844 and 15579.68 ± 5352.216 hr.ng/mL for Cetrotide®, MS1, MS2, MS3 and MS4 formulations respectively. When compared with Cetrotide® SC injection the exposures achieved by the microsphere formulations are of the order MS4 > MS3 > MS2 > MS1 formulations. Therefore based on the results of the study it can be concluded that PLGA and PLA microsphere formulations of cetrorelix can be of clinical relevance helping the patients to avoid multiple injections during their therapy.

Keywords: *Cetrorelix; Poly-lactic-coglycolic acid (PLGA); Polylactic acid (PLA); Subcutaneous; Pharmacokinetics; Long acting release (LAR)*

Corresponding Author:

Prof. Y. Narasimha Reddy,
Head, Dept of Pharmacology
UCPSc, Kakatiya University
Warangal, Telangana
India-506009
Email:ynrku@yahoo.co.in

QR code

Please cite this article in press as Y. Narsimha Reddy et al, ***Pharmacokinetic Evaluation of PLGA & PLA Based Long Acting Release (LAR) Microsphere Formulations of Cetrorelix In Rats****, Indo Am. J. P. Sci, 2017; 4(02).*

INTRODUCTION:

Therapeutic drugs formulated with PLA and PLGA polymers are reported to be biocompatible and doesn't exhibit any local or systemic untoward reactions and were used for therapeutic applications (1, 2). The interest in utilizing the biodegradable and biocompatible polymers for preparing formulations which could control and prolong the duration of action of the drug has increased in recent times. The main reason attributable to this could be due the fact that these polymers need not to be removed from the invivo system at the end of therapy or treatment duration and undergo degradation into physiologically occurring compounds which can be eliminated from the invivo system (4). Further encapsulating the drug in a biodegradable polymer increases the biological availability of the drug. In the recent times PLGAs as an injectable drug delivery system evolved as an advanced drug delivery system which can be utilized to deliver varied type of drugs such as small and large molecules for ex peptides, proteins and DNA/RNA. Many long acting release (LAR) formulations comprising PLGA/PLA as drug delivery systems are available in the market (e.g., Lupron Depot®, Zoladex®, Decapeptyl®, Eligard®, Enantone®, Trenantone®, Risperdal Consta® and Profact®) (5-14). Advantages posed by these injectable long acting release delivery systems are reduced frequency of dosing and total dose administered, lower side effects which would increase further the patient compliance.

Cetrorelix has been identified as a potent luteinizing hormone-releasing hormone (LH-RH) receptor antagonist free of edematogenic side effects (15). Cetrorelix competitively inhibits LH-RH at receptor in the pituitary gland which results in the suppression of ovarian and testicular functions. Recently cetrorelix was granted in European Union and United states as Cetrotide® (0.25mg and 3mg) for the inhibition of premature LH surges in women undergoing controlled ovarian stimulation (16). Inspite of its many uses, the clinical applications of cetrorelix are limited by frequent injections that are required to ensure an adequate control of the disease. In a study by Badrawi et al. [17], the cost of medication per cycle and per pregnancy was shown to be higher in a GnRH antagonist such as cetrorelix. It's been reported that multiple-dose protocols are now the standard and single-dose protocols are rarely used (18).

Therefore, to obtain a long-term therapeutic effect, LAR depot formulation is required in patients. In our current study we have developed PLGA & PLA microsphere formulations to achieve the sustained release invivo and avoid multiple injections. In this study, we evaluate the comparative pharmacokinetics of cetrorelix from PLGA and PLA microspheres and investigate the pharmacokinetic parameters. Therefore the objectives of this study were the following

1) To study the release of cetrorelix from PLGA & PLA microsphere LAR formulations
2) To compare the plasma cetrorelix levels of PLGA & PLA microsphere LAR formulations and Cetrotide® injection following subcutaneous administration
3) To compare in vivo pharmacokinetic drug absorption behavior

MATERIALS & METHODS:

Materials:

Cetrorelix acetate was procured from G. C. Chemie Pharmie Ltd (Mumbai). 50:50 PLGA copolymers (MW 10 777–31 281), 75:25 PLGA (MW 11161), a PLA homopolymer (MW 9489), 85:15 PLGA (MW 17903) and Polyvinyl alcohol (PVA) (average MW, 30 000-70 000) was obtained from Sigma Aldrich Chemicals Pvt Ltd (Bangalore, India). The hydrophilic resomers (H) had free carboxyl end groups, whereas the hydrophobic ones were capped with long-chain alkyl alcohols. Excipients and other Solvents were of analytical grade and purchased from commercial sources; dialysis tubes (Tube-O-Dialyzer®) and a Spectra/Por® CE dialysis membrane (molecular weight cut-off, or MWCO, 300 000 Da) was procured from G-Biosciences (St. Louis, MO, U.S.A). Testosterone ELISA Kits were procured from Biocompare (South San Francisco, CA 94080, USA).

Preparation of Cetrorelix Microspheres:

Cetrorelix acetate PLGA and PLA microspheres with drug loading were prepared by o/w dispersion method with solvent extraction/evaporation. Briefly, the dispersed phase consisted of cetrorelix acetate dissolved in methanol and mixed with polymer solution which was further dissolved in methylene chloride. The dispersed phase prepared above was slowly added to the continuous phase consisted of 0.35%w/v solution of PVA at pH 7.2. Then the mixture was stirred at 5500rpm in a Silverson L4R homogenizer (Silverson Machines Ltd, Waterside, UK). For extraction/evaporation of the solvents the mixture was continuous stirred at $40^{O}C$ for 1 hr. Vacuum filtration was used to recover the final hardened microspheres and then they were washed with water. Vacuum drying for 48 hr was done to

remove any residual solvents, water from microspheres

Microsphere Characterization

Methylene chloride was used to dissolve the microspheres and using 0.1M acetate buffer (pH 4.0) the extraction of peptide was done. Concentration of peptide was measured using high-performance liquid chromatography (HPLC).

High-Performance Liquid Chromatography Method for Drug Content Assay

The Agilent 1200 Series HPLC System (Agilent, USA) consisted of a pump and a UV detector was used to perform the HPLC analysis. Kromasil® C18, 250 × 4.60 mm, 5µm column (Dikma Technologies, China) was used. A gradient elution method with mobile phases consisting of A (0.1% (v/v) TFA in water) and B (0.1% (v/v TFA in acetonitrile) pumped into the HPLC system as 80:20 (A:B) to 40:60 (A:B) over 25 minutes, at 1 mL/min flow rate. UV absorbance was measured at 220nm.Every time the injection volume was maintained at 20 µL.

The encapsulation efficiency of the microspheres was calculated as.

Encapsulation Efficiency (% w/w) = [(actual drug content) / (nominal drug content)] x 100

***In Vitro* Release Study**

The in vitro release study of cetrorelix acetate loaded PLGA and PLA microspheres was done as per method reported earlier. Briefly, 10mg of drug loaded microspheres were added to a 5mL tube consisting of a mixture of 2-ml phosphate buffer solution (10mM, pH 7.4) containing 0.02% Tween 80 and 0.02% Tween 20 and suspended thoroughly. The tube was placed in a 50 °C water bath and shaken at 160 rpm horizontally. The tube was centrifuged at 3500rpm for 10min and then 2.0mL of supernatant was removed for determination of % cetrorelix released at different time points. An equal volume of fresh phosphate buffer was added back.

Animal Study

The study was conducted in compliance with Institutional Animal Ethics Committee (IAEC) requirements. The protocol has been approved by the Institutional Animal Ethics Committee (IAEC) of JVR Bio Life Sciences Pvt Ltd. All the ethical practices as laid down in the CPCSEA guidelines for animal care was followed during the conduct of the study. The animal experiments were performed at JVR Bio Life Sciences Pvt Ltd (Hyderabad, India). Male Sprague Dawley rats (n = 6) weighing approximately 300 g were divided into 5 different groups. Cetrotide® (cetrorelix acetate for injection), MS1, MS2, MS3 and MS4 were administered through subcutaneous route at the lower back section of the neck. The microsphere formulations MS1-4 were reconstituted in a liquid vehicle consisting of (1% carboxy methyl cellulose and 2% mannitol). 0.25mL of blood samples were collected from the tail vein at different time points into a 1mL centrifuge tubes containing K2EDTA as anticoagulant. Plasma was separated by centrifuging blood samples at 3500 rpm for 10 min. under refrigeration (2-4 °C) within 30 minutes of sampling. The obtained plasma samples was separated into a pre-labeled tubes and stored at -70±10 °C until analysis. Plasma samples collected were used to measure the cetrolrelix concentrations.

Instrumentation and Chromatographic Conditions

The Agilent 1200 Series HPLC System (Agilent, USA) consisted of a pump and a UV detector was used to perform the HPLC analysis. Kromasil® C18, 250 × 4.60 mm, 5µm column (Dikma Technologies, China) was used to perform chromatographic separation. A mixture of acetonitrile–water–trifluoroacetic acid (35:65:0.1, v/v/v) was used as the mobile phase which was degassed before use and the flow rate was set as 0.2mL/min. HPLC was coupled to an Applied Biosystems Sciex Qtrap 5500 mass spectrometer (Applied Biosystems Sciex, Ontario, Canada) using electrospray ionization (ESI). Nitrogen was used as nebulizer and drying gas at flow rate of 30 and 300L/hr respectively. Source temperature was maintained at 120^{O}C.Capillary voltage was set at 3 kV. Multiple reaction monitoring (MRM) at unit resolution involved transitions of the protonated forms of m/z 1429,1543 and 1657 for cetrorelix and 1586,1700 and 1813 for Brominated cetrorelix IS in the positive ion mode. Optimized MS conditions were described as follows: curtain gas, gas 1 and gas 2 (all nitrogen) with 35, 55 and 55 units, respectively; dwell time with 100 ms; ion spray voltage with 5500 V; declustering potentials with 90 V for cetrorelix and 100 V for brominated cetrorelix.

Sample extraction

Sample analysis was conducted following the methodology reported by Niwa et al (19). Briefly, to 50µL of plasma, 5 µL of IS was added. It was vortex mixed diluted with methanol–water (10:90, v/v, 10 ml) and then passed through Sep-Pak Vac C8 cartridge (125 A pore size, 500 mg) which was activated with 20 ml of methanol and rinsed with 20 ml of water previously. The cartridge was washed with methanol–water (10:90, v/ v, 10 ml and 50:50, v/v, 10 ml), and then I.S. and cetrorelix were eluted with methanol–water–trifluoroacetic acid (90:10:0.1,

v/v/v, 10 ml). Under reduced pressure the eluate was evaporated to dryness. The residue was then dissolved in mobile phase (0.1 ml) and was further mixed by vortex. The entire mixture was transferred to Ultrafree-MC/LCR (0.2 mm pore size, 0.2 cm) and it was filtered by centrifuging at 3500 g for 20 min at 4^{O}C. Finally 40µL of the filtrate was injected into the column

Pharmacokinetic Data Analysis:

Pharmacokinetic (PK) parameters were calculated by non-compartmental analysis (NCA) by using Phoenix™ WinNonlin® Version 6.4 (Certara, USA) based on the individual plasma concentration. Pharmacokinetic parameters including peak plasma concentration (C_{max}), time to reach the peak plasma concentration (T_{max}), half-life ($T_{1/2}$), AUC_{0-t}, AUC_{0-inf}, Vd, CL, and MRT was estimated. The area under the concentration-time curve (AUC) was estimated by linear trapezoidal method and the apparent elimination rate constant (Kel) was calculated by the least squares regression analysis.

RESULTS AND DISCUSSION:

The invitro release of cetrorelix is shown in figure-1. For MS-1 during the first 15 days only 24% of the drug was released from the microspheres and then 50% release was after 23 days and in another 23 days (ie 36days) ~90% of the drug got released. Whereas for MS-2, only 7~% of the drug was released within 1 day and the microspheres continued to release at a steady rate of 1.7% per day, completing 50% after 28 days and by day-36 ~70% of the drug was released. In contrast MS-3 microsphere formulation the release rate was much slower which can be observed from the figure that only ~5% of the drug was released during the first 10days and during the first month achieved the invitro release of ~16% within 30days and by day-56 ~50% of the drug was released. For the MS-4 microsphere formulation released only 16% in vitro in the first 14 days, but subsequently the release rate stabilized at 0.38% per day and achieving about ~26% release by day-50, the slower release from PLA continued until 100days by then it has released ~48% of the drug and finally by day-120 it achieved the ~57% release.

Table-1: Characterization of Cetrorelix microsphere formulations

Formulation	Polymer Type	Mw (Da)	Drug load (wt %)	EE (%)
MS1	PLGA50:50	28022	13.6	82
MS2	PLGA75:25	11161	10.6	83
MS3	PLGA85:15	17903	15.3	89
MS4	PLA	9489	18.5	82

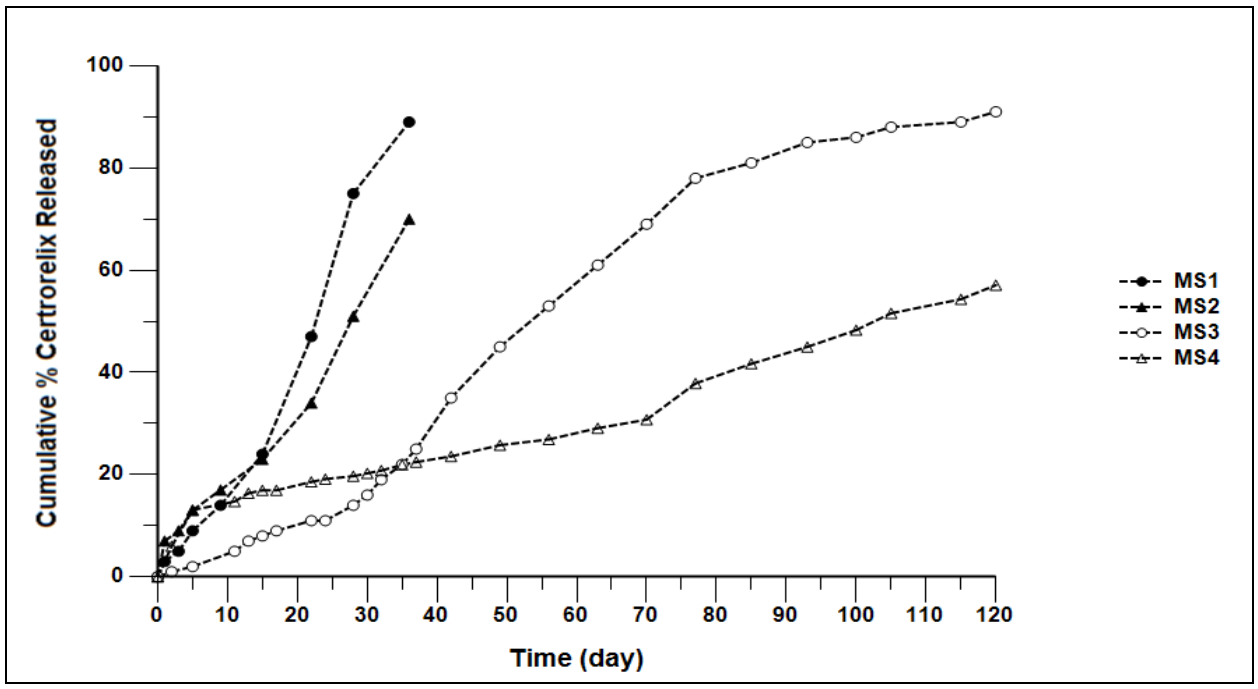

Fig 1: Cumulative % Cetrorelix released of MS1, MS2, MS3 and MS4 microsphere formulations

As shown in the figure-2 below a single subcutaneous administration of cetrorelix injection to rats at 0.25 mg/kg provided peak maximum concentration (C_{max}) of 79.274 ± 17.734 ng/mL at 1.5 hr (T_{max}). Plasma cetrorelix concentrations in rats after administration of 0.25mg/kg microsphere formulations are provided in figure-3. From the figure-3 on SC injection of MS1, MS2, MS3 & MS4 microspheres it can be observed that there is an initial rise observed in plasma cetrorelix level's which is of 23.812 ± 8.554 ng/mL, 150.701 ± 51.772 ng/mL, 57.581 ± 19.781 ng/mL and 164.466 ± 56.512 ng/mL at the median T_{max} of 6hr. The MS1 microspheres showed the highest concentration at 6 hours and a second smaller peak occurred on day 4; The MS2 microspheres had the highest initial release (C_{max} of 150.701 ng/mL at 6 hours) and maintained levels above 1 ng/mL for more than 22 days. Whereas for MS3 and MS4 formulations maintained higher plasma levels than did the 2 other formulations MS1 and MS2 during the first 10 days; for most of the study, the levels were below 3 ng/mL. This shows to achieve a rapid and longer duration of castration in rats, it is important to achieve initially higher levels (above 10 ng/mL for the first 10-14 days), and to suppress testosterone for longer duration may be months it appears that levels as low as 3 ng/mL are enough. Therefore all the microsphere formulations showed an initial elevation of cetrorelix levels after administration and peaked at 6 days post dose administration.

This kind of biphasic drug release mechanism from microspheres has been observed and reported in many papers (20-24). Briefly, initially the drug loaded onto the polymer diffuses from the surface or close to the surface of the polymer after this drug diffuses out of the porous polymer matric due to the hydration of polymer followed by the bulk degradation of the polymer resulting in the sustained release of the drug. Erosion-controlled release of a luteinizing hormone analogue from a PLGA polymer was reported to exhibit biphasic kinetics over 2 months when injected intramuscularly to rhesus monkeys (25), and the triphasic pattern was reported in the in vivo absorption study of octreotide pamoate (20).

Table-1: Mean (±SD) Pharmacokinetic parameters following single SC administration of Certrotide®, MS1, MS2, MS3 and MS4 formulations at 0.25mg/kg to male Sprague Dawley rats

Treatment	Dose (mg/kg)	T_{max} * (hr)	C_{max} (ng/mL)	AUC_{0-t} (hr.ng/mL)	AUC_{0-inf} (hr.ng/mL)	$T_{1/2}$ (hr)	$AUC_{0-t, MS}$/ $AUC_{0-t\ ,\ SC}$
Cetrotide®	0.25	1.5	79.274 ± 17.734	628.059 ± 127.706	640.492 ± 133.839	6.92	-
MS 1	0.25	6	23.812 ± 8.554	4814.860 ± 1729.582	5632.151 ± 2023.167	231.81	7.7
MS 2	0.25	6	150.701 ± 51.772	8324.062 ± 2859.633	8453.135 ± 2903.975	223.02	13.3
MS 3	0.25	6	57.581 ± 19.781	10414.69 ± 3577.844	10442.550 ± 3587.415	234.53	16.6
MS 4	0.25	6	164.466 ± 56.512	15579.68 ± 5352.216	17123.980 ± 5882.742	564.80	24.8

*Median

The exposure (AUC_{0-t}) achieved by the microsphere formulations are of the order 628.059±127.706, 4814.86 ± 1729.582, 8324.062 ± 2859.633, 10414.69 ± 3577.844 and 15579.68 ± 5352.216 hr.ng/mL for Cetrotide®, MS1, MS2, MS3 and MS4 formulations respectively. When compared with Cetrotide® SC injection the exposures achieved by the microsphere formulations are of the order MS4 > MS3 > MS2 > MS1 formulations. In a study by Schwann et al, 2000 (26) the population pharmacokinetic / pharmacodynamics modelling of cetrorelix was studied and the pharmacokinetics of cetrorelix following SC administration in Wistar rats at a dose of 0.25mg/kg reported C_{max} of 68.6 ng/mL and AUC_{0-inf} of 619 hr.ng/mL whereas the pharmacokinetic parameters following similar dose administration through SC route in the current study performed by us was similar to the ones reported earlier.

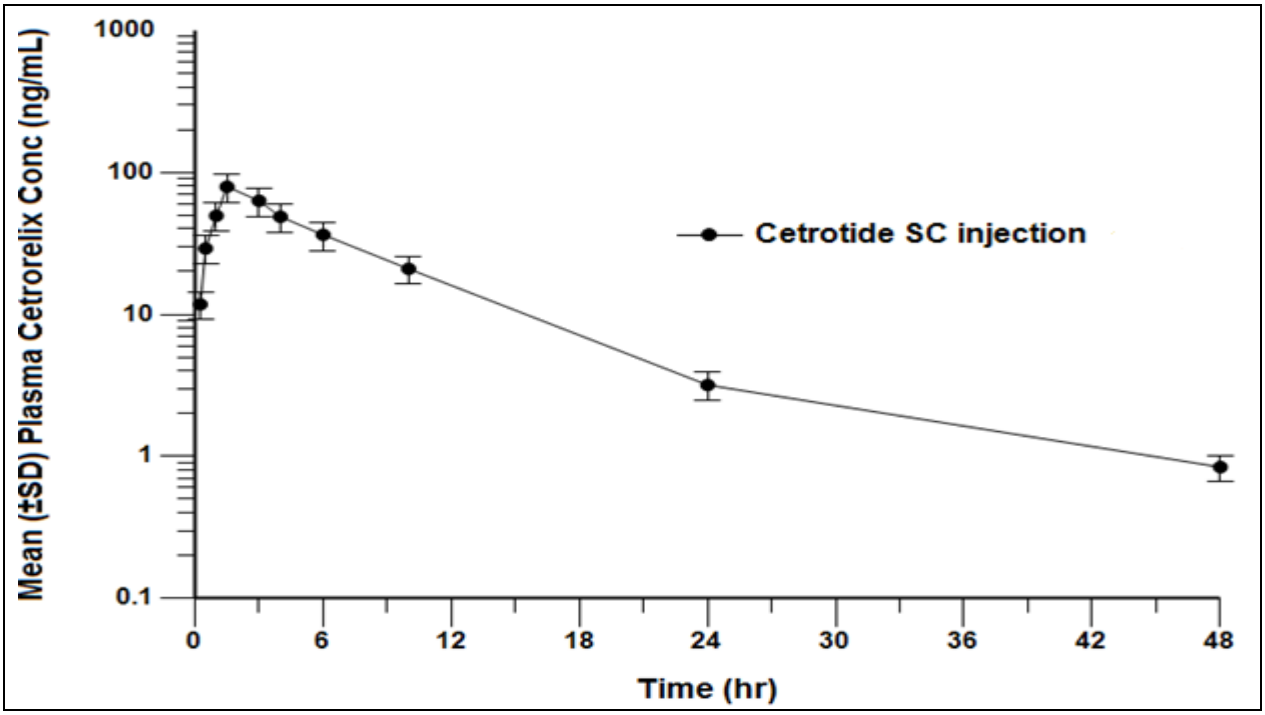

Fig 2: Mean Plasma Cetrorelix concentrations following single dose administration of 0.25mg/kg Cetrotide® SC injection through SC route to male Sprague Dawley rats

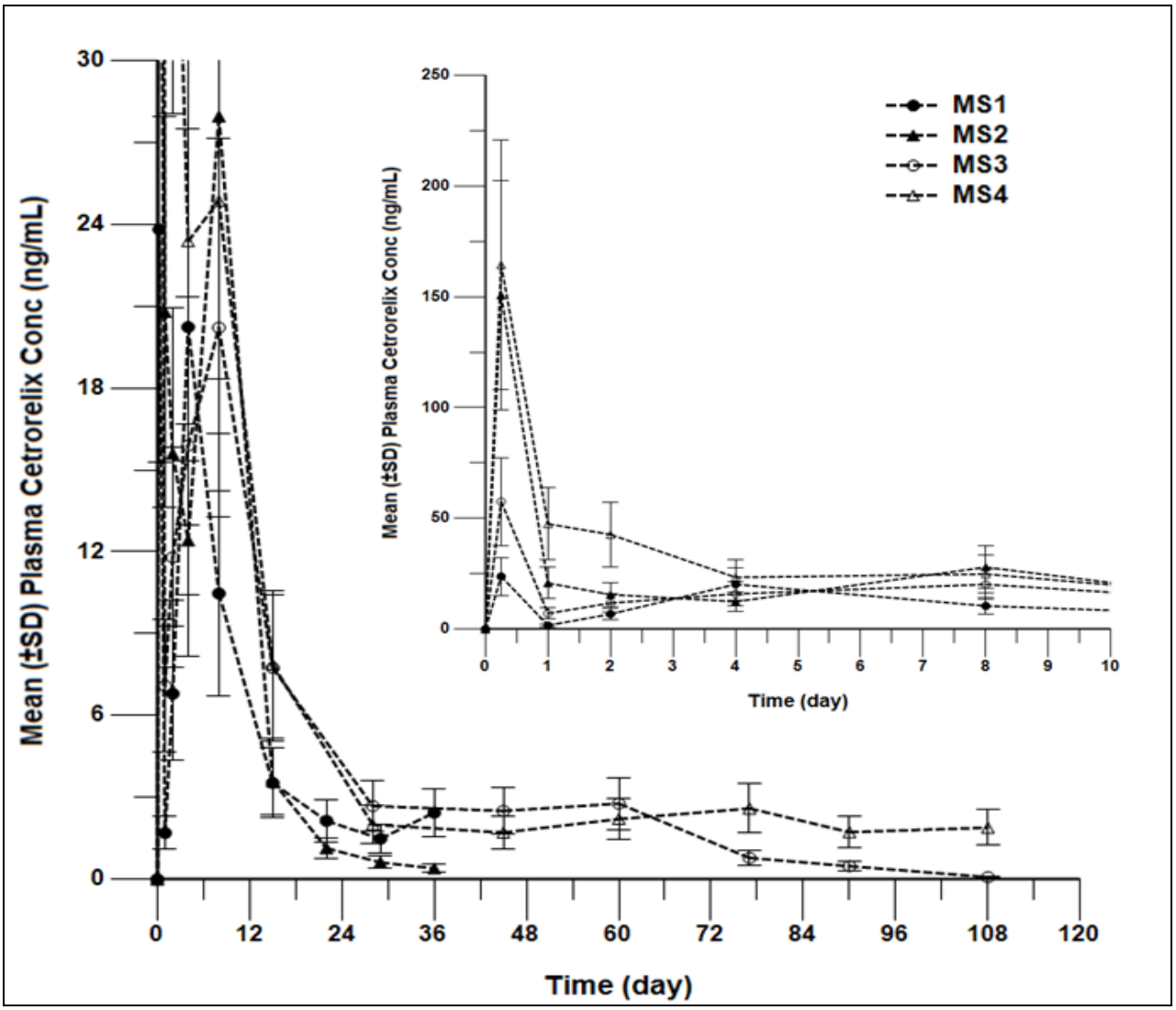

Fig 3: Mean Plasma Concentrations of Cetrorelix following SC administration of MS1, MS2, MS3 and MS4 microsphere formulations at 0.25 mg/kg to Male Sprague Dawley rats

Invivo fraction cetrorelix absorbed from microsphere formulations calculated by Loo-Riegelman method is shown in the figure-4 below. The in vivo release of MS1 microsphere formulation started immediately after administration and was rapid during the first 15 days (77% release) and T50% release invivo was 7 days. After the initial 25% release, it can be observed that in vivo and in vitro release rates were similar, even though the in vitro release profile remained delayed by approximately 2 weeks for the entire study. Whereas for MS2 microsphere formulation even faster in vivo release was seen ~32% in 1 day and T50% release was achieved by ~5days and within 15 days ~91% invivo release was observed and total~99% release was achieved within 36days. In contrary MS4 formulation released ~22%invivo within 1 day and ~50% within 8 days and by day 15 another 13% of the drug ie 63% was released demonstrating that the invivo release from MS4 formulation was slower and continued at the same rate till 108 days whereas MS3 formulation was slower in the initial time period releasing only ~10% within 1day and achieving T50% within 15days and thereafter the invivo release was faster than MS4 formulation achieving 100% release by day 108. The rationale behind the higher initial release observed invivo than compared to invitro system can be attributed to the higher acidic environment in the microsystem of the biodegradable polymer caused due to increased H^+ concentration at the interface of plasma protein and polymer (27-28).

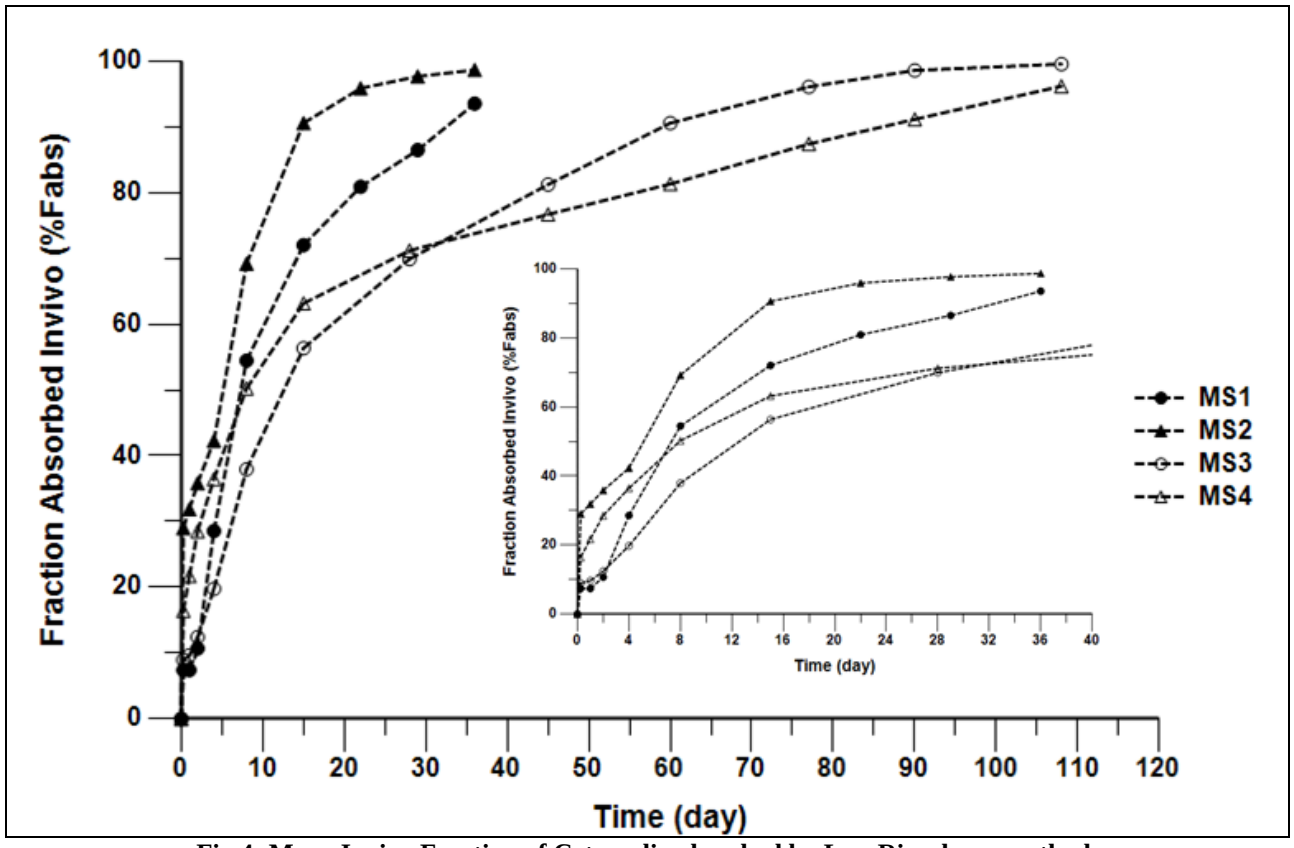

Fig 4: Mean Invivo Fraction of Cetrorelix absorbed by Loo-Riegelman method

CONCLUSIONS:

In the current study the prepared cetrorelix injectable PLGA and PLA based micropshere formulations could sustain the release of drug for 30-120 days, which would be further beneficial for treatment of several indications such as benign or malignant hormone-dependent diseases, to postpone the LH surge IVF (in vitro fertilization) protocols where multiple dosing is of standard therapeutic practice. Therefore the results of the study highlights the fact that a long acting release microsphere injectable formulations could be prepared with PLGA and PLA polymers which could reduce the frequency of injections to be taken by the patients during the course of their treatment

REFERENCES:

1. Ignatius AA, Claes LE. In vitro biocompatibility of bioresorbable polymers: poly (L, DL-lactide) and poly (L-lactide-co-glycolide). Biomaterials, 1996; 17(8):831–9.

2. Shive MS, Anderson JM. Biodegradation and biocompatibility of PLA and PLGA microspheres. Adv Drug Deliv Rev, 1997; 28 (1):5–24.

3. Kang J, Lambert O, Ausborn M, Schwendeman SP. Stability of proteins encapsulated in injectable and biodegradable poly (lactide-co-glycolide)-glucose millicylinders. Int J Pharm, 2008; 357 (1–2):235–43.

4. Abouelfadel Z, Crawford ED. Leuprorelin depot injection: patient considerations in the management of prostatic cancer. Therapeut Clin Risk Manag, 2008; 4(2):513–26.

5. Kundi SA, Wazir S, Afzal S, Afzal N, Qayum I, Forsling ML. Release of vasopressin during suppression of oestrous cycle in rat by zoladex and hypovolemic challenge. J Ayub Med Coll Abbottabad, 2005; 17 (4):63–6.

6.Horvath JE, Bajo AM, Schally AV, Kovacs M, Herbert F, Groot K. Effects of long-term treatment with the luteinizing hormone releasing hormone (LHRH) agonist Decapeptyl and the LHRH antagonist Cetrorelix on the levels of pituitary LHRH receptors and their mRNA expression in rats. Proc Natl Acad Sci USA, 2002; 99 (23):15048–53.

7. Berges R, Bello U. Effect of a new leuprorelin formulation on testosterone levels in patients with advanced prostate cancer. Curr Med Res Opin, 2006; 22(4):649–55.

8. Sophocleous AM, Zhang Y, Schwendeman SP. A new class of inhibitors of peptide sorption and acylation in PLGA. J Control Release, 2009; 137 (3):179–84.

9.Kemp SF, Fielder PJ, Attie KM, Blethen SL, Reiter EO, Ford KM. Pharmacokinetic and pharmacodynamic characteristics of a long-acting growth hormone (GH) preparation (nutropin depot) in GH-deficient children. J Clin Endocrinol Metab, 2004; 89(7):3234–40.
10.Cook DM, Biller BM, Vance ML, Hoffman AR, Phillips LS, Ford KM. The pharmacokinetic and pharmacodynamics characteristics of a long-acting growth hormone (GH) preparation (nutropin depot) in GH-deficient adults. J Clin Endocrinol Metab, 2002; 87(10):4508–14.
11.Gefvert O, Eriksson B, Persson P, Helldin L, Bjorner A, Mannaert E, et al. Pharmacokinetics and D2 receptor occupancy of long-acting injectable risperidone (Risperdal Consta) in patients with schizophrenia. Int J Neuropsychopharmacol, 2005; 8(1):27–36.
12.Irani J, Mottet NA, Salomon L, Oba R. Randomized, double blind study comparing efficacy and tolerance of venlafaxine vs medroxyprogesterone acetate vs cyproterone for hot flushes in men under leuprorelin 11.25 mg (Enantone) for prostate cancer. J Urol, 2009; 181(4):231–231.
13.Grischke EM, Fehm T, Solomayer E, Hillger H, Wallwiener D. Effect of a 3 month depot injection of leuprorelin acetate (trenantone (R)) on pre-menopausal breast cancer—results of a post-marketing surveillance study. Geburtshilfe Frauenheilkd, 2008; 68(10):1008–12.
14.Rhee YS, Park CW, DeLuca PP, Mansour HM. Sustained-release injectable drug delivery systems. Pharm Tech, 2010; 11: 16–22.
15.Bajusz S, Kovacs M, Gazdag M, Bokser L, Karashima T, Csernus VJ, Janaky T, Guoth J and Schally AV . Highly potent antagonists of luteinizing hormone-releasing hormone free of edematogenic effects. Proc Natl Acad Sci USA, 1988; 85:1637–1641.
16.Cetrotide® 0.25mg injection Label
17.Badrawi A, Al-Inany H, Hussein M, Zaki S, Ramzy A: Agonist vs. antagonist in ICSI cycles: a randomized trial and cost-effectiveness analysis. Middle East Fertil Soc J, 2005; 10: 49-54.
18.Copperman AB, Benadiva C. Optimal usage of the GnRH antagonists: a review of the literature. Reproductive Biology and Endocrinology, 2013, 11-20. DOI: 10.1186/1477-7827-11-20
19.Makoto N, Keiko E, Kouwa Y. Measurement of the novel decapeptide cetrorelix in human plasma and urine by liquid chromatography–electrospray ionization mass spectrometry. Journal of Chromatography B, 1999;729: 245–253
20.Comets E, Mentre F, Nimmerfall F, Kawai R, Mueller I, Marbach P. Nonparametric analysis of the absorption profile of octreotide in rabbits from long-acting release formulation OncoLAR. J Control Release, 1999; 59(2):197–205.
21.Cleland JL. Solvent evaporation processes for the production of controlled release biodegradable microsphere formulations for therapeutics and vaccines. Biotechnol Prog, 1998; 14(1):102–7.
22.Lewis KJ, IrwinWJ, Akhtar S. Development of a sustained-release biodegradable polymer delivery system for site-specific delivery of oligonucleotides: characterization of P (LA-GA) copolymer microspheres in vitro. J Drug Target, 1998; 5(4):291–302.
23.Bodmer D, Kissel T, Traechslin E. Factors influencing the release of peptides and proteins from biodegradable parenteral depot systems. J Control Release, 1992; 21(1–3):129–37.
24.Chen X, Ooi CP, Lye WS, Lim TH. Sustained release of ganciclovir from poly (lactide-co-glycolide) microspheres. J Microencapsul, 2005; 22(6):621–31.
25.Sanders LM, Kent JS, McRae GI, Vickery BH, Tice TR, Lewis DH. Controlled release of a luteinizing hormone-releasing hormone analogue from poly (D,L-lactide-co-glycolide) microspheres. J Pharm Sci, 1984; 73(9):1294–7.
26.Schwann M, Nelamangala VN, Derendorf H. Population Pharmacokinetic/Pharmacodynamic Modeling of Cetrorelix, a Novel LH-RH Antagonist, and Testosterone in Rats and Dogs. Pharmaceutical Research, 2000, 17, 3,328-335.
27.Makino K, Ohshima H, Kondo T. Potential distribution across a plasma protein-coated poly (L-lactide) microcapsule surface. J Microencapsulation, 1990; 7 (2):199-208.
28.Makino K, Ohshima H, Kondo T. Effects of plasma proteins on degradation properties of poly (l-lactide) microcapsules. Pharm Res, 1987; 4 (1):62-65.