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A novel long-acting biodegradable depot formulation of anastrozole for breast cancer therapy

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ABSTRACT

The purpose of the present study was to fabricate PLGA 50:50 and PLA microspheres for controlled delivery of anastrozole. The microspheres were prepared by oil-in-water (o/w) emulsion/solvent evaporation technique and evaluated for particle size and encapsulation. The optimised formulations were studied for solid state characterization, *in vitro* release and pharmacokinetic studies. The maximum encapsulation efficiency for PLGA 50:50 and PLA microspheres with 40:1 polymer - drug ratio was observed to be 78.4 ± 2.5 and 87.7 ± 2.6%. The solid state characterization confirmed dispersion of drug at the molecular level in the polymeric matrix. Microspheres were spherical in shape with a very smooth surface texture. Drug release was found to be in a sustained fashion, releasing constantly up to 720 h (30 days) for PLGA and 60 days for PLA microspheres. The pharmacokinetic study data revealed that the intramuscular administration of PLA microspheres showed improved pharmacokinetic profile as compared to PLGA microspheres, and therefore this formulation can be considered as the best optimised formulation with sustained exposure of the drug *in vivo* compared to other microspheres. From experimental results, PLA microspheres demonstrate the feasibility of employing biodegradable depot polymeric microspheres of anastrozole for long-term treatment of breast cancer.

1. Introduction

Breast cancer is a devastating disease that affects women and is the second leading cause of death worldwide. Approximately 2,32,670 breast cancer incident cases were reported in the USA for the year 2014. It has the maximum cumulative financial burden of care relative to other cancers in female patients [1,2]. The urbanisation and radical changes in the lifestyle were found to be some of the reasons for the increase in the occurrence of breast cancer, especially in the developing countries. Cancer research over the years led to the development of early detection techniques and the effective therapies that had led to significant reductions in breast cancer-related deaths. However, breast cancer remains a major cause of death especially in regions with limited access to the screening and effective therapies. The lack of effective screening is one of the prime factors for the detection of late-stage cancer. Therefore, prevention of breast cancer remains a major global public health priority [2,3]. Intense advances in medical sciences during the past decade have revolutionised the cancer therapy. Multiple-drug regimen therapy is popular in the treatment of most of the cancers. Novel drug delivery system has promising potential to mitigate cancer trajectory. Estrogen and its receptors are among the most relevant prognostic and predictive factors for breast cancer carcinogenesis and therapeutic targeting [4]. In post-menopausal women, the principal source of circulating estrogen is the conversion of adrenally-generated androstenedione to estrone by aromatase enzyme, with further conversion to estradiol. Treatment of breast cancer has included efforts to decrease estrogen levels, by hormonal therapy, which is considered as one of the preferred treatment, as it has a beneficial tolerability profile compared with cytotoxic chemotherapy [5]. Anti-estrogen therapy utilises aromatase inhibitors that act by inhibiting the enzyme aromatase that plays the catalyst role in the synthesis of estrogen. Anastrozole [ANS] of triazole class is one such potential aromatase inhibitor belonging to Biopharmaceutical Classification System (BCS) Class III presently available

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Table 1
Different composition of PLGA and PLA microspheres.

PLGA microspheres	PLGA-01	PLGA-02	PLGA-03	PLGA-04	PLGA-05	PLGA-06	PLGA-07	PLGA-08
Anastrozole (mg)	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
PLGA (mg)	25.0	50.0	75.0	100.0	150.0	200.0	200.0	200.0
Polyvinyl alcohol (mL)								
0.3% *w/v*	–	–	–	–	–	–	–	50.0
0.5% *w/v*	50.0	50.0	50.0	50.0	50.0	50.0	–	–
1.0% *w/v*	–	–	–	–	–	–	50.0	–
PLA microspheres	PLA-01	PLA-02	PLA-03	PLA-04	PLA-05	PLA-06	PLA-07	PLA-08
Anastrozole (mg)	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
PLA (mg)	25.0	50.0	75.0	100.0	150.0	200.0	200.0	200.0
Polyvinyl alcohol (mL)								
0.5% *w/v*	–	–	–	–	–	–	–	100.0
1.0% *w/v*	100.0	100.0	100.0	100.0	100.0	100.0	–	–
2.0% *w/v*	–	–	–	–	–	–	100.0	–

as 1 mg oral tablet with a half-life of 41–48 h. As we reported earlier, the duration of breast cancer therapy should be prolonged (>31 months) until tumour cell regression occurs completely. Also, conventional anastrozole administration shows gastrointestinal (GI) disturbances (incidence 39–43%) with oral administration along with the other side effects such as skeletal complications, anaemia, vaginal bleeding *etc.* [6].

Considering all the above factors, in the present study an attempt was made to prepare microspheres of anastrozole with PLGA 50:50 [poly(lactide-*co*-glycolide)] and PLA [poly(lactic-acid)] and compared their *in vivo* behavior in a rat model. This drug delivery system is anticipated to reduce the side effects associated with the conventional cancer therapy [Arimidex®] by reducing the frequency of dose administration, thereby improving patient compliance. These polymers, being highly biocompatible and biodegradable undergo hydrolytic degradation and slowly release the drug for a prolonged period of time with sustaining plasma concentration without adverse cellular and tissue responses. The rate of drug release can be controlled by adjusting the lactide:glycolide molar ratio. Injectable microspheres encapsulating a wide variety of drugs ranging from small-molecular-weight molecules to peptide hormones, antibiotics, and chemotherapeutic agents in various concentrations have been studied widely as depot formulations [7,8]. Presently, there are limited literatures available on sustained delivery of anastrozole, some of them are, anastrozole-loaded PLGA nanoparticles (NPs) for sustained drug release as an alternate to conventional cancer therapy [9], transdermal patches of anastrozole for site-specific delivery [10], extended release of anastrozole, by dendrimer-based stealth nanoparticles [11] and anastrozole microparticles as alternative therapy for long-term treatment of breast cancer [12]. In our earlier attempt, the efficacy of chitosan microspheres of anastrozole was investigated [13]. In addition, we used lipids and biodegradable polymers as drug carriers for the development of nanomedicines, confirmed the long-time circulation of anastrozole for sustained action [6,14]. Considering that, PLGA and PLA microspheres have been widely used for many years with successful rates for implantable controlled drug delivery [15,16]. Hence in current study, PLGA and PLA microspheres of anastrozole were prepared using surfactant; polyvinyl alcohol (PVA) as emulsifier and a stabilising agent. The effect of polymer to drug ratio and surfactant concentration on encapsulation efficiency, particle size and *in vitro* drug release were studied. Further optimised formulations were subjected for FTIR study, DSC analysis, XRD, surface morphology, stability studies and *in vivo* pharmacokinetic study in Wistar rats.

2. Materials and methods

Anastrozole [ANS] (99.5% purity) was obtained as a gift sample from Sun Pharmaceuticals Pvt. Ltd., Baroda, India. PLGA, poly (lactide-*co*-glycolide) 50:50 and PLA, poly (lactic-acid) were procured from Boehringer Ingelheim, Germany. Polyvinyl alcohol (PVA) was purchased from Sigma-Aldrich, USA. All other chemicals and reagents used were either of analytical or HPLC grade.

2.1. PLGA microspheres

Classically, PLGA and PLA microspheres were prepared by oil in water (o/w) emulsion technique which consists of a volatile organic phase with dissolved polymer and the drug to be encapsulated, emulsified in an aqueous phase surfactant phase followed by solvent evaporation. A surfactant was included in the aqueous phase to prevent the organic droplets from coalescing once they are formed. The droplets formed *via* physical means, organic solvent leaches out of the droplet into the external aqueous phase before evaporating at the water-air interface. The solvent used should be completely or almost immiscible in water such that two-phase system can be easily obtained.

In this study, PLGA microspheres loaded with anastrozole were prepared by a simple o/w emulsion technique, followed by solvent evaporation as described by Zidan et al. [17]. The compositions of various formulations are depicted in Table 1. The drug (5 mg) was dissolved in 2 mL of dichloromethane (DCM) in which specified amounts of PLGA was dissolved. This oil phase was added dropwise using 3 mL syringe with 22-gauge needle to 50 mL of PVA solution, (stabiliser/emulsifier) which was stirred using a magnetic stirrer (Remi Equipments, Mumbai, India) at a speed of 1000 rpm for 2 h to achieve emulsification followed by complete solvent evaporation. The resulting microspheres obtained were collected from the PVA solution by filtration under vacuum using 1.2 μm membrane filters. The microspheres were washed twice with distilled water and dried in a vacuum desiccator at room

Table 2
Percentage yield, percentage encapsulation efficiency of anastrozole-loaded PLGA microspheres ($n = 3$).

Formulations	Percentage yield (%)	Encapsulation efficiency (%)	Mean particle size (μm)
PLGA-01	59.1 ± 1.2	41.0 ± 2.1	42.3 ± 1.5
PLGA-02	50.1 ± 1.6	48.3 ± 2.5	56.7 ± 1.3
PLGA-03	61.0 ± 1.8	56.7 ± 3.3	72.8 ± 1.8
PLGA-04	76.8 ± 2.3	64.6 ± 3.6	80.1 ± 1.6
PLGA-05	84.4 ± 2.5	72.1 ± 2.3	85.7 ± 1.2
PLGA-06	88.5 ± 3.4	78.4 ± 1.5	88.9 ± 3.2
PLGA-07	86.2 ± 2.9	70.3 ± 2.0	115.1 ± 1.2
PLGA-08	82.8 ± 3.1	74.7 ± 1.2	86.8 ± 1.4