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Injectable PLGA Adefovir microspheres; the way for long term therapy of chronic hepatitis-B

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ABSTRACT

For patient convenience, sustained release Adefovir Poly-d,l-lactic-*co*-glycolic acid (PLGA) microspheres were formulated to relieve the daily use of the drug which is a problem for patients treated from chronic hepatitis-B. PLGA microspheres were prepared and characterized by entrapment efficiency, particle size distribution and scanning electron microscopy (SEM). *In-vitro* release and *in-vivo* studies were carried out. Factors such as drug: polymer ratio, polymer viscosity and polymer lactide content were found to be important variables for the preparation of PLGA Adefovir microspheres. Fourier transform infrared (FTIR) analysis and differential scanning calorimetry (DSC) were performed to determine any drug-polymer interactions. One way analysis of variance (ANOVA) was employed to analyze the pharmacokinetic parameters after intramuscular injection of the pure drug and the selected PLGA microspheres into rats. FTIR and DSC revealed a significant interaction between the drug and the polymer. Reports of SEM before and after 1 and 24 h release showed that the microspheres had nonporous smooth surface even after 24 h release. The entrapment efficiency ranged between 55.83 and 86.95% and *in-vitro* release studies were continued for 16, 31 and 90 days. The pharmacokinetic parameters and statistical analysis showed a significant increase in the $T_{max,}$ AUC_{0-t} and MRT, and a significant decrease in the C_{max} of the tested formulation ($p < 0.05$). Results demonstrated that PLGA Adefovir microspheres could be used for long-term treatment of chronic hepatitis-B instead of the daily dose used by the patient.

1. Introduction

Chronic Hepatitis B virus infection is a global public health problem, so suppression of the replication of hepatitis B virus is the main effective mechanism of the antiviral drugs used in the treatment of chronic hepatitis B which cause liver cirrhosis and hepatocellular carcinoma (Liver, 2017; D'souza and Foster, 2004; Marcellin et al., 2003).

Adefovir dipivoxil is a prodrug of Adefovir, which makes inhibition and termination of the replication of virus B (Izzedine et al., 2004). Oral administration of a daily dose (10 mg) of Adefovir dipivoxil may continue for several years or lifelong (Sokal et al., 2008). So, injectable sustained release formulations like biodegradable polymeric microspheres were developed to prevent progression of the disease, particularly to cirrhosis, liver failure, and hepatocellular carcinoma and also the sustained virological response is also increased by extending treatment duration (Tang et al., 2014).

Poly (d,l-lactic-*co*-glycolic acid) (PLGA) is one of the most interesting polymers in the field of controlled drug delivery systems, which were approved by the FDA, as it is biodegradable, undergoes erosion after long time and achieves sustained drug release (Danhier et al., 2012; Makadia and Siegel, 2011).

LUPRON DEPOT® is one of the marketed drugs loaded PLGA microspheres. Leuprolide acetate microspheres are commercial products used for the treatment of prostate cancer (Soloway et al., 2002). Also, RISPERDAL CONSTA® (risperidone loaded PLGA microspheres) is indicated for the maintenance treatment of schizophrenia (Eerdekens et al., 2004). Such products can be intramuscularly or subcutaneously administered at 1 month or even 6 month intervals.

Adefovir is available in the market as tablets but not as parenteral sustained formulation (Baker, 2005). So, the aim of this study was to formulate PLGA loaded Adefovir microspheres to attain a prolonged period of release as possible (more than one month), also to reduce the side effects, drug toxicity, the frequency of administration, improve the patient compliance and also the bioavailability of the drug (Han et al., 2016). Some variable parameters were performed to evaluate the formulated microspheres such as entrapment efficiency determination, particle size distribution, scanning electron microscopy, *in-vitro* and *in-vivo* drug release profile. Fourier transform infrared (FTIR) analysis and

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differential scanning calorimetry (DSC) were also performed. Selected formulation from the *in-vitro* studies was used to determine the pharmacokinetics of the drug in rats.

2. Materials and Methods

2.1. Materials

Adefovir was kindly supplied by Eva Pharma., Egypt. PLGA of different viscosity grades and different ratios of lactide and glycolide were purchased from LACTEL International Absorbable Polymers, 2200 River-chase Center, Suite 501 Birmingham, USA. Polyvinyl alcohol (PVA) (molecular weight of 70,000:100,000 Da), dibasic potassium hydrogen phosphate, hydrochloric acid, monobasic sodium hydrogen phosphate and methylene chloride were purchased from El-Nasr Pharmaceuticals Chemicals Co., Egypt. Methanol and acetonitrile (Fisher chemical® HPLC gradient grade) were also purchased. All other chemicals were of analytical grade and used as received. Preparation of buffer and its dilutions were done with Millie-Q demineralized double-distilled water.

2.2. Preparation of Adefovir loaded PLGA microspheres

PLGA microspheres loaded with Adefovir were prepared by emulsion solvent evaporation method using distilled water as continuous phase containing PVA as an emulsifier. PVA solution (0.5%) was prepared in distilled water by heating to facilitate the solubility of PVA, and then allowed to cool at room temperature. The drug and polymer were weighed and dissolved in 20 ml methylene chloride at room temperature. The above organic phase was slowly added to 100 ml of 0.5% PVA solution at room temperature and emulsified by Heidolph PZP stirrer at 1600 rpm for 5 h. Microspheres formed were filtered, washed with water and dried overnight at room temperature (Goyal et al., 2011; Han et al., 2016; Nila et al., 2014). Formulations were prepared as shown in Table 1.

2.3. Estimation of Entrapment Efficiency of Adefovir Microspheres

Microspheres equivalent to 10 mg of the drug were taken for evaluation. The amount of drug entrapped was estimated by dissolving the microspheres in methylene chloride and then extracting with aliquots of 0.1 N hydrochloric acid by agitation in mechanical stirrer. Methylene chloride was evaporated then the solution was filtered through Whatman filter paper in 100 ml volumetric flask and the volume was adjusted to 100 ml using 0.1 N hydrochloric acid. The solution was diluted suitably and analyzed for drug content spectrophotometrically at λ_{max} (260 nm) after construction a calibration curve of Adefovir in 0.1 N hydrochloric acid with linearity range (5–40 µg/ml) by using GENESYS 10S spectrophotometer, USA. 0.1 N hydrochloric acid was used as a blank. The percent entrapment efficiency is calculated using the following equation (Sabry, 2013).

% Entrapment Efficiency = (Actual content/Theoretical content) × 100.

2.4. Particle Size Determination

Microspheres (50 mg) were suspended in distilled water (5 ml) containing 2% w/v of tween 80 to prevent microsphere aggregation. The above suspension was sonicated in a water bath and the particle size was expressed as volume mean diameter in micrometer using laser diffraction technique (Mastersizer 2000 Ver. 5.6).

2.5. Scanning Electron Microscopy Study (SEM)

Morphology was characterized by scanning electron microscopy using JEOL-T330A scanning microscope (Japan). Dry samples were placed on an aluminium plate and coated with gold. Pictures of microparticles were randomly taken. Other two samples were prepared by keeping them into 100 ml of Sörensen phosphate buffer and kept in a shaker at 37 ± 0.5 °C; one sample for 1 h and the other for 24 h. The microparticles were separated from the media and frozen at −80 °C for 4 h. The frozen samples were then lyophilized for 24 h in a freeze drier. The dried samples were sputter coated with gold and then SEM micrographs were obtained.

2.6. In-vitro Release Study

The dissolution of Adefovir pure powder and its release from the prepared microparticles were performed using the dialysis bag method. Microspheres equivalent to 10 mg of Adefovir were placed into cellulose dialysis bags (Molecular weight of 12,000:14000 Da) and then suspended in 100 ml of Sörensen phosphate buffer pH 7.4 in a closed bottle (Liu and Lv, 2014). The bottles were shaken at 50 rpm and 37 ± 0.5 °C. Each sample was run in triplicate. Aliquots of 3 ml were taken from each bottle at 0.5, 1, 2, 3, 4, 5, 6, 8 and 24 h then at 2, 3, 5, 7, 10, 13, 16, 21, 31, 41, 45, 55, 62, 70, 80 and 90 days. Samples were analyzed for the drug content spectrophotometrically at 260 nm against Sörensen phosphate buffer pH 7.4 as a blank. The dissolution medium was replaced with fresh medium to maintain a sink condition. Then, the average of the standard error of mean values was calculated (Ahmed et al., 2012).

2.7. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra were obtained on a Perkin-Elmer 1600 FTIR spectrophotometer using KBr disk method. The scanning range was 400–4000 cm^{-1} and the resolution was 1 cm^{-1}.

Table 1
Composition and entrapment parameters of Adefovir-loaded PLGA microspheres.

Batch no.	Formulation variables			Entrapment parameters		
	Polymer type	Drug: polymer ratio	Polymer inherent viscosity dL/g	Actual content (mg)	Theoretical content (mg)	Entrapment efficiency (%)
1	PLGA (50:50)	1:4	1.05	5.66	10	58.33
2	PLGA (50:50)	1:7	1.05	7.22	10	72.20
3	PLGA (50:50)	1:10	1.05	8.45	10	84.58
4	PLGA (50:50)	1:13	1.05	8.69	10	86.95
5	PLGA (50:50)	1:10	0.55–0.75	5.58	10	55.83
6	PLGA (65:35)	1:10	0.55–0.75	5.70	10	57.00
7	PLGA (75:25)	1:10	0.55–0.75	6.04	10	60.40
8	PLGA (85:15)	1:10	0.55–0.75	6.55	10	65.50

SDs did not exceed 1.5% of the reported value.