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Hydrolytic degradation and drug release properties of ganciclovir-loaded biodegradable microspheres

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Abstract

The in vitro hydrolytic degradation of ganciclovir (GCV)-loaded biodegradable microspheres of poly(D,L-lactide) and poly(D,L-lactide-co-glycolide) polymers were studied. Microspheres of size 120 ± 40 μm were prepared using an oil-in-water emulsification/solvent evaporation technique. The effects of polymer molecular weight, lactide (LA) to glycolide (GA) ratio and GCV payload on the degradation and drug release profiles were investigated in vitro in phosphate-buffered solution (pH 7.0) at 37 °C. GCV accelerated the hydrolysis process of the low (5–7 wt.%) GCV-loaded microspheres due to a basic catalytic effect, giving a larger degradation rate, k', compared with blank and high (18–20 wt.%) GCV-loaded microspheres. In the high GCV-loaded microspheres, hydrolysis of the polymer backbone occurred with little and/or no autocatalytic effect, resulting in a smaller k' compared with low GCV-loaded microspheres. This was due to pores and microchannels created at the surface following the initial burst release, which increased water uptake and the dissolution and diffusion of GCV and degradation products from the matrix. The rate of hydrolytic degradation was also affected by the LA to GA ratio. For polymers of similar LA to GA ratio, those with a higher degree of blockiness had faster hydrolytic degradation rates irrespective of the initial molecular weight. The release profile had a biphasic pattern, which closely followed the degradation profile of the polymer. The time taken for the complete release of GCV was controlled by the diffusion phase and was dependent on the hydrolytic degradation rate of the polymers.

Keywords: Poly(lactide-co-glycolide); Poly(D,L-lactic acid); Microsphere; Hydrolysis; Drug release

1. Introduction

In order to avoid the inconvenience of surgical insertion of large implants, injectable biodegradable and biocompatible polymeric particles like microspheres, microcapsules, nanocapsules and nanospheres can be employed for the controlled release of drugs [1]. These polymeric devices generally release drugs by diffusion and/or by chemical mechanisms such as degradation of the polymer, or the bonds between the drug and a polymer backbone [2].

The most widely used and studied biodegradable polymers for medical devices are the polyesters poly(lactic acid), poly(glycolic acid) and their copolymers [3], due to the extensive safety profile based on their use as sutures [4]. By far the largest research effort utilizing poly(D,L-lactide-co-glycolide) (PLGA) in controlled drug delivery is in microparticulate systems [5–9]. These formulations, designed to be administered subcutaneously, orally or transmucosally, have the tremendous advantage of supplying a continuous amount of drug over a long period of time (from weeks to months). PLGA microspheres containing ganciclovir (GCV) for intravitreal administration developed using an oil-in-oil emulsion technique have been reported to release the drug in vitro, and in vivo in rabbit eyes for at least 42 days [10,11].

It is generally accepted that the hydrolysis of most polyester proceeds according to the reaction [12]:

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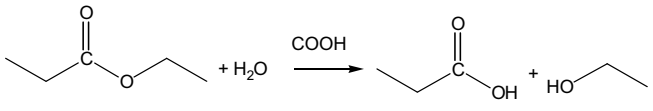

The kinetics of this reaction are given by

$$\frac{d[COOH]}{dt} = k'[ester][H_2O][COOH] = k[COOH] \quad (1)$$

where [COOH], [ester] and [H_2O] are the concentrations of carboxyl end groups, ester and water in the polymer matrix respectively, and it is assumed that in the early stages of the reaction the concentration of water and ester are constant. By assuming that $[COOH] = 1/M_n$, it can thus be shown that

$$\ln M_n = \ln M_{n,0} - kt \quad (2)$$

where $M_{n,0}$ is the initial number-average molecular weight of the polymer and k is the rate constant of the biodegradation process.

A bulk erosion mechanism is responsible for the main degradation of biodegradable polyesters, where random chain scission on the linkage of ester bonds in the polymer backbone proceeds homogeneously throughout the matrix. The bulk degradation pathway has three major features in the polymer erosion profile [13]. The first stage is the incubation stage, where there are no changes in the mass and molecular weight (M_w) of the polymer, which reflects the time required for water penetration into the polymer matrix. The second stage is the induction stage, where the M_w decreases and a rapid increase is observed in the degree of matrix hydration followed by saturation. The third and final stage is polymer erosion. In this stage, weight loss is observed.

Grizzi et al. [14] reported that large devices of biodegradable polyesters degraded via a heterogeneous mechanism, where degradation proceeded more rapidly in the center than at the surface. This was attributed to the autocatalytic action of the carboxylic acid end groups of degradation products that were trapped in the matrix. Due to this heterogeneous mechanism, the smaller the polymer size, the slower the degradation rate.

The biodegradation rate of polyesters is dependent on several factors, such as the molar ratio of the lactic and glycolic acids in the copolymer chain, the molecular weight of the polymer, the degree of crystallinity and the T_g of the polymer [15–17]. Additional factors include temperature, additives in the polymeric matrix, additives in the surrounding medium, pH, buffering capacity, size and processing history, quenching or annealing, steric hindrance, and porosity [18].

Cytomegalovirus (CMV) retinitis is the most frequently encountered HIV-related ocular opportunistic infection. It is caused by CMV, a common virus in the herpes family which, if left untreated, will generally cause blindness within 6 months [19]. Ganciclovir is a nucleoside analog which inhibits herpes virus DNA polymerase. The drug is virustatic against cytomegalovirus. It is a white to off-white crystalline powder with the chemical name of 9-[[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl]guanine. Ganciclovir is a polar hydrophilic compound with a solubility of 2.6 mg ml^{-1} in water at 25 °C and an *n*-octanol/water partition coefficient of 0.022. The pK_a (acid dissociation constant) values for GCV are 2.2 and 9.4 [20]. Within cells, GCV becomes mono-, di- and triphosphorylated (it is the triphosphate that interferes with viral DNA synthesis) [20].

The purpose of this study is to investigate the effect of GCV payload, polymer molecular weight and lactide (LA) to glycolide (GA) ratio on the hydrolytic degradation kinetics and drug release profiles of poly(D,L-lactide) (PDLLA) and PLGA microspheres. These GCV-loaded microspheres are intended for intravitreal administration for treatment of cytomegalovirus retinitis. Ganciclovir-loaded microspheres of PDLLA and PLGA polymers were degraded in phosphate-buffered solution (PBS) (pH 7.00) at 37 °C for various lengths of time. Thermal analysis (differential scanning calorimetry (DSC)) and gel permeation chromatography (GPC) were employed to follow the changes in the sample morphology with degradation. Changes to the pH of the degrading medium and mass loss measurements were also carried out. Control samples made of blank microspheres were also prepared and tested for morphological changes at the end of each immersion period.

2. Experimental methods

2.1. Materials

The anti-viral drug used was GCV purchased from Roche Products Limited (Welwyn Garden City, UK) in the form of Cymevene®. Poly(D,L-lactide-co-glycolide) 75:25, with an intrinsic viscosity (i.v.) of 0.94 dl g^{-1} and an average molecular weight of 130 kDa, was purchased from Purac Far East. Poly(D,L-lactide-co-glycolide) 75:25, i.v. of 0.24 dl g^{-1}, average molecular weight of 23 kDa, poly(D,L-lactide-co-glycolide) 50:50, i.v. of 0.50 dl g^{-1}, average molecular weight of 37 kDa, poly(D,L-lactide-co-glycolide) 50:50, i.v. of 0.22 dl g^{-1}, average molecular weight of 11 kDa, and poly(D,L-lactide), i.v. of 0.22, average molecular weight of 20 kDa were purchased from Absorbable Polymer Technologies, Inc. Methylene chloride from Merck Chemicals Ltd (KGaA, Darmstadt, Germany) and poly(vinyl alcohol) (87–89% hydrolyzed, average molecular weight 13,000–23,000), from Aldrich Chemical Company Inc. (Milwaukee, WI), were used as received. The samples were degraded in PBS, pH 7.0, at 20 °C, obtained from Merck Chemicals Ltd (KGaA, Darmstadt, Germany).

2.2. Methods

2.2.1. Preparation of microspheres

The GCV-loaded polymer microspheres were prepared using an oil-in-water (O/W) emulsion technique [21].