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Formulation and characterization of microspheres loaded with imatinib for sustained delivery

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ABSTRACT

The aim of this study was the development of imatinib-loaded poly(D,L-lactide-*co*-glycolide) (PLGA) microspheres with high loading efficiency which can afford continuous release of imatinib over a prolonged period of time. Imatinib mesylate loaded PLGA microspheres with a size of 6–20 μm were prepared by a double emulsion ($W_1/O/W_2$) method using dichloromethane as volatile solvent. It was found that the microspheres were spherical with a non-porous surface; imatinib loading efficiency (LE) was highly dependent on the pH of the external water phase (W_2). By increasing the pH of W_2 phase above the highest pK_a of imatinib (pK_a 8.1), at which imatinib is mainly uncharged, the LE increased from 10% to 90% (pH 5.0 versus pH 9.0). Conversely, only 4% of its counter ion, mesylate, was retained in the microspheres at the same condition (pH 9.0). Since mesylate is highly water soluble, it is unlikely that it partitions into the organic phase.

We demonstrated, using differential scanning calorimetry (DSC), that imatinib was molecularly dispersed in the polymeric matrix at loadings up to 8.0%. At higher drug loading, imatinib partially crystallized in the matrix. Imatinib microspheres released their cargo during three months by a combination of diffusion through the polymer matrix and polymer erosion.

In conclusion, we have formulated imatinib microspheres with high LE and LC. Although we started with a double emulsion of imatinib mesylate, the obtained microspheres contained imatinib base which was mainly molecularly dispersed in the polymer matrix. These microspheres release imatinib over a 3-month period which is of interest for local treatment of cancer.

1. Introduction

Protein tyrosine kinases (PTKs) contribute in signal transduction pathways that regulate various cellular processes such as growth, metabolism, differentiation, adhesion and apoptosis. Deregulation of PTK activity has been associated with the pathogenesis of various cancers as well as other inflammatory diseases (Chute and Himburg, 2013; Dolman et al., 2012; Falke et al., 2015; Wallace and Gewin, 2013). Imatinib is a multi-targeted tyrosine kinase inhibitor (TKI) that is used as molecularly targeted therapy in different types of cancer. It acts through competitive inhibition of the ATP binding site of the PTKs, thus blocking autophosphorylation and subsequent intracellular signal transduction (Govindarajan et al., 2012; Peng et al., 2005; Ruan et al., 2013). Imatinib is now the standard treatment for patients with chronic myeloid leukemia as well as gastrointestinal stromal tumors. It is marketed as Gleevec® which is a film-coated tablet formulation that contains imatinib mesylate equivalent to 100 or 400 mg of imatinib free base for oral administration (Henkes et al., 2008).

Recently, a new way of administration of imatinib has been described in which imatinib-loaded polymeric microspheres were injected in close proximity to the tumor (Benny et al., 2009; Karal-Yilmaz et al., 2013). Benny et al. evaluated the local

Abbreviations: PLGA, poly(D,L-lactide-*co*-glycolide); M_w, molecular weight; RO, reverse osmosis; $W_1/O/W_2$, water in oil in water; W_1, inner water phase; W_2, outer water phase; LE, loading efficiency; LC, loading capacity; T_g, glass transition temperature; T_m, melting temperature; SEM, scanning electron microscopy; TGA, thermal gravimetric analysis; DSC, differential scanning calorimetry; DCM, dichloromethane; PVA, polyvinylalcohol; THF, tetrahydrofuran; PBS, phosphate buffered saline; TL, theoretical loading; GPC, gel permeation chromatography.

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tumor inhibition of imatinib-loaded PLGA microspheres in a glioblastoma xenograft mice model (Benny et al., 2009). A single dose of imatinib-loaded microspheres corresponding to 1.5 mg imatinib was injected intracranially at the site of the tumor and resulted in a 79% reduction in the tumor volume 14 days post injection. In another study of Karal-Yilmaz et al., imatinib-loaded PLGA microspheres were studied for their inhibition of angiogenesis in craniopharyngioma (a type of brain tumor) (Karal-Yilmaz et al., 2013). A more recent publication of the same group reported on the development of imatinib-loaded polystyrene-*g*-poly(lactide-*co*-glycolide) microspheres for local sustained delivery of imatinib (Kukut et al., 2014).

These studies clearly show that imatinib-loaded PLGA microspheres hold potential for local inhibition of cancer. However, the physicochemical properties of these microspheres have not been studied well. For example, an ideal microparticles formulation should have reasonably high drug loading efficiency (LE), loading capacity (LC), and sustained (preferably zero-order) release of the loaded drug for desired period of time (Ye et al., 2010). The high LE and LC are critical, especially for expensive and less potent drugs. Imatinib is one of the exceptionally expensive cancer drugs for the treatment of chronic myeloid leukemia (Experts in Chronic Myeloid Leukemia, 2013). Therefore, decreasing the drug loss during the formulation and improving its LE is of great importance. In the previous studies, maximum LE and LC which was achieved for imatinib-loaded PLGA microspheres was about 57% and 0.23%, respectively. In addition to the low LE and LC, the initial drug release (burst) was rather high in the previous studies (Benny et al., 2009; Karal-Yilmaz et al., 2013). The present study therefore aims at developing an efficient procedure for loading imatinib into PLGA microspheres and to study the effect of formulation parameters such as pH of external water phase, volume of inner water phase, and theoretical drug loading on microsphere characteristics for developing microspheres with sustained (preferably zero-order) release of imatinib.

2. Materials and methods

2.1. Materials

Poly(D,L-lactide-*co*-glycolide) (PLGA, 50:50 lactide:glycolide ratio, end-capped, intrinsic viscosity 0.4 dL/g) was obtained from Purac, the Netherlands. Imatinib mesylate was obtained from LC Laboratories, USA. Polyvinyl alcohol (PVA; M_w 30,000–70,000; 88% hydrolyzed) was obtained from Sigma–Aldrich, Germany. Dichloromethane (DCM), acetonitrile and tetrahydrofurane (THF) were purchased from Biosolve (Valkenswaard, The Netherlands).

2.2. Preparation of imatinib-loaded microspheres

Imatinib-loaded microspheres were prepared using a double emulsion solvent evaporation technique ($W_1/O/W_2$) as described in literature (Benny et al., 2009). Briefly, inner water phase (1 mg imatinib mesylate in 250 μl reversed osmosis (RO) water) was added to 500 μl of DCM in which 200 mg PLGA was dissolved. The inner water phase (W_1) was emulsified into the polymer solution using an IKA homogenizer (IKA Labortechnik Staufen, Germany) for 30 s at maximum speed (30,000 rpm). This primary emulsion (W_1/O) was subsequently emulsified at maximum speed (30,000 rpm) for 30 s in 1 ml buffer with different pH (pH 5.0 [0.25 M sodium acetate buffer], pH 7.0 [0.25 M sodium potassium phosphate buffer] and pH 9.0 [0.25 M Tris hydrochloride buffer]) also containing 1% PVA. The obtained $W_1/O/W_2$ emulsion was then transferred into 40 ml of the same PVA-containing buffer and stirred for 3 h using a magnetic stirrer (500 rpm, 3 h) at room temperature (formulations, F1–F3, Table 1). For other formulations, the primary emulsion (W_1/O) was emulsified in 6 ml of Tris buffer pH 9.0 PVA 1% as external water phase. After 40 min, the emulsion was transferred into 44 ml of 1% PVA phosphate buffer (pH 7.0) under magnetic stirrer (500 rpm, 3 h). The formed microspheres were centrifuged (Laboratory Centrifuge, 4K 15, Germany) at 4000 × *g* for 3 min and washed 2 times with 100 ml tween 20 (0.025%) followed by two times washing with 100 ml RO water and lyophilized. The obtained microspheres were stored at −25 °C. Besides the pH of W_2, variables in this study were the volume of W_1 ranging from 5 to 350 μl and theoretical drug loading (weight of initial drug/weight of both drug and polymer × 100) ranging from 0.4% to 19% w/w. All the microspheres batches were prepared in triplicate.

2.3. Preparation of imatinib free base

Imatinib free base was prepared as follows: about 100 mg imatinib mesylate was added to 1 ml of pH 9.0 Tris buffer and the solution was vortexed for 10 min. The precipitated imatinib base was separated from mesylate salt by centrifugation (13,000 rpm, 5 min) and washing with 3 ml RO water. The imatinib base (precipitant) was then freeze dried overnight and stored at room temperature.

2.4. Characterization of the microspheres

The average size and size distribution of the microspheres were measured using an Accusizer 780 (Optical Particle Sizer, Santa Barbara, California, USA). The volume weighted mean diameter (vol–wt mean) of microspheres is reported as particle size and the

Table 1
Characteristics of imatinib-loaded PLGA microspheres. Microspheres were prepared using external W_2 phases of different pH values. The concentration of PLGA in DCM was 23% (w/w) and the internal W_1 volume was 20% in all formulations. Data are expressed as mean ± SD (n = 3).

Formulation[a]	W_2 (pH)	TL[b] (wt%)	Recovery[c] (%)	Particle size[d] (μm)	Span[e] value	Imatinib LC[f] (%)	Imatinib LE[g] (%)
F1	5.0	0.41	63 ± 2	20 ± 2	1.4 ± 0.1	0.042 ± 0.006	10 ± 2
F2	7.0	0.41	67 ± 3	20 ± 2	1.2 ± 0.1	0.226 ± 0.007	54 ± 2
F3	9.0	0.41	67 ± 4	22 ± 1	1.2 ± 0.2	0.378 ± 0.002	90 ± 4
F4	9.0[a]	0.41	72 ± 3	16 ± 1	1.4 ± 0.2	0.378 ± 0.006	90 ± 2
F5	9.0[a]	9.50	60 ± 4	6 ± 1	1.4 ± 0.1	8.1 ± 0.7	86 ± 7
F6	9.0[a]	19.0	59 ± 4	9 ± 1	1.6 ± 0.2	16.0 ± 0.4	84 ± 3

Mean ± SD values were calculated from the data of three independent batches and represent reproducibility between batches.
[a] The microspheres F4–F6 were prepared with the external water phase of pH 9.0 for 40 min and subsequently transferred to pH 7.
[b] TL: theoretical drug loading.
[c] Recovery of microspheres as percentage of drug and polymer starting weight.
[d] Particle size expressed as volume weighted mean diameter.
[e] Span value = (d90 − d10)/d50 which reflects the polydispersity within an individual batch.
[f] LC: loading capacity of imatinib.
[g] LE loading efficiency of imatinib expressed as free base.