RELEASE OF ^{125}I -rhBMP-2 FROM FOAMED POLY-(D,L)-LACTIDE

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Abstract:

Composite tablets of human recombinant bone morphogenetic protein 2 (rhBMP-2) and poly-(D,L)-lactide (PDLLA) (1-2 mg rhBMP-2/g PDLLA) were prepared by foaming of PDLLA with supercritical CO₂. Release kinetics of rhBMP-2 were measured by a continuous flow and a batch dilution method at 22 °C and 50 °C respectively. In both cases a two-phase first-order release was found. Release half-lives of the sustained release phases (87-90% of total) decreased significantly from 348 days at 22 °C to 86 days at 55 °C i.e. by a factor of 4 indicating a coupling with PDLLA hydrolysis. The released rhBMP-2 was biologically active.

Keywords: polylactide, foaming with supercritical CO₂, rhBMP-2, release kinetics, rate-constants

Introduction

In the preparation of bioactive implant materials either by the adsorption or encapsulation of protein growth factors a bioadapted drug delivery is one of the major objectives. Release kinetics of a substance from a polymer substrate can be described by the following equation (see ref. [1]):

$$-\frac{d[\text{P} \cdot \text{PLA}]}{dt} = k_1 [\text{P} \cdot \text{PLA}] - k_2 [\text{P}] [\text{PLA}] \quad (1)$$

where k_1 and k_2 are the rate constants of dissociation and association (i.e. readsorption) respectively. $[\text{P}]$, $[\text{PLA}]$ and $[\text{P} \cdot \text{PLA}]$ denote the concentration of protein, polylactic acid and the protein-polylactic acid complex respectively. Under the condition that the system is far from equilibrium, the second term (readsorption) becomes zero and eq. 1 reduces to a first order reaction, which on integration yields the exponential equation:

$$[\text{P} \cdot \text{PLA}] = C e^{-k_1 t} \quad (3)$$

where $C = [\text{P} \cdot \text{PLA}]_0$ i.e. the initial concentration of the protein-polylactic acid complex at time zero. First-order kinetics have also been shown for the degradation of the polymers poly(lactic-co-glycolic acid) (PLGA) [2] and PLA [3]. If the protein is released into a reservoir within the polymer and its diffusion to the bulk is restricted by pores, then linear zero-order kinetics are observed [4]. The major techniques for eliminating readsorption are dilution [1,5] and release under continuous flow conditions as will be shown below. As a model system

^{125}I -rhBMP-2 incorporated into PDLLA tablets by foaming with supercritical CO₂ (scCO₂) [6] will be employed.

Methods

Self-prepared rhBMP-2 [7,8] (activity equivalent: K_{0.5}~5-20 nM [8,9]) was employed and labelled with ^{125}I -rhBMP-2 [7]. 1 g defined PDLLA granules with CaCO₃ was suspended in 1.6 ml ^{125}I -BMP-2 solution and intensely homogenized [6]. During the mixing procedure a slurry formed which was lyophilized to a powder and fluidized in teflon molds (10 x 5 x 2 mm openings) for 16 h at 45 °C and 100 bar, depressurization 1-2 min, yielding composite tablets 10 x 5 x 2 mm, weight: ~38 mg, vol. ~0.106 cm³.

In method 1 (continuous flow) the tablets were placed in a flow cell (**Fig. 1**) at room temperature (~22 °C). To wash out released protein, cells were perfused with PBS (pH 7.4) at a flow-rate of ca. 10 mL/h by a multiple channel peristaltic pump (205 S, Fa. Watson-Marlow, Willmington, USA) for 40 days. At specified times, the flow cell with the tablet, was measured in a γ -counter. After the experiments the empty flow cells were corrected for non-specifically adsorbed ^{125}I -rhBMP-2 during release. Non-specifically retained rhBMP-2 comprised ca 6-7% of the released ^{125}I -rhBMP-2. In method 2 (batch dilution) ^{125}I -rhBMP-2 was released from the ^{125}I -rhBMP-2/PDLLA tablets into 1.5 mL PBS-buffer (pH 7.4) in vials on a in a heated shaker at 50 °C for 40 days. At the indicated times the tablets were washed two times with 1.5 mL fresh PBS, resuspended in 1.5 mL and counted in a γ -counter. Curves were fitted by the PC program Prism 4 for Windows [8]. Calculated half-lives were corrected for the spontaneous decay of ^{125}I ($t_{1/2}$ =60d).

Fig. 1: Flow cell in continuous flow method. The tablet was placed in the chamber (vol. 1.5 mL). Scale: 4 cm.

Results

Fig. 2 and **Fig. 3** show the double exponential decay of ^{125}I -rhBMP-2 /PDLLA tablets at 22 °C and 50 °C respectively. The derived rate constants are shown in **Table 1** together with half-lives of the burst and the sustained release phases. The decay rate increases 4-fold at 50 °C. There was no indication of a significant readsorption.

Table 1: Comparison of the calculated kinetic data of the releasing kinetics*.

Temperature $^\circ\text{C}$	incorporated ^{125}I -rh-BMP-2 [mg/g PDLLA]	Spans		Rate-constants		Half-lives		P_{runs}	r^2
		Span a	Span b	Burst phase $k_{-1}^a [\text{s}^{-1}] \times 10^5$	Sustained rel. ph. $k_{-1}^b [\text{s}^{-1}] \times 10^8$	$t_{1/2}^a [\text{d}]$	$t_{1/2}^b [\text{d}]$		
~ 22	1.880 ± 0.086	1.700	0.168	14.56 ± 1.23	2.3 ± 0.0063	0.055 ± 0.0046	348 ± 1.0	0.319	0.97
50	0.745 ± 0.014	0.651	0.089	6.45 ± 0.98	9.3 ± 0.279	0.124 ± 0.019	85.5 ± 2.6	0.2086	0.99

*The release of ^{125}I -rh-BMP-2 can be fitted according the following equation : $y = \text{Span } a \cdot e^{-k_1 \cdot t} + \text{Span } b \cdot e^{-k_2 \cdot t} + \text{Plateau}$ (Plateau = 0). Results are stated as (mean $\pm$ SEM, $n=3$). The burst phase is marked as a, main phase as b. Spans = Amount of released ^{125}I -rh-BMP-2 for each phase: burst phase 10-13%, main phase 87-90%

Discussion

The experiments demonstrate first-order release kinetics of rhBMP-2 from foamed PDLLA tablets in agreement with PLA hydrolysis [3]. A temperature increase from 22°C to 50°C increases the dissolution rate of ^{125}I -rhBMP-2/PDLLA tablets (possibly by PDLLA hydrolysis) by a factor of 4.

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