European Journal of Pharmaceutics and Biopharmaceutics 84 (2013) 456–463

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

Studies on the lipase-induced degradation of lipid-based drug delivery systems. Part II – Investigations on the mechanisms leading to collapse of the lipid structure

Martin Schwab [a], Cushla M. McGoverin [b], Keith C. Gordon [b], Gerhard Winter [a], Thomas Rades [c], Julia Myschik [a,*], Clare J. Strachan [c]

[a] Ludwig-Maximilians-University Munich, Department of Pharmacy, Munich, Germany
[b] Department of Chemistry and MacDiarmid Institute, University of Otago, Dunedin, New Zealand
[c] School of Pharmacy, University of Otago, Dunedin, New Zealand

ARTICLE INFO

Article history:
Received 20 July 2012
Accepted in revised form 21 December 2012
Available online 4 February 2013

Keywords:
Lipid-based depot systems
Biodegradation
Erosion
Extrudates
Implants
Lipase

ABSTRACT

It has recently been found that lipid composition appears to have a major influence on the rate of lipase-induced degradation of lipid-based extended release drug delivery systems (microparticles, compressed implants and extrudated implants). Previously, we have found that during lipase incubation, depending on the lipid used, lipidic extrudates can lose their physical strength and collapse generating lipid particles in the μm-range. The aim of this study was to characterise the processes leading to collapse of solid lipid-based drug delivery systems during *in vitro* lipase incubation. Compressed lipid implants were used as model systems. Free fatty acids (FFA) generated in the incubation experiments were derivatised and subsequently analysed via reversed phase-HPLC in order to characterise the degradation behaviour of single lipid components (glyceryltrilaurate (D112), glyceryltrimyristate (D114), glyceryltripalmitate (D116) and glyceryltristearate (D118)) used for the preparation of compressed lipid implants. Further, Raman spectroscopy/microscopy, differential scanning calorimetry, scanning electron and light microscopy were used to investigate the physical and chemical changes in the implants upon lipase incubation. This study revealed that the lipid component D112 plays a major role in the degradation and erosion processes occurring during lipase incubation of lipid implants. The production of D112/lauric acid mixtures, with a melting point below human body temperature, leads to lipid matrices melting and losing their physical integrity.

1. Introduction

Recently, there has been an increasing interest in parenterally administered lipid-based drug delivery systems for the formulation of proteins due to their outstanding performance in stabilising incorporated protein molecules [1,2]. However, there is currently little known about the fate of lipid-based depot devices in the human body. In most of the publications dealing with triglyceride-based drug delivery systems, biodegradation and bioerosion *in vivo* have not been reported [3,4]. Nevertheless, biodegradation and bioerosion are key to the use of these drug delivery systems. As lipases play a crucial role in the metabolic system by hydrolysing triglycerides to produce free fatty acids (FFAs), which serve as a major source of energy, human lipase activity in the subcutaneous fat tissue and the serum could affect the structure and the stability of lipid-based drug delivery systems [5–7].

In a recent study, we conducted *in vitro* lipase incubation studies with different lipidic delivery systems (microparticles, compressed implants and extrudated implants) and investigated the chemical degradation rate of these systems. We reported that for some of the investigated systems, mainly extrudates, there was a tendency for the samples to disintegrate during lipase incubation resulting in suspended particles in the μm-range [8]. In particular, extrudates containing glyceryltrilaurate (D112) exhibited a higher degree of disintegration upon incubation than those containing triglycerides with longer fatty acid chain lengths. In the present study, the physical and chemical degradation behaviours of implants prepared from single lipid components (triglycerides with different chain lengths) during incubation have been analysed, as models for drug depot systems. Raman spectroscopy/microscopy, scanning electron microscopy, differential scanning electron microscopy, as well as HPLC analysis have been used to gain insight into the physical and chemical mechanisms behind the process of lipid matrix breakdown.

* Corresponding author. Ludwig-Maximilians-University Munich, Department of Pharmacy, Pharmaceutical Technology and Biopharmaceutics, Butenandtstr. 5, D-81377 Munich, Germany. Tel.: +49 89 2180 77024; fax: +49 89 2180 77020.
E-mail address: julia.myschik@cup.uni-muenchen.de (J. Myschik).

0939-6411/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ejpb.2012.12.023

2. Materials and methods

2.1. Materials

All lipids (glyceryl-tristearate (D118), -tripalmitate (D116), -trimyristate (D114), -trilaurate (D112) and the corresponding fatty acids: stearic acid, palmitic acid, myristic acid and lauric acid) used in matrix preparation were purchased from Sasol GmbH, Witten, Germany. Lipases, lipoprotein lipase (LPL) from *Pseudomonas* sp. and lipase from *Rhizoma oryza* (RO) and all other chemicals were purchased from Sigma–Aldrich, Deisenhofen, Germany.

2.2. Methods

2.2.1. Lipid implant preparation

Tablet-shaped implants were compressed from each of the triglyceride components, or from mixtures of two of the single components using one of three following methods: gently grinding components in an agate mortar together with liquid nitrogen; co-melting both components; or producing layered tablet-shaped implants. Unless otherwise stated, implants with a diameter of 13 mm were compressed with 2 tons for 30 s using a hydraulic press.

2.2.2. Incubation

Implants were incubated at 37 °C in phosphate buffered saline (PBS; isotonic 0.01 M phosphate buffer pH 7.4) with and without lipase (lipoprotein lipases and RO lipase). The lipase concentration and time of incubation varied and are described for each experiment below and summarised in Table 1.

The physical and chemical changes of the implants and incubation medium during incubation were investigated using the following techniques.

2.2.3. Characterisation

2.2.3.1. Morphology changes. Implants of two masses were compressed: 50 mg and 500 mg. The 50 mg implants were formed using the lipid components, D118 D116, D114 and D112, individually. These implants had a diameter of 5 mm and an average height of 2.3 mm; implants were incubated at 37 °C in 2.0 mL of isotonic 0.01 M phosphate buffer, pH 7.4 containing 100 U of lipoprotein lipase and RO lipase. The 500 mg implants were prepared from D112 alone, or as a mixture with D118 in a ratio of 80:20 (D118/D112), which was gently ground in an agate mortar prior to compression. These implants had a diameter of 13 mm and a height of 3.7 mm. The implants were incubated in 5 mL of PBS containing 440 U lipoprotein lipase at 37 °C. Experiments were performed in triplicate. The morphological changes of the implants during incubation were analysed using visual inspection.

The 500 mg implants were also analysed using scanning electron microscopy and digital microscopy during swelling and erosion. After predetermined time points, the implants were withdrawn from the medium, washed three times in 20 mL water, blotted to remove excess water and then weighed (W_1). The implants were then dried in a vacuum chamber at reduced pressure (100 mbar at 25 °C) for 24 h. Implants were then visually characterised using a scanning electron microscope (Jeol JSM-6500F, Jeol Inc., Peabody, USA) and a digital microscope (VHX-600 digital microscope, Keyence, Osaka, Japan, 20× magnification). Implants analysed using the digital microscope were then weighed (W_2) using an analytical balance (model UMX2, Mettler–Toledo, Greifensee, Switzerland). W_0 is defined as the initial weight of the implants prior to incubation. Swelling (S), defined as the percentage increase in weight due to water uptake of the lipid, was determined at each time point from the following equation [8]:

$$S\ (\%) = \frac{W_1 - W_0}{W_0} \times 100 \quad (1)$$

Erosion is defined as the mass loss of the initial implant weight; the percentage of erosion processes (ES) was calculated from the following equation:

$$ES\ \ (\%) = 100 - \left(\frac{100}{W_0} \times W_2\right) \quad (2)$$

The residual buffer media, containing the main degradation products, was extracted and analysed using the methods described in the section below. The content of free fatty acids (FFAs) released in the buffer media was used to calculate the percentage of degradation (D) as outlined in the following section.

2.2.3.2. Analysis of incubation medium. To investigate the lipase-induced degradation of compressed lipid, implants consisting of 50 mg of single components (D112, D114, D116 and D118) were incubated as described above. Buffer medium was completely removed and new buffer solution was added to the incubated samples every 3 days. In this experiment, the release of FFA in the buffer medium was quantified using Eq. (3).

Free fatty acids (FFAs) were extracted from the incubation medium after using the modified Dole-extraction method [9] resulting in a FFA-solution for derivatisation. Phenacyl esters of fatty acids were prepared by the method described by Wood and Lee [10]. RP-HPLC was used to quantify the different FFA in solution. A Thermo Separation Products HPLC system (Thermo Fisher Scientific, Inc. Waltham, USA) equipped with a LiChrospere RP C18 column

Table 1
Experimental conditions used for implant analyses.

Parameter investigated	Implant weight	Composition	Diameter/ height	Incubation volume and time	Lipoprotein lipase/RO lipase	Analysis technique
Morphological changes	50 mg	D112; D114; D116; D118	5 mm/ 2.3 mm	2.0 mL for 30 days; exchange of buffer every 3 days	100 U	Visual inspection
	500 mg	D112 alone; D118/ D112 (ratio 80:20)	13 mm/ 3.7 mm	5 mL for 8 h	440 U	Visual inspection and SEM
Raman analysis of FFA production	50 mg	D112; D114; D116;D118	5 mm/ 2.3 mm	2 mL for 2.5 h	100 U	Raman microscopy
Raman analysis of the erosion process	50 mg + 50 mg (layered structure)	D118 (bottom layer) D112 (top layer)	Approx. 3.3 mm	25 mL for 42 h	350 U	*In situ* Raman spectroscopy
Thermal analysis	500 mg	D118/D112 (80:20)	13 mm/ 3.7 mm	5 mL for 3 days	100 U	DSC
	50 mg	D112	5 mm/ 2.3 mm	2 mL for 2.5 h	100 U	DSC