European Journal of Pharmaceutics and Biopharmaceutics 70 (2008) 690–694

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

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

Note

Correlation of in vivo and in vitro release data for rh-INFα lipid implants

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ARTICLE INFO

Article history:
Received 11 January 2008
Accepted in revised form 9 May 2008
Available online 5 June 2008

Keywords:
Protein release
Lipid implants
Computer modeling
In vivo–in vitro correlation

ABSTRACT

Previous in vitro experiments had shown that rh-INFα releasing tristearin implants feature promising properties making them an excellent tool for the delivery of therapeutic proteins. Sustained release for periods up to one month could be achieved, associated with high protein stabilization. The objective of this study was to investigate for the first time the in vivo release properties of these implants in rabbits and to establish an in vivo–in vitro correlation. Computer modeling was used to simulate rh-INFα serum levels based on pharmacokinetic data. Protein serum concentrations on therapeutically relevant nearly constant levels could be detected for 9 days. Modeling revealed that in vivo release correlated closely with the release monitored in vitro.

1. Introduction

Since pharmaceutical proteins given by injection must be repeatedly administered in short intervals to reach and maintain therapeutic useful concentrations in the blood plasma, low patient compliance and high hospital costs are resulting. To overcome these problems polymer-based delivery systems for therapeutic proteins have been developed. Although application of these new delivery devices is very promising most of the used polymer systems such as poly(lactic-coglycolic acid) (PLGA) [1] show some major drawbacks, i.e., interference with the protein stability like protein–polymer interaction, pH-shift and interface formation during formulation [2]. Recently, a new model depot system for the controlled delivery of rh-INFα has been developed in our working group [3]. This implant consists of a lipid component, rh-INFα, hydroxypropyl-β-cyclodextrin (HP-β-CD) or trehalose as a stabilizer and PEG 6000 as a release modifier. The use of lipids known as matrix materials with a high biocompatibility [4,5] allows to generate implants capable of a sustained protein release. The application of solvent-free processing techniques like compressing the lipid mass satisfies the necessity for both a high biocompatibility and a high protein stabilization. For long-time storage especially HP-β-CD can provide a high stability of the embedded therapeutic protein within the matrix [6].

In vitro release experiments have shown a continuous release over 30 days with a close to linear release phase for the first 2 weeks. Overall protein liberation up to 95% can be achieved within 4 weeks.

This controlled release is a consequence of the interaction of PEG with the incorporated rh-INFα, leading to a reversible precipitation of the protein within the matrix, hence the resulting retardation of dissolution of the INFα strongly contributes to the controlled release [7]. The aim of this work was to elucidate whether the release profile of the implants in vitro can be verified in vivo, i.e., in rabbits. We wanted to confirm whether the release mechanism was under total physiochemical control, independent of enzymes and other components of body fluids and mechanical stress. Further, we wanted to prove the expected excellent biocompatibility of the lipid implants.

2. Materials and methods

Rh-interferon α-2a (rh-IFNα, Roche Diagnostics, Penzberg, Germany; protein conc. 1.7 mg/ml in a 25 mM acetate buffer of pH 5.0, 120 mM sodium chloride) was lyophilized in a 1:3 ratio with hydroxypropyl-β-cyclodextrin (HP-β-CD, Merck, Darmstadt, Germany).

Tristearin (Dynasan 118) was purchased from Condea Chemie, Witten, Germany and polyethylene glycol 6000 (PEG 6000) is a product from Clariant, Gendorf, Germany.

All other materials (from Merck) were of high purity grade.

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doi:10.1016/j.ejpb.2008.05.010

2.1. Implant manufacturing

Implants were prepared by using a 5 ton hydraulic press (Maassen, Eningen, Germany).

The implant components – tristearin powder 80%, PEG 6000 10% and INFα-lyophilisate 10% – were ground in an agate mortar. The resulting mixture was compressed with a pressure of 2 tons for 30 s. The obtained implants had an average weight of 50 mg and an average height of approximately 2.3 mm. The drug load of the implant was 2.5% of the implant weight, accounted for the rh-INFα dose.

2.2. Animal experiment setup

In order to investigate the rh-IFNα release rate of the delivery device, lipid implants were administered to a group of five young female rabbits. Rabbits were chosen as experimental animals, because in contrast to rats or mice rabbits show a distinctive subcutaneous fat tissue which is quite similar to the human one. Under ketamin/xylazin anaesthesia one implant per animal was placed subcutaneously between the omoplates and the insertion site was sewn up. Samples of approx. 0.5–1 ml blood – gained from the ear vein – were taken daily for the first week, afterwards every three days.

Blank values were taken before the implantation.

To gain basic pharmacokinetic data, another group of three rabbits received an injection of unretarded rh-INFα solution (10^7 IU, i.e 37 μg rh-INFα per kg bodyweight).

Samples were taken every hour for at least 9 h, and after 12 and 24 h.

2.3. In vivo release studies

Blood samples were centrifuged and the resulting serum was frozen at −80 °C until analysis.

Analysis was performed with a 96-well INFα-ELISA (Bender MedSystems, Vienna, Austria).

Rh-INFα in the rabbit blank serum solutions of known concentrations (8.0–1600.0 pg/mL) were used to generate calibration curves. Analysis was conducted according to the ELISA test protocol. The absorbance of the colored product was measured using a CS-930-1PC plate reader (Shimadzu, Kyoto, Japan). The detection limit of the rh-INFα-ELISA was 4 pg/ml.

2.4. Rabbit anti-rh-INFα-antibody determination

Repeatedly administered injections of rh-INFα were reported to cause antibody generation in rabbits [8]. As no rabbit anti-rh-INFα-antibody-ELISA was available spiking experiments with serum samples were conducted in order to investigate whether rabbit antibodies against rh-INFα were present in the serum.

Aliquots of the samples drawn on day 9 or later were analyzed by INFα-ELISA and showed no detectable amount of rh-INFα. An exact amount (1.600 or 3.200 ng/mL) of rh-INFα solution was now added to these rabbit serum samples, and the mixture was incubated for 1 h at 40 rpm in a horizontal shaker. After such spiking, the recovery of the added rh-INFα was determined via the rh-INFα-ELISA kit from Bender.

It was expected that the antibodies generated in the rabbits against the recombinant human protein can also capture the added rh-INFα, and the analysis would therefore lead to a low recovery of the added rh-INFα.

2.5. Histological studies

At the end of the animal experiment (after 28 days) the implants were surgically removed. Slices of the implant and surrounding tissue were stained with a hematoxylin and eosin (H and E) stain. Image data were collected through a Leica DFC 320 camera (Leica Microsystems, Wetzlar, Germany) mounted on an Orthoplan microscope (Leica, Wetzlar, Germany).

2.6. In vitro release studies

Studies were conducted by incubating the implants ($n = 3$) at 37 °C in TopPac® vials containing 2.0 ml isotonic 0.01 M phosphate buffer, pH 7.4. The samples were shaken at 40 rpm (Certomat® IS, Braun Biotech International, Melsungen, Germany). Samples were taken daily the first 7 days, afterwards every 3 days. Sample volumes were replaced with fresh buffer. Analysis was conducted using a Thermo Separation Products HPLC system equipped with a Tosoh TSK-Gel G3000 SWxl column. 120 mM disodium hydrogen phosphate dehydrate, 20 mM sodium dihydrogen phosphate and 4 g/L sodium chloride, adjusted with hydrochloric acid to a pH of 5.0 were used as mobile phase. The flow rate was set to 0.6 mL/min, UV detection was performed at 210 nm wavelength.

2.7. Computer modeling

To model virtual rh-INFα serum levels, a single compartment model was used. Basic pharmacokinetic data and data gained by the in vitro release studies were collected to calculate the virtual rh-INFα blood levels by using the in vitro release curve as input function and the basic pharmacokinetic (PK) data for modeling distribution and elimination.

Input functions were created using an Inverted Gaussian (IG) function covering the experimentally obtained data [9]. The invasion rate of the rh-INFα into the rabbit blood described by the IG-function of the control group was neglected and therefore was not included into the final modeling. Variables of the IG-functions were chosen to allow a good fit on the particular curve. Any other data necessary for PK-calculations and modeling, i.e., volume of distribution and bioavailability of rh-INFα were provided by the literature [10].

Calculations were conducted with a self-programmed program and obtained data transferred into Microsoft Excel® for diagram generation. The pharmacokinetic analysis program WinNonlin® was used to determine the half-life and the elimination rate of rh-INFα.

3. Results and discussion

3.1. Pharmacokinetic data

The reference group with non-retarded rh-INFα-solution applied as s.c. injection provided the basic PK-data. The Cmax was reached after 2.5 h. Rh-INFα in serum was eliminated with an average terminal elimination half-life of 3.5 h ($k_{el} = 0{,}2$). After 12 h no rh-INFα was detectable any longer. The rh-INFα serum curve gained from the reference group is shown in Fig. 1. The modeled curve covering the rh-INFα serum curve depends on two functions: an IG-function describing the inflow and a first-order kinetics curve describing the elimination of rh-INFα used for PK-data determination.

The IG-function was calculated using the following formula [9]:

$$Y = \mathrm{Gain}^{*}\sqrt{\frac{\mathrm{MT}}{2^{*}\pi^{*}\mathrm{cv}^{2*}t^{3}}} * \exp - \left(\frac{(t - \mathrm{MT}^{2})}{(2^{*}\mathrm{cv}^{2*}\mathrm{MT}^{*}t)}\right)$$

where MT = 2.423, $cv^2 = 1.908$ and Gain-value = 9.209.

Goodness-of-fit was calculated using the following formula: