International Journal of Pharmaceutics 517 (2017) 175–184

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Research Paper

Ex vivo encapsulation of dexamethasone sodium phosphate into human autologous erythrocytes using fully automated biomedical equipment

Giovanni Mambrini[a], Marco Mandolini[a], Luigia Rossi[a,b], Francesca Pierigè[b], Giovanni Capogrossi[a], Patricia Salvati[a], Sonja Serafini[a], Luca Benatti[a], Mauro Magnani[a,b,*]

[a] EryDel SpA, via Sasso 36, 61029, Urbino (PU), Italy
[b] Department of Biomolecular Sciences, University of Urbino "Carlo Bo", via Saffi 2, 61029, Urbino (PU), Italy

ARTICLE INFO

Article history:
Received 28 July 2016
Received in revised form 14 November 2016
Accepted 5 December 2016
Available online 7 December 2016

Keywords:
Carrier erythrocytes
Red cell loader
Glucocorticoids
Ataxia telangiectasia
Bioreactors

ABSTRACT

Erythrocyte-based drug delivery systems are emerging as potential new solutions for the release of drugs into the bloodstream. The aim of the present work was to assess the performance of a fully automated process (EDS) for the ex-vivo encapsulation of the pro-drug dexamethasone sodium phosphate (DSP) into autologous erythrocytes in compliance with regulatory requirements. The loading method was based on reversible hypotonic hemolysis, which allows the opening of transient pores in the cell membrane to be crossed by DSP. The efficiency of encapsulation and the biochemical and physiological characteristics of the processed erythrocytes were investigated in blood samples from 34 healthy donors. It was found that the processed erythrocytes maintained their fundamental properties and the encapsulation process was reproducible. The EDS under study showed greater loading efficiency and reduced variability compared to previous EDS versions. Notably, these results were confirmed using blood samples from Ataxia Telangiectasia (AT) patients, 9.33 ± 1.40 and 19.41 ± 2.10 mg of DSP (mean ± SD, n = 134) by using 62.5 and 125 mg DSP loading quantities, respectively. These results support the use of the new EDS version 3.2.0 to investigate the effect of erythrocyte-delivered dexamethasone in regulatory trials in patients with AT.

1. Introduction

Dexamethasone is a highly potent and long-acting glucocorticoid that has been widely used for more than 40 years in children, adolescents and adults to treat acute and severe inflammatory, immunological diseases and allergies. However, its short half-life requires frequent administrations resulting in high Cmax (maximum concentration of a drug achieved after dosing) and high dosages, leading to toxic side effects such as osteoporosis, glaucoma, diabetes and high blood pressure (Da Silva et al., 2006; Nishimura and Ikuyama, 2000), especially when long-term therapy is required and more fragile patients are exposed. A rational approach to overcome these limitations is to develop a controlled-release formulation able to provide prolonged release of low dose dexamethasone over time. To date, a series of control release formulations of dexamethasone have been proposed, including nanocarriers, liposomes and physical method-based formulations;however, the majority of these formulations were designed for local treatments rather than sustained drug release in circulation (El Kechai et al., 2016; Goodfriend et al., 2016; Jansook et al., 2016; Ramtin et al., 2016; Robinson et al., 2016). Delivery systems able to release dexamethasone systemically are limited. Recently, a transdermal iontophoresis capable of producing constant dexamethasone plasma levels from 1 to 5 h was validated in rats (Cázares-Delgadillo et al., 2016). In the last few years the possible use of cell-based drug delivery systems has emerged as a

Abbreviations: AT, ataxia telangiectasia; DSP, dexamethasone sodium phosphate; EDS, EryDex system; EDS-EP, EDS end product; GLP, good laboratory practice; GMP, good manufacturing practice; HCT, hematocrit; HD, healthy donor; HGB, hemoglobin; HPLC, high-performance liquid chromatography; HV, healthy volunteer; LLOQ, lower limit of quantification; MCH, mean cellular hemoglobin; MCHC, mean cellular hemoglobin concentration; MCV, mean cellular volume; MDD, Medical Device Directive; PhEur, European Pharmacopoeia; PLT, platelets; RBC, red blood cell; RC, Lred cell loader; RDW, red cell dispersion width; WB, whole blood; WBC, white blood cell.

* Corresponding author at: Department of Biomolecular Sciences, University of Urbino "Carlo Bo" via Saffi 2, 61029, Urbino (PU), Italy.
E-mail addresses: giovanni.mambrini@erydel.com (G. Mambrini), marco.mandolini@erydel.com (M. Mandolini), luigia.rossi@uniurb.it (L. Rossi), francesca.pierige@uniurb.it (F. Pierigè), giovanni.capogrossi@erydel.com (G. Capogrossi), salvatipatricia@gmail.com (P. Salvati), sonja.serafini@erydel.com (S. Serafini), luca.benatti@erydel.com (L. Benatti), mauro.magnani@uniurb.it (M. Magnani).

http://dx.doi.org/10.1016/j.ijpharm.2016.12.011
0378-5173/

safe and effective alternative to the administration of pharmaceuticals at long (monthly) intervals. Among these delivery techniques, human red blood cell-based delivery systems are the most advanced (Wu et al., 2016). Magnani et al. (1998) have reported an erythrocyte-based delivery system able to carry out a slow and sustained release of dexamethasone into the bloodstream. This system, consisting of a dedicated medical device, encapsulates the pro-drug dexamethasone sodium phosphate (DSP) into the patient's erythrocytes, where it is slowly converted to dexamethasone. The erythrocytes are then re-infused into the patient, and the dexamethasone is released into his/her bloodstream for approximately 30 days or more (Rossi et al., 2001). A number of clinical investigations have shown the feasibility of this approach, which uses 50 mL of the patient's whole blood only, and its potential benefits for the patients (Bossa et al., 2013; Bossa et al., 2008; Castro et al., 2007; Lucidi et al., 2006; Annese et al., 2005; Rossi et al., 2004). Recently, this device has been greatly improved and has evolved from the research stage into the EryDex System (EDS), a novel CE marked fully automated and user friendly delivery system developed by EryDel S.p.A (Urbino, Italy). The EDS has been classified by the FDA as a combination product. In the European Union (EU), the RCL, the EryKit_01 and process solutions are CE marked Class IIb medical devices in compliance with the EC Council Directive MDD 93/42/EEC. The first fully automated version of the EDS was used in a phase II study (IEDAT-ERY01-2010) in 22 ataxia telangiectasia (AT) patients (Chessa et al., 2014). AT is a rare autosomal recessive disorder with onset in the first years of life. Neurological degeneration is the major contributor to the severe outcome of the disease, and most AT subjects die in the second decade of life, although some individuals survive longer (Boder, 1985). In the IEDAT-ERY01-2010 study, patients received monthly EDS infusions for 6 months and showed overall statistically significant improvement in neurological symptoms, the primary end point, and in various secondary measures. However, large inter-subject variability in the DSP dose was observed pointing to the need to improve the encapsulation procedure. This effort led to the development of the EDS process version 3.2.0 described herein.

The aim of the present work was to test the EDS process version 3.2.0 in a series of ex-vivo and in-vivo studies to assess the reproducibility of DSP encapsulation and the biochemical and physiological characteristics of processed erythrocytes.

2. Materials and methods

2.1. Whole blood (WB) samples

Blood was collected from 34 healthy donors in the Italian blood donor registry (registered A.V.I.S. donors) after obtaining approval from the San Salvatore Hospital Blood Bank in Pesaro, Italy (ethical commitee nr. Prot. 12/20.10.2010). Blood donations had a volume of approximately 400–450 mL, including a fixed volume (63 mL) of citrate phosphate dextrose as an anticoagulant. Each donation was microbiologically tested (HIV, HBV, HCV-free blood) and qualified as non-reactive. All donations had complete blood counts in the normal range for blood donors. No further exclusion criteria were applied. The whole blood samples were processed between 2 and 7 h from withdrawal and kept at 4 °C before use. Volume was adjusted appropriately to compensate for anticoagulant-induced changes in hematocrit (HCT) and differences between subjects, to approximate an HCT physiological value of roughly 40% in all procedures, with the exception of 5 procedures conducted with HCT increased to roughly 50% and to test the influence of this parameter on DSP encapsulation. Only 50 mL of whole blood is required to perform the EDS process. A subset of experiments was performed with leukodepleted blood samples. Leukodepletion was obtained using a leukodepletion filter BioR 01 BS PF VP (Fresenius Kabi, Bad Homburg, Germany)connected to a 50 mL sterile syringe without plunger. Roughly 100 mL of WB was filtered in order to obtain about 80 mL of leukodepleted blood.

2.2. Dexamethasone sodium phosphate (DSP) solution

DSP solution (the investigated drug product) was formulated at 25 mg/mL in water for injection (WFI). No excipients, buffers or preservatives were used. Each single use glass ampoule contained 250 mg in 10 mL of water for injection (manufactured by an aseptic filtration and filling process) and no other excipients. DSP loading quantities of 50, 62.5, 75, 125, 200 and 250 mg (obtained with 2.0; 2.5; 3.0; 5.0; 8.0; 10.0 mL of DSP solution, respectively) were always diluted with 11 mL of water for injection in a syringe just before use in the EDS: the drug preparation was injected in contact with red blood cells (RBCs) via an injection port when prompted by the EDS software.

The DSP solution is produced in accordance with CGMP and sterile procedures by the Laboratorio Farmacologico Milanese S.r.l. (LFM, Caronno Pertusella, VA, Italy).

2.3. EryDex system (EDS) process summary and process solutions

Three sterile product-specific process solutions were used in the EDS process. The solutions facilitate encapsulation in the erythrocytes of DSP. All three sterile process solutions contain only inactive ingredients and are CE marked for exclusive use with the EDS process. EryDel is the manufacturer.

Erythrocytes were swollen and their pores were "opened" in two steps using two process solutions (hypotonic solutions 1 and 2), which were progressively more hypotonic. DSP loading amount was added after the second more hypotonic solution (hypotonic solution 2). Osmotic pressure was then restored by a hypertonic solution referred to as PIGPA hypertonic solution, which "reseals" the erythrocytes (closing the pores and encapsulating the DSP). Non-encapsulated drug was removed by extensive washing with injectable saline solution. Drug-loaded erythrocytes, ready for infusion into the subjects, kept in an infusion bag, namely the EDS end product (EDS-EP). The EDS-EP volume was determined with a calibrated scale (model EG4200-2NM, Kern, Balingen, Germany) by the differential weight of the full and empty final infusion bag and its value was independent from the DSP loading quantity.

The EDS-EP is usually infused (within 30 min) at the end of the EDS process to the same donor-subject (autologous use).

2.3.1. Hypotonic solution 1

Hypotonic solution 1 is a sterile non-pyrogenic solution used in an early step in the EDS process to produce a small reduction in osmolality, which causes RBC swelling. Hypotonic solution 1 has an osmolality of 180 mOsm/kg and pH range between 4.5 and 7.0.

2.3.2. Hypotonic solution 2

Hypotonic solution 2 is a sterile non-pyrogenic solution used in the EDS process to produce a greater reduction in osmolality, causing RBCs to swell further. Hypotonic solution 2 has an osmolality of 120 mOsm/kg and pH range between 4.5 and 7.0.

2.3.3. PIGPA hypertonic resealing solution

The PIGPA Hypertonic Solution is added to the EDS process to reseal RBC membrane pores, thereby restoring the initial osmolality and ATP levels and entrapping DSP. Each single use sterile non-pyrogenic vial of PIGPA hypertonic solution was formulated at pH 7.4 ± 1.2 at a target osmolality of 3785 ± 285 mOsm/kg.