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Semi-mechanistic Pharmacokinetic/Pharmacodynamic model of three pegylated rHuEPO and ior®EPOCIM in New Zealand rabbits

G. Reynaldo-Fernández[a], J. Solozábal[b], D. Amaro[b], E.M. Fernández-Sánchez[a,1], L. Rodríguez-Vera[a], M. Bermejo[c], V. Mangas-Sanjuan[d,f,*], I.F. Troconiz[e]

[a] Department of Pharmacy, Institute of Pharmacy & Foods, University of Havana, Havana, Cuba
[b] Center of Molecular Immunology, Cuba
[c] Engineering: Pharmacy and Pharmaceutical Technology Area, Miguel Hernandez University, Spain
[d] Pharmacy and Pharmaceutical Technology Area, University of Valencia, Spain
[e] Pharmacometrics & Systems Pharmacology, Department of Pharmacy and Pharmaceutical Technology, School of Pharmacy and Nutrition, University of Navarra, Pamplona, Spain
[f] Institute of Molecular Recognition and Technological Development (IDM), Joint Centre of Polytechnic University of Valencia and University of Valencia, Spain

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ABSTRACT

Marketed formulations of erythropoietin (EPO) ior®EPOCIM, MIRCERA® and two newly developed pegylated-EPO analogues (PEG-EPO 32 and 40 kDa) formulations were intravenously administered to New Zealand rabbits. A semi-mechanistic Pharmacokinetic/Pharmacodynamic (PK/PD) model describing in a simultaneous and integrated form the time course of reticulocytes, red blood cells and hemoglobin was built to account for the time course of hematopoiesis stimulation after erythropoietin administration. Data analysis was performed based on the population approach with the software NONMEM version 7.3. Erythropoietin disposition of each of the administered formulations was best described with a two compartment model and linear elimination. Different formulations show different clearance and apparent volume of distribution of the central compartment but share estimates of inter-compartmental clearance and apparent peripheral volume of distribution. A semi-mechanistic model including cell proliferation, maturation, and homeostatic regulation provided a good description of the data regardless the type of erythropoietin formulation administered. The system-, and drug-related parameters showed consistency and differed across formulations, respectively. A single IV administration of PEG-EPO 32 and 40 kDa formulations in New Zealand rabbits achieves a median change of 27% and 22% on RET levels, and of 47% and 63% on RBC and HGB levels, respectively compared to MIRCERA®. The administration of new branched PEG-chains formulations improves PK and PD properties *of EPO*, in terms of increasing elimination half-lives and pharmacological activity on RET, RBC and HGB compared to commercially available formulations (ior®EPOCIM and MIRCERA®).

1. Introduction

Erythropoiesis is the physiological process involved in the production of mature red blood cells (RBC) from a multipotent hematopoietic stem cell in the bone marrow. In the hematopoietic cascade, a cell undergoes a series of stages, reaching the formation of reticulocytes (RET) and finally mature RBC, which are released to the blood stream. Both cell types are responsible for transporting oxygen to body tissues thanks to the presence of hemoglobin (HGB). Erythropoietin (EPO) is an endogenous glycoprotein hormone (30.4 kDa) secreted by the kidneys. It stimulates the production of RBC as a result of the binding to its receptor presented on the surface of erythroid precursor cells located in the bone marrow (Jelkmann, 1992), and maintains the steady-state levels of erythroid cells and oxygen homeostasis.

Recombinant human erythropoietin (rHuEPO) is structurally very similar to endogenous EPO (Egrie, 1990), showing equivalent *in vitro* and *in vivo* biological activity to EPO (Egrie et al., 1985) and with a plasma half-life of 6–8 h in humans (Jelkmann, 2004). It was first produced after gene cloning (Jacobs et al., 1985; Lin et al., 1985) and has been mainly used to treat anemic patients with chronic renal failure and patients receiving chemotherapy and radiation. ior®EPOCIM is a rHuEPO approved by the Center for State Control on the Quality of

* Corresponding author at: Pharmacy and Pharmaceutical Technology and Parasitology Department, School of Pharmacy, University of Valencia, Av/Vicent Andres Estelles, s/n. 46100, Burjassot, Valencia, Spain.
E-mail address: victor.mangas@uv.es (V. Mangas-Sanjuan).
[1] Deceased 21st December 2016.

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Nonstandard abbreviations

−2LL	−2 × log (likelihood)
DDE	delayed differential equations
EPO	erythropoietin
ESA	erythropoiesis-stimulating agent
GOF	goodness-of-fit plots
HGB	hemoglobin
IIV	inter-individual variability
IV	intravenous
MTT	mean transit time
ODE	ordinary differential equations
OFV	objective function value
PD	pharmacodynamics
PEG-EPO	pegylated-erythropoietin
PK/PD	Pharmacokinetic/Pharmacodynamics
PK	pharmacokinetics
RBC	red blood cells
RET	reticulocytes
rHuEPO	recombinant human erythropoietin
RSE	relative standard error
RUV	residual unexplained variability
pc-VPC	prediction-corrected visual predictive check

Drugs and Medical Devices (CECMED) of Cuba in 1998 (Pucaj et al., 2014). Due to the chronic nature of the above mentioned diseases, development of long-acting erythropoiesis-stimulating agents (ESA) would benefit patient compliance and clinical outcome. Since the studies of human EPO have indicated that increase of the plasma half-life is more relevant in affecting EPO clinical efficacy as compared to the increase of receptor binding affinity (Egrie and Browne, 2001), new EPO analogs have been developed after modifying the original molecule by (i) either pegylation (PEG-EPO) (Jolling et al., 2005; Jolling et al., 2004; Macdougall, 2005; Macdougall et al., 2006; Macdougall et al., 2008), where Pegzyrepoietin alfa (MIRCERA®) showed a serum half-life of 130 h in chronic kidney disease patients following IV administration, or (ii) including sialic acid residues by glycoengineering (darbepoietin alfa) (Egrie and Browne, 2001; Macdougall et al., 1999), where the number of sialic acid residues incorporated are indirectly related to its clearance, increasing the half-life in the range of 20–88 h (Dewamitta et al., 2013; Glaspy et al., 2005). More recently, a peptidic erythropoiesis receptor agonist (synthetic dimeric peptide linked to PEG) have shown greater half-life (22.9–129.2 h) compared to rHuEPO in preclinical studies in monkeys (Woodburn et al., 2008a; Woodburn et al., 2008b; Woodburn et al., 2009) with a reduced immune response, as it lacks of a sequence homology to EPO. Thus, the structural modification of rHuEPO has a clear impact in the development of new EPO analogs in terms of efficacy/safety balance. Two new PEG-EPO formulations (PEG-EPO 32 kDa and PEG-EPO 40 kDa) have been developed in our laboratory, incorporating branched polyethylene glycol polymer chains to the rHuEPO structure. This novel alternative in the design of PEGylated formulations of rHuEPO might increase the steric hindrance, causing a slower binding to EPO receptor and, subsequently, reducing internalization and degradation (Sinclair, 2013). As a part of the pre-clinical characterization, we aimed in the current evaluation to establish their exposure-effect relationship in New Zealand rabbits after IV administrations, including in the experimental setup the marketed formulations ior®EPOCIM and MIRCERA® and assess the impact of new branched PEG-EPO formulations versus the marketed PEG-EPO formulations on the PK/PD endpoints.

To fulfill the above mentioned objective a semi-mechanistic Pharmacokinetic/Pharmacodynamic (PK/PD) model was built to describe simultaneously the time course of RTC, RBC, and HGB based on computational frameworks accounting for the time course of hematopoiesis stimulation after rHuEPO (Agoram et al., 2006; Ait-Oudhia et al., 2010a, 2010b; Doshi et al., 2010; Gaudard et al., 2003; Krzyzanski et al., 2008; Krzyzanski et al., 2006; Perez-Ruixo et al., 2005; Ramakrishnan et al., 2003; Woo et al., 2008; Woo et al., 2006; Wu et al., 2015; Yan et al., 2012).

2. Material and methods

2.1. EPO formulations

Commercial formulation of rHuEPO (ior®EPOCIM, nominal concentration 10,000 I.U./mL) was obtained from Center of Molecular Immunology, (CIMAB, S.A., Cuba). Commercial MIRCERA® was furnished by Roche in precharged syringes of 100 μg/0.3 mL. Experimental formulations of branched PEG-chains were developed by Center of Molecular Immunology and the Center of Genetic Engineering and Biotechnology, La Havana (Cuba): 32 kDa-PEG-rHuEPO (PEG-EPO 32 kDa) and 40 kDa-PEG-rHuEPO (PEG-EPO 40 kDa). Each formulation in homogenous solution was packaged in sterile ampoules at a concentration of 100 μg/mL.

2.2. Study design

The experimental protocol (see below) was approved by Institutional Animal Use and Care Committee and all procedures were conducted in compliance with Caring for Animals for Better Science Directive of the European Union (2010) (Commission, 2010), the ARRIVE guidelines for animal experimentation (McGrath et al., 2010) and in accordance with the Guide for the Care and Use of Laboratory Animals.

2.2.1. Animals

Male New Zealand rabbits weighing 1.5–2.3 kg were obtained from the National Center of Laboratory Animal Production (CENPALAB), La Havana (Cuba). At the start of dosing, the age of the animals was 24–28 weeks. The health and quality of all animals was certified. Rabbits were placed in individual cages under controlled conditions. Temperature was 19.5 °C, humidity was 71% and the light/dark cycle was 12 h/12 h. Food and water were given *ad libitum*.

2.2.2. Drug administration and sample collection

Forty animals were randomly allocated in eight groups of five rabbits each. Four groups (I–IV) were used for the pharmacokinetic (PK) study providing levels of EPO in serum, and the rest (V–VIII) for the pharmacodynamic (PD) study, in which the three response endpoints were measured in peripheral blood.

A single 10 μg/kg intravenous bolus dose of ior®EPOCIM, MIRCERA®, PEG-EPO 32 kDa or PEG-EPO 40 kDa was injected into the left marginal vein of the ear to each animal in groups I & V, II & VI, III & VII, and IV & VIII, respectively after an overnight fasting. All formulations analyzed in this article contained the same amount of rHuEPO (10 μg/kg). Food and water were resumed *ad libitum* 2 h after administration.

Blood samples (1 mL) were drawn at the several times points after drug administration (Table 1). All experiments were carried out at the same time of the day to avoid differences between groups and animals due to circadian variations.

For the PK study, blood (1 mL) was placed in Eppendorf® tubes and let stand for 1 h and for the PD study, blood (1 mL) was placed in Eppendorf® tubes containing 10 μL EDTA (10%) as anticoagulant.