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SHORT COMMUNICATION

Stability of erythropoietin repackaging in polypropylene syringes for clinical use

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KEYWORDS

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Abstract *Introduction:* Epoetin alfa (Eprex®) is a subcutaneous, injectable formulation of short half-life recombinant human erythropoietin (rHuEPO). To current knowledge there are no published studies regarding the stability of rHuEPO once repackaging occurs (r-EPO) for clinical trial purposes. *Materials and methods:* We assessed EPO concentration in Eprex® and r-EPO syringes at 0, 60, 90, and 120 days after repackaging in polypropylene syringes. R-EPO was administered to 56 patients taking part in a clinical trial in Friedreich Ataxia. Serum EPO levels were measured at baseline and 48 h after r-EPO administration. *Results:* No differences were found between r-EPO and Eprex® syringes, but both globally decreased in total EPO content during storage at 4 °C. Patients receiving r-EPO had similar levels in EPO content as expected from previous trials in Friedreich Ataxia and from pharmacokinetics studies in healthy volunteers. *Discussion:* We demonstrate that repackaging of EPO does not alter its concentration if compared to the original product (Eprex®). This is true both for repackaging procedures and for the stability in polypropylene tubes. The expiration date of r-EPO can be extended from 1 to 4 months after repackaging, in accordance with pharmacopeia rules.

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1. Introduction

Erythropoietin (EPO) is a 34 KDa glycoprotein, synthesized by the kidney that stimulates erythropoiesis acting on erythrocyte precursors in the bone marrow (Jelkmann, 1992). Recombinant Human Erythropoietin (rHuEPO) is used for treatment in anemic patients with chronic renal failure in whom the endogenous production of EPO is impaired. It is used in patients with chemotherapy-induced anemia (Galli et al., 2015), reduces the need for blood transfusions in patients that undergo surgery, and can also be used for patients at risk for perioperative transfusions with anticipated significant blood loss (McGirr et al., 2014).

Epoetin alfa (Eprex®) is a subcutaneous, injectable formulation and short half-life recombinant human erythropoietin. It is produced by recombinant DNA technology in Chinese Hamster Ovarian cells.

There are no published studies regarding the stability of EPO, once repackaging occurs. Even the stability of the protein in polypropylene tubes has never been tested.

In our study, Eprex® was aseptically repackaged in 1 mL polypropylene syringes and stored at 4 °C for up to 120 days, and quantitative tests were performed. Repackaged EPO (r-EPO) was administered to patients taking part to a clinical trial, and serum levels were measured in order to confirm the clinical utility of repackaging procedures.

2. Materials and methods

2.1. Preparation of syringes

Commercially available Eprex® 40,000 IU/1 mL was purchased from Janssen-Cilag (Milano, Italy), a subsidiary of Johnson & Johnson (New Brunswick, NJ, USA). Each Eprex® batch used for tests had at least 18 months of shelf life before expiration date. Eprex® was delivered to "Farmacia Ettore Florio snc", an ISO 9001:2008 pharmacy, at controlled temperature.

We performed drug-repackaging procedures following the Italian pharmacopeia rules. For each preparation, Eprex® syringes were manually emptied in a sterile glass container under a laminar flow hood. Eprex® was aspirated in 1 mL syringes (BD Micro-fine, 29G × 12.7 mm, Becton Dickinson) for a total volume of 1 mL (40,000 IU of EPO) or 0.75 mL (30,000 IU of EPO), based on clinician's request. Environment air was checked during repackaging procedure using a portable particle counter (Solair 3100, Lighthouse, Fremont, CA, USA).

The whole process was performed slowly in order to avoid the introduction of air bubbles during the transfer phase. The syringes were then sealed in vacuum sterile bags in order to maintain sterility. Ten percent of each production, with a minimum of four syringes, was kept aside for microbiological sterility testing, as prescribed by the pharmacopeia sterility assay 2.6.1. This was performed at the Experimental Medicine Department of the Second University of Naples, Italy.

2.2. Stability assay

For stability evaluation, eight 1 mL r-EPO syringes, from the same batch preparation, were stored at 4 °C for future assays for each time-point. In parallel, the same amount of syringes of Eprex® was retained at the moment of repackaging for comparison. Both Eprex® and r-EPO syringes were stored at 4 °C up to 120 days.

The stability of r-EPO and EPREX® was assessed by enzyme-linked immunosorbent assay (ELISA) to detect human EPO (Quantikine IVD Erythropoietin ELISA, DEP00; R&D systems, Minneapolis USA) was used to assess the EPO content, following manufacturer's instructions.

The content of the syringes was expelled into 2-mL Eppendorf tubes. Two serial dilutions of 1:100 and a final dilution of 1:40 were performed in order to obtain a final dilution of 1:400000. This matched the assay range of 2, 5–200 mIU/mL, with an expected final concentration of 100 mIU/mL. Results from ELISA measurements were multiplied by the dilution factor for final results analysis.

2.3. Clinical trial design

The trial was approved form the local Ethics Committee (256/11), registered at www.clinicaltrials.gov (NCT01493973), and EUDRACT (2011-006156-37). The trial was performed in accordance with the Declaration of Helsinki, European guidelines CPMP/ICH/135/95, and Italian law D. M.15/07/1997. All patients gave written informed consent before any activity was linked to the clinical trial.

r-EPO was administered to patients taking part in a randomized, placebo-controlled trial, to assess the effect of EPO on exercise capacity in Friedreich Ataxia (FRDA) patients (Saccà et al., 2016). The trial is part of an extensive effort in identifying drugs able to ameliorate the biochemical deficits of FRDA (Boesch et al., 2007, 2008, 2014; Saccà et al., 2011). Briefly, 56 patients were randomized to receive r-EPO (1200 IU/kg body weight subcutaneously) or equivalent dose of placebo in a 1:1 ratio. Serum samples were obtained immediately before the administration of r-EPO and 48 h later. Samples were frozen at −80 °C until analysis. Serum concentrations of EPO were measured using the same ELISA kit as specified. Further details on the trial can be obtained at www.clinicaltrials.gov (NCT01493973).

2.4. Statistical analysis

For stability assays, data were analyzed using a General Linear Model for repeated measures (GLM-RM). Within-subjects factor was time, and four time-points were set. These included baseline, 60, 90, and 120 days. Production method (Eprex® or r-EPO) was set as the between-subjects factor. Simple repeated measure contrast was used with baseline as the reference category. We calculated estimated marginal means for the production and time interaction. Bonferroni correction was applied for all repeated analysis. P values less than 0.05 were considered significant. For serum EPO concentration, we used a GLM-RM to analyze the effect of time and r-EPO administration in the same way as the stability assay. In addition, the effect of repackaging-to-administration time was set as a covariate in order to test its effect on serum EPO levels. Statistical analysis was performed using SPSS version 22.0.0.1 running on MAC OSX 10.10.4.