Comparison between Erythropoietin Alfa Biosimilars and Reference Product in Anemic Patients of Chronic Kidney Disease

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Abstract

Patent expiration for Erythropoietin alfa in Europe in 2004 led to development of biosimilar Erythropoietin alfa, recombinant human Erythropoietin (rhuEPO) is used for treatment of anemia due to renal failure. Our study aimed to evaluate clinical efficacy of two biosimilars from different manufacturers relative to innovator product Eprex (Cilag. AG. Switzerland). Clinical efficacy is assessed as a function of therapeutic equivalence of a biosimilar and innovator product through a parallel, randomized single blind study in 35 patients with anemia result from chronic kidney disease and had not received EPO previously. The primary efficacy endpoint was the serum EPO level which measured by enzyme immunoassay (ELISA) during 120 hours after administration of a single 4000IU dose. The secondary endpoints were the hematological parameters (the hemoglobin, hematocrit, total serum iron levels and reticulocyte) before and after treatment. Results from this study confirmed presence of significant difference between reference Erythropoietin (EPO) alfa group (Eprex) and biosimilar Erythropoietin alfa Groups (A) in terms of serum EPO profile but biosimilar B has similar EPO profile to Eprex and no significant difference with Eprex in clinical efficacy and hematological parameters.


Introduction

BIOLOGICAL products derived from recombinant DNA (rDNA) technology are used in treatment of several diseases, recombinant human erythropoietin, for example. This treatment is relatively expensive in many developing countries so it has not been available to all patients who need it. Therefore a less expensive forms of these drugs are developed. In 1990 several pharmaceutical companies started in developing what was termed 'biosimilars' (in European Union) or 'follow-on products' (in United States and Japan) and called “subse-quent-entry biologic” (in Canada). Patents for many biological have either expired or are about to expire. Due to this expiration, the biosimilars are developed onto market [1,2].

Biosimilars are defined as biological products similar, but not identical, to reference products that are submitted for separate marketing approval following patent expiration of the reference products. Unlike generic pharmaceuticals, there is a strong correlation between the manufacturing processes of biopharmaceuticals and the characteristics of the final product. Small changes in the manufacturing process of biological products can affect both efficacy and safety. Comparability testing used to ensure that biosimilars have similar quality, efficacy and safety profiles as the innovator product. Several assays are available to evaluate the physicochemical and biological properties of biosimilars. These assays are important to evaluate the similarities and differences of biosimilar against innovator product [3-5].

Erythropoietin (EPO) is an endogenous glycoprotein of 165 amino acids with molecular mass 30-35kDa that is synthesized by the interstitial cells in the kidney (peritubular capillary and tubular epithelial tubule) and locally by some other tissues, including the brain, in response to low oxygen in blood (hypoxia) [6]. The kidney secreted EPO to stimulate the growth and maturation of erythrocyte precursors (erythropoiesis) in red bone marrow and spleen by promoting their survival through protecting from apoptosis and also it affects neuronal protection during hypoxic conditions [7,8].

After human erythropoietin gene was cloned, the synthetic forms of erythropoietin were produced with DNA technology. Recombinant EPO formu-
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Recombinant human EPO has been used since the 80’s in the treatment of anemia associated to renal disease. Reports in hemodialysis patients indicate an effective erythropoiesis increment, ceasing or decrease of transfusion frequency and quality of life improvement [9].

Immunogenicity is the major safety concern with development of biosimilars. Many factors have increased the immunogenicity of biopharmaceutical products including product related factors (aggregation, formulation, and glycosylation), patient’s related factors, route of administration, and storage and duration of treatment. The replacement of endogenous protein with biological may cause risk of stimulation the immune system to develop anti-product antibodies (Abs) that may cross-react with endogenous protein [10,11].

The objective of this study was to assess the efficacy of biosimilar alfa rHuEPO versus innovator alfa rHuEPO after administration subcutaneously for the treatment of anemia inpatients with CKD.

Subjects and Methods

35 patients 22-65 years old, both sexes with anemia (hemoglobin level <10gm/dl) result from chronic kidney disease and were not on hemodialysis. The study was performed according to the guideline for the use of human subject’s materials and Institution Review Board (IRB) ethics committee approval and written; informed consents were obtained from all the persons involved. All patients were selected from Kasr EL-Aini Hospital during a time from Jan 2013 to Oct. 2014 and classified into three groups according to type of used erythropoietin in treatment.

Eprex-group included: 15 anemic patients (8 male and 7 female) were treated with Eprex.

Group-A included: 10 anemic patients (5 male and 5 female) were treated with biosimilar-A.

Group-B included: 10 anemic patients (2 male and 8 female) were treated with biosimilar-B.

The exclusion criteria were: Previous EPO treatment, pregnancy, cancer, hormonal treatment (except thyroid hormone and insulin), bone marrow aplasia and signs of active bleeding.

EPO formulations:

Erythropoietin innovator product (Eprex, Cilag. AG, Switzerland) was presented in prefilled syringe containing 4000IU of human recombinant (rHuEPO), polysorbate 80, 2.192 mg NaCl, 0.580mg NaH2PO4, 2H2O, 1.115mg NaH2PO4·H2O, 2.50mg glycine, and water for injection to complete 0.4ml.

Biosimilar products are formulations of recombinant human erythropoietin (biosimilar-A and biosimilar-B) were obtained from the Egyptian market and purchased from different manufacturers. The commercial name of biosimilar products was not disclosed for confidential purposes. They were in vials containing 4000 IU of human recombinant (rHuEPO) (produced in Chinese Hamster Ovarian (CHO) cells at CIGB, Havana), 2.5mg serum human albumin, 0.2mg polysorbate 20, 2.9mg NaCl, 4.6mg NaH2PO4·2H2O, 9.9g NaH2PO4 and water for injection to complete 1mL.

Study design:

Subjects were distributed according to a computer generated random table. They received subcutaneously a single dose of 4000IU of EPO formulations (Eprex or biosimilar A or B) in a parallel design. The study was double blinded. As the presentation of the products differed, blinding was kept by loading biosimilar-A vials in syringes coded with the patient’s numbers and the nurses that administered the products didn’t participate in the rest of the study.

Sampling:

5ml venous blood samples were withdrawn before and after EPO administrations then divided as follows:

A- 2ml blood on EDTA for Hb, HCT and reticulocyte count before injection and after treatment.

B- 3ml of blood were left for 10 minutes at water bath to clot and then centrifuged at 760 x g for 10 minutes. Serum divided into aliquots and stored at (–20ºC) in defrosting freezer for the following determination:

1- Serum total iron before injection and after treatment.

2- Serum EPO before injection (T0) and after administration of EPO blood samples were collected from each patient at the following interval hours: T6, T12, T24, T48, T72, T96, and T120 for follow-up level of EPO.

Laboratory evaluations:

Hb and HCT were measured by using Medonic cell analyzer CA620/530, Sweden. Reticulocytes were determined by using brilliant cresyl blue stain. Serum iron was determined by iron ferrozine colorimetric end point method using a commercial kit supplied from (LINEAR CHEMICALS S.L.
Barcelona, Spain) according to the method of Carter [12]. Serum EPO was determined by using enzyme immunoassay (ELISA) technique using a commercial Kit supplied from (Quantikine® IVD®, R & D Systems Inc, Minneapolis, USA) according to the method of Cotes [13].

Statistical analyses:

Statistical Package for Social Science (SPSS) version 16.0 was used for analysis of data. Data was summarized as mean ± Standard Deviation (SD). t-test was applied for analysis of quantitative data and Chi square test for analysis of qualitative data. Independent t-test was used for analysis of 2 quantitative data and used for detection of significant difference. The Levene’s test used for Equality of variances. To compare the laboratory tests in different groups the analysis of variance (ANNOVA) was used.

Results

The demographic and baseline characteristics of the patients and causes of renal failure are shown in (Table 1). The groups were balanced regarding sex (8 male and 7 female VS 5 male and 5 female, 2 male and 8 female, innovator, biosimilar A and biosimilar B, respectively). Twenty three patients had specified the cause of renal failure, hypertension. The hypothesis of homogeneity between the groups was accepted.

| Table 1: Descriptive statistical of demographic and characteristics of the patients. |
|------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Variables              | Eprex group     | Group A         | Group B         | Total           | p               |
| Number                 | 15              | 10              | 10              | 35              |                 |
| Age (years)            | 43.8±10.8 (25-65) | 50.4±12.9 (30-65) | 47.2±15.6 (22-65) | 46.6±12.8 (22-65) | 0.460*          |
| Weight (Kg)            | 62.6±6.4 (50-75) | 69.6±4.2 (63-75) | 62.4±7.7 (45-67) | 64.5±6.4 (45-75) |                 |
| Sex:                   |                 |                 |                 |                 | 0.021*          |
| Male                   | 8 (53.3%)       | 5 (50%)         | 2 (20%)         | 15 (42.9%)      |                 |
| Female                 | 7 (46.6%)       | 5 (50%)         | 8 (80%)         | 20 (57.1%)      |                 |
| Causes of CKD:         |                 |                 |                 |                 | 0.398^          |
| Hypertension           | 10 (66.6%)      | 7 (70%)         | 6 (60%)         | 23 (65.7%)      |                 |
| Diabetes               | 1 (6.6%)        | 1 (10%)         | 1 (10%)         | 3 (8.5%)        |                 |
| Kidney stones          | 1 (6.6%)        | 1 (10%)         | 1 (10%)         | 3 (11.4%)       |                 |
| Urinary infection      | 2 (13.3%)       | 1 (10%)         | 1 (10%)         | 4 (11.4%)       |                 |
| Glomerulonephrities    | 1 (6.6%)        | 0 (0%)          | 1 (10%)         | 2 (5.7%)        |                 |

Data are summarized as mean ± standard deviation (range) or number of patients (%).

*: ANNOVA test.

^: Chi-square test.

Overall mean ± SD hemoglobin at baseline (before treatment) were 8.3 ± 1.2g/dL VS 8.9±1.2g/dL and 8.0±1.5g/dL and mean hematocrit 25.6±4.3% VS 27.0±3.5% and 23.5±4.4 for innovator, biosimilar A and biosimilar B, respectively. There were no significant differences between groups at baseline since the hemoglobin (p=0.164) and hematocrit point of view (p=0.082).

After treatment mean ± SD hemoglobin and hematocrit were 9.7±1.4g/dL VS 9.3±1.5g/dL and 8.4±1.4g/dL and mean hematocrit 30.8±5.2% VS 28.5±4.4% and 26.1±3.6 for innovator, biosimilar A and biosimilar B, respectively. There were no significant differences between groups (p=0.133 and p=0.167 for hemoglobin and hematocrit, respectively).

After treatments mean of reticulocyte count increments from 1.76±0.33 to 4.06±0.87, 1.48±0.79 to 3.3±0.49 and 1.94±1.91 for innovator, biosimilar A and biosimilar B, respectively. There were highly significant differences between groups (p=0.004) for reticulocyte before treatment and (p=0.000 for reticulocyte after treatment).

Total serum iron level before treatment for innovator group ranged from 37-78 µg/dl with a mean± SD (53.8±12.2) µg/dl while in biosimilar A group ranged from 39-50 µg/dl with a mean ± SD (43.4±14.1) µg/dl and in biosimilar B group ranged from 21-54 µg/dl with a mean ± SD (46.4±10.7) µg/dl. There was no significant difference (p=0.253) between groups before receiving the treatment.
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After treatment Total serum iron level for innovator group ranged from 54-87 µg/dl with a mean ± SD (67.7±12.2) µg/dl while in biosimilar A group ranged from 50-62 µg/dl with a mean ± SD (57.6±4.5) µg/dl and in biosimilar B group ranged from 45-62 µg/dl with a mean ± SD (55.8±5.1) µg/dl. There was slightly significant difference (p=0.044) between all studied groups as shown in (Table 2).

Results of serum EPO profiles:

Serum EPO concentration in Eprex and different biosimilars groups were determined as follow before treatment (T0) and after 6, 12, 24, 48, 72, 96 and 120 hours interval as shown in Fig. (1). Serum EPO concentration in the innovator (Eprex) increased slowly from baseline (T0=13.2±3.66) mIU/ml till reaching to the maximum concentration after 24 hour (Tmax) where (T24=55.96±11.41) mIU/ml after that the concentration of EPO had returned gradually to initial value after 120 hours (T120=16.13±5.07) mIU/ml while in the biosimilar A, serum EPO concentration were sharply increased from baseline (T0=11.57±1.56) mIU/ml till reaching to the maximum concentration after 12 hour (Tmax) where (T12=77.2±1.62) mIU/ml after that the concentration of EPO were sharply declined at (T24=37±1.61) mIU/ml then returned gradually to initial value after 120 hours (T120=11.94±1.58) mIU/ml and in the biosimilar B serum EPO concentration profile has similar graduation as Eprex group where increased slowly from initial concentration (T0=11.38±3.97) mIU/ml till reaching to the maximum concentration after 24 hour (Tmax), where (T24=47.28±14.89) mIU/ml after that the concentration of EPO had returned gradually to initial value after 120 hours (T120=11.41±3.96) mIU/ml.

Table (2): Comparison of laboratory data and maximum response of serum EPO between reference group and different biosimilars groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Eprex group</td>
<td>Group A</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>6.2-10.1</td>
<td>6.8-10.2</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>8.3±1.2</td>
<td>8.9±1.2</td>
</tr>
<tr>
<td>HCT (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>19.2-32.6</td>
<td>20.9-31.1</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>25.6±4.3</td>
<td>27.±3.5</td>
</tr>
<tr>
<td>Reticulocyte (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>1.2-2.2</td>
<td>0.6-2.8</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>1.76±0.3</td>
<td>1.4±0.79</td>
</tr>
<tr>
<td>T.T.S. Iron (µg/dl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>37-78</td>
<td>39-50</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>53.8±12.2</td>
<td>43.4±4.1</td>
</tr>
</tbody>
</table>

Data were summarized as mean ± standard Deviation (SD).
p: ANOVA between groups at 95% confidence interval.

Table (3): Comparison of maximum response of serum erythropoietin between reference group and different biosimilars groups after single administration of 4000IU in 35 patients.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Eprex-group (15)</th>
<th>Group-A (10)</th>
<th>Group-B (10)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt;-T&lt;sub&gt;0&lt;/sub&gt;</td>
<td>42.7±8.2</td>
<td>65.6±0.15</td>
<td>35.9±11.8</td>
<td>0.000</td>
</tr>
<tr>
<td>T&lt;sub&gt;120&lt;/sub&gt;-T&lt;sub&gt;0&lt;/sub&gt;</td>
<td>2.93±1.82 (0.000)</td>
<td>0.37±0.14 (0.000)</td>
<td>0.33±1.04 (0.000)</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Data were summarized as mean ± Standard Deviation (SD).
Paired tests vs. baseline were done and comparison between groups at each time by ANOVA tests (with Bonferrony).
T<sub>max</sub>: Time to reach maximum response.
Discussion

Renal anemia can be effectively managed by the administration of rHuEPO, which is able to increase hemoglobin levels and has been associated with significant improvements in the cardiovascular status of patients with CKD [14].

Erythropoiesis stimulating agents are effective in the management of anemia, but they substantially add to the overall treatment costs. Economizing treatment is, therefore, of significant importance for both payers and clinicians. The introduction of biosimilars to the market is a controversial matter in nowadays. The two recombinant rHuEPO (reference and biosimilar) could be similar from the physicochemical point of view, but it is possible found some differences in the strains or manufacturing process that could have clinical significances in terms of safety and efficacy [15-17].

The results from the present study show that subcutaneously administered rHuEPO (innovator and biosimilars) were effective for the control and management of anemia in CKD patients. During the period of the results monitoring we found that the hematological parameters (Hb, reticulocytes and total serum iron) were increasing significantly with variable range, this was because erythropoietin administration activates the erythropoiesis process in bone marrow producing reticulocytes which differentiated into Red Blood Cells (RBCs). The majority of the body’s iron stored in RBCs is available for recycling at the end of the lifespan of the RBCs. When erythropoiesis is suppressed, in particular in chronic inflammatory the uptake of recycled iron into the developing RBCs will be limited. In these circumstances, iron released at the end of the lifespan of RBCs will be deposited in the storage iron pool. The process of RBCs production (erythropoiesis) requires about 30-40mg iron each day to produce some 6g of new Hb. In renal disease, the failure of the erythropoietin mechanism can be readily and directly remedied; rHuEPO therapy can replace the missing erythropoietin, but this will be negated if iron supply to the erythroid marrow falls short of demand [18]. Iron comes from macrophages to the bone marrow to be incorporated into the protoporphyrin IX forming the heme group of hemoglobin in erythrocytes increasing hemoglobin. Our findings were similar to those reported by other authors [19].

Regarding serum profiles in the present work, the baseline of serum erythropoietin concentration was not zero and when we compared between all studied groups we found there were no statistically significant ($p=0.324$) in the baseline erythropoietin concentrations (T0) between groups (innovator and biosimilars-A & B).

These results were in agreement with those reported by [19]. After subcutaneous administration of alfa erythropoietinproducts, the time point ($T_{max}$) was within the 12-24 hours reported for EPO, although a great variability is reported for this this variable in renal patients [20]. The mean of $T_{max}$ in the studied Group A which receiving the same dose 4000 IU showed significant difference and the curves weren't overlapped while Group B did not differ significantly. There is highly significant difference ($p=0.001$) found between groups at time point ($T_{max}-T_0$) which indicate the difference between time required to reach maximum concentration and EPO concentration before treatment and also highly significant difference ($p=0.000$) at point (T120-T0).

The differences in serum EPO profiles between the innovator product and biosimilars may be due to the difference in biochemical composition of biosimilars than innovator and the changes in manufacturing process.

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The variability in the efficacy between biosimilars and innovator product may be due to the changes in manufacturing process of reference product than biosimilars which affect both efficacy and safety of product these were agreement with those reported by [22]. The data of our study demonstrate that rHuEPO biosimilar B is an acceptable alternative to the innovator product. The sharply increased or decreased in EPO concentration profile of biosimilar products may be affecting on erythropoiesis mechanism in the bone marrow.
Conclusion:

During the comparability study between innovator and biosimilar products the biosimilar A has differences in serum EPO profiles but biosimilar B is the more similar and is nearly to innovator. The biosimilar product B is safe in clinical practice and is effective and stable as innovator product. Biosimilars offer a welcome opportunity to reduce treatment costs of renal anemia.

- There are similarities in the biological indicators between innovator and biosimilar product, the reticulocyte counts, serum iron, and hemoglobin increase during the treatment.

References


أدى انتهاء براءة اختراع الإريثروبيوتين ألفا في أوروبا في عام 2004 لتطوير وصناعة بدائل حيوية لمستشفى الإريثروبيوتين الذي يستخدم لعلاج الأنيميا وقفر الدم الناتج عن الفشل الكلوي. الهدف الرئيسي من دراستنا هو تقييم الفعالية السريرية لمستشفى الإريثروبيوتين المختلفين ومقارنتهما المستحضر الأصلي وهو (الإريثروبفت من إنتاج شركة سيلاج-أج-سويسرا) ويتم تقييم الفعالية السريرية بوصفها وظيفة من الكفاءة العلاجية للمستشفى الأصلي والبدائل الحيوية من خلال دراسة أجريت على 20 مريض معاني من الأنيميا وقفر الدم الناتج من مرض الكلى المزمن ولم يثقبوا علاج الإريثروبيوتين سابقا. وقد تم قياس مستوى الإريثروبيوتين في مصل الدم باستخدام الطريقة الإندوهرمومتري مانحة قبل علاج هؤلاء المرضى وبعد علاجهم مستحضر الإريثروبيوتين (الأصلي والبدائل الحيوية) بجرعة 500 وحدة نوكليتة خلال 120 ساعة من بداية العلاج، وأيضا قياس الهيموجلوبين، الهيماتوكريت، مجموع مستويات الحديد في مصل الدم وعدد الخلايا الشبكية قبل وبعد العلاج، وقد أثبتت نتائج هذه الدراسة وجود فرق كبير بين مجموعة المرضى الذي يتم علاجهم بالمستشفى الأصلي (الإريثروبفت) والمجموعة التي يتم علاجها بالبدائل الحيوية من حيث قراءات مستوى الإريثروبيوتين في مصل الدم بينما البدائل الحيوية يشبه المستحضر الأصلي في قراءات مستوى الإريثروبيوتين B في مصل الدم والعوامل الدموية مثل (الهيموجلوبين، الهيماتوكريت، مستوى الحديد في مصل الدم وعدد الخلايا الشبكية).