Pharmacokinetics and Pharmacodynamics of Recombinant Human EPO-Fc Fusion Protein In Vivo

Xunlong Shi1,3, Jianjun Yang2, Haiyan Zhu1, Li Ye1, Meiqing Feng1, Jiyang Li1, Hai Huang1, Qun Tao2, Dan Ye2, Lee-Hwei K. Sun3, Bill N. C. Sun3, Cecily R. Y. Sun3, Guizhen Han4, Yuanyuan Liu4, Minghui Yao4, Pei Zhou1, Dianwen Ju1*

1 Department of Biosynthesis, School of Pharmacy, Fudan University, Shanghai, China, 2 Shanghai Meiyer Biotech Institute, Shanghai, China, 3 PharMab (Shanghai), Inc., Shanghai, China, 4 Department of Pharmacology, School of Medicine, Fudan University, Shanghai, China

Abstract

In this study, the in vivo pharmacokinetics and pharmacodynamics of a novel recombinant human erythropoietin (rhEPO) Fc fusion protein, rhEPO-Fc, were studied in both rodents and rhesus monkeys. Animal models of anemia induced by irradiation, cyclophosphamide and partial renal ablation were used to evaluate therapeutic effects of rhEPO-Fc. We have demonstrated that serum half-life of rhEPO-Fc was 29.5 to 38.9 h at doses of 8, 25, 80 μg/kg in rhesus monkeys and 35.5 to 43.5 h at doses of 16, 50, 160 μg/kg in rats. In anemia animal models, rhEPO-Fc dose-dependently (7.5–30.0 μg/kg in mice, 5.4–21.4 μg/kg in rats and 5.0–10.0 μg/kg in rhesus monkeys) increased reticulocyte level, followed by an increase of RBC count, hemoglobin and hematocrit levels. At reduced intervention frequency of weekly treatments, rhEPO-Fc showed similar hematopoietic effects as compared with rhEPO given three times a week. These results indicated that rhEPO-Fc could potentially be used in treatment of anemia and warrants future clinical trials.

Introduction

Erythropoietin (EPO) is a glycoprotein that stimulates the production of erythrocytes [1,2]. It promotes proliferation, differentiation and maturation of erythroid progenitor cells, and inhibits their apoptosis [3]. In clinical practice, recombinant human EPO (rhEPO) has been used in treatment for anemia associated with chronic renal failure [4], cancer chemotherapy [5], HIV infection [6], and a number of other pathological conditions [7,8].

Since initial clinical usage of rhEPO-α in the 1980s, clinicians quickly recognized the need of frequent administration as one of major drawbacks of the drug. This imposes a burden on both patients and health care providers, as in vivo half-lives of rhEPO-α and rhEPO-β administered intravenously or subcutaneously in humans are only about 8.5 and 17 hours respectively [9,10]. Thus, there has been a longstanding need to develop recombinant EPO analogs with longer in vivo half-lives.

Attempts have been made to genetically or chemically modify the structure of native EPO protein to either slow down its in vivo metabolism or improve its therapeutic properties [11,12]. To extend the half-life, EPO has been chemically conjugated with other moieties to increase its molecular weight. For instance, polyethylene glycol conjugated (PEGylated) EPO has a much higher molecular weight and is protected from being cleared from circulation and therefore has a longer plasma half-life. However, PEGylation may alter the protein structure resulting in unanticipated changes of function and specificity of EPO moiety [13,14]. Other strategies have also been reported to increase the molecular weight of EPO, such as linking EPO molecule to a carrier protein (e.g., human albumin), or forming a homo-dimer of two EPO molecules using linking peptides (3- to 17-amino acids) or chemical cross-linkers [15–18].

To develop a “controlled release” of EPO, we have previously developed a novel recombinant human EPO fused with an Fc domain from a modified human IgG2 without CDC and ADCC function [19,20]. In the present report, its pharmacokinetics and pharmacodynamics were studied. The erythropoietic effects were investigated in various rodent as well as nonhuman primate anemia models.

Materials and Methods

Materials

Recombinant human EPO (rhEPO, 30.4 kD, 98% purity) was purchased from 3SBio Inc. China. The novel EPO fusion protein, rhEPO-Fc (118 kD, ~60,000 IU/mg) was supplied by Shanghai...
Pharmacokinetics and Pharmacodynamics of rhEPO-Fc

Cyclophosphamide induced anemia in rhesus monkeys

Cyclophosphamide is an alkylating nitrogen mustard which is clinically used to treat numerous types of cancer and certain autoimmune disorders. Bone marrow suppression, including anemia, is one of the major adverse effects [23,24]. To induce anemia, rhesus monkeys were given intravenously twice a day at a dose of 50 mg/kg cyclophosphamide on two consecutive days. Two days after the second injection, monkeys (n = 4 per group) were subcutaneously injected with rhEPO (2.5 μg/kg, 3 times per week), rhEPO-Fc (5.0, 10.0 μg/kg/weekly) or PBS for seven weeks. Blood samples were collected at 0–49 days during treatment period and additional 4 times weekly after treatment ended. RBC, hemoglobin, hematocrit, reticulocyte, blood platelet and leukocyte were determined as described above.

Statistical Analysis

Pharmacokinetics and pharmacodynamics data were analyzed using one-way ANOVA, and a p value ≤0.05 was considered statistically significant.

Results

Pharmacokinetics

To evaluate pharmacokinetic behavior of the rhEPO-Fc fusion protein in vivo, both single and repeated rhEPO-Fc injections were conducted in both rats and rhesus monkeys. Circulating levels of rhEPO-Fc following single injections were measured by ELISA. The mean serum concentration-time curves were shown in Fig. 1A and Fig. 1B for rhesus monkeys and rats, respectively. The pharmacokinetics parameters were summarized in Table 1. In rhesus monkeys (Fig. 1A and Table 1, the Cmax and AUC0–168 h positively correlated with dosage. With increasing doses of rhEPO-Fc, Cmax and AUC0–168 h correspondingly increased, but Tmax, CL and half-life did not show obvious dose-dependent effects. The serum half-life of rhEPO-Fc was 29.5 to 38.9 h.

In rats (Fig. 1B and Table 1), the similar dose-dependent correlations of Cmax and AUC0–168 h were also observed. The half-life of rhEPO-Fc was 35.5 to 43.5 h.

In rhesus monkeys receiving repeated injections, there was no rhEPO-Fc detected before the 2nd, 3rd and 4th injection (Fig. 1C). After the 4th injection, the serum concentration-time curve of rhEPO-Fc did not change significantly, similar to that of the 1st injection. This indicated that the repeated injections did not change the pharmacokinetic behavior of rhEPO-Fc.

rhEPO-Fc alleviates anemia induced by irradiation

Next, we tested whether rhEPO-Fc have hematopoietic effects in animal anemia models. We first evaluated the effects of rhEPO-Fc on anemic mice induced by total body irradiation (TBI).

As shown in Fig. 2A, TBI induced severe anemia in C57BL/6 mice. The mean RBC count of PBS control mice decreased to the lowest level (4.9×1012/L, 54% of baseline) on day 7 post irradiation and recovered to 7.2×1012/L (80% of baseline) four weeks later. In mice treated with rhEPO-Fc (7.5, 15.0, 30.0 μg/kg, weekly), rhEPO-Fc treatments attenuated reduction of RBC in a dose-dependent manner. The RBC counts on day 7 were 6.2×1012/L, 5.7×1012/L, 6.1×1012/L in mice treated with rhEPO-Fc at doses of 7.5, 15 and 30.0 μg/kg, decreased to the lowest level on day 9 and then recovered. On day 13 and 27, RBC counts of mice treated with 15 and 30.0 μg/kg rhEPO-Fc were significantly higher than those in PBS control group.

Hemoglobin and hematocrit (Fig. 2B and 2C) were also significantly lowered after irradiation similar to RBC (Fig. 2A).
Treatment with rhEPO-Fc also dose-dependently alleviated the reduction of hemoglobin and hematocrit ($p<0.05$, compared with PBS control). At the weekly dosing of 30.0 µg/kg, rhEPO-Fc exerted similar effects on RBC, hemoglobin and hematocrit, compared with rhEPO (7.5 µg/kg, 3 times a week).

After irradiation, reticulocyte counts of PBS control mice reduced to the lowest level on day 3, then rapidly reached the highest level on day 13 and then decreased gradually. The rhEPO-Fc treatment showed dose-dependent stimulating effects on reticulocytes, which significantly elevated at doses of 15.0, 25.0, and 50.0 µg/kg.

### Table 1. Pharmacokinetic parameters of rhEPO-Fc following single subcutaneous injection in rhesus monkeys and rats.

| Species   | Dose (µg/kg) | N  | Cmax (IU/L) | Tmax (h) | AUC0-168 (IU h/ml) | CL (ml/h/kg) | Half-life (h) |
|-----------|--------------|----|-------------|----------|--------------------|--------------|---------------|
| Rhesus monkey | 8            | 6  | 2193.1±902.3 | 7.5±3.0  | 56.3±19.0          | 0.4±0.2      | 38.9±16.3     |
|           | 25           | 6  | 12186.1±2695.5 | 8.0±2.2   | 335.0±81.1         | 0.2±0.1      | 29.5±4.2      |
|           | 80           | 6  | 26772.4±13066.6 | 6.7±1.6   | 837.0±295.2        | 0.3±0.1      | 33.1±14.1     |
| Rat       | 16           | 12 | 899.6±141.9  | 21.7±5.7  | 39.0±5.9           | 0.5±0.1      | 38.6±22.9     |
|           | 50           | 12 | 1522.5±209.1 | 24.0±0.0  | 692.2±6.7          | 0.5±0.3      | 35.3±8.9      |
|           | 160          | 12 | 22191.4±4422.3 | 23.0±17.4 | 1238.0±116.5       | 0.3±0.3      | 43.5±32.0     |

*aMaximal drug concentration.

*bTime of maximal drug concentration.

*cArea under the curve (0–168 h).

*dClearance.

doi:10.1371/journal.pone.0072673.t001

Figure 1. Pharmacokinetics of rhEPO-Fc with single and repeated injections. Mean serum rhEPO-Fc concentration versus time after single subcutaneous injection in rhesus monkeys (Fig. 1A), rats (Fig. 1B) and repeated subcutaneous injections in rhesus monkeys (Fig. 1C) were plotted. Data were mean±SD for six rhesus monkeys or twelve rats per group. doi:10.1371/journal.pone.0072673.g001
30.0 μg/kg on day 9 and day 13 as compared with those in rhEPO-treated mice \((p \leq 0.05)\) (Fig. 2D).

These data demonstrated that rhEPO-Fc dose-dependently attenuated reduction of RBC, hemoglobin and hematocrit, rapidly elevated levels of reticulocyte in anemia induced by TBI in mice.

rhEPO-Fc alleviates anemia induced by partial renal ablation

The erythropoietic effect of rhEPO-Fc was also evaluated in anemia induced by partial renal ablation in rats. The mean RBC count decreased to the lowest level \((5.5 \times 10^{12}/L, 76\% \text{ of baseline})\) on day 19 after partial nephrectomy, and remained at this level throughout the entire experiment (Fig. 3A). However, when treated with rhEPO-Fc \((5.4, 10.7, 21.4 \text{ μg/kg weekly})\), on day 19, RBC counts significantly increased \((7.9 \times 10^{12}/L, 7.8 \times 10^{12}/L, 8.5 \times 10^{12}/L)\) in animals treated with all three doses of rhEPO-Fc. In anemic rats treated with rhEPO-Fc at doses of 10.7 and 21.4 μg/kg, the RBC counts were restored to the sham operation levels, significantly higher than those of PBS-treated anemic rats \((p \leq 0.05)\) in the course of treatment. At the end of the treatment, RBC counts of rats treated with rhEPO-Fc \((21.4 \text{ μg/kg weekly})\) reached similar levels, as compared with those in mice treated with rhEPO \((5.4 \text{ μg/kg, 3 times a week})\).

Similar dose-dependent stimulating effects on hemoglobin and hematocrit were also observed (Fig. 3B and 3C). During rhEPO-Fc therapy with weekly doses of 10.7 and 21.4 μg/kg, hemoglobin and hematocrit levels were restored to the sham operation level. The effects of rhEPO-Fc \((21.4 \text{ μg/kg})\) were similar to those of rhEPO treatment \((5.4 \text{ μg/kg, 3 times a week})\).

After the partial renal ablation surgery, reticulocytes of PBS-treated rats rapidly increased and changed in fluctuation. At the dose of 21.4 μg/kg, rhEPO-Fc exerted the strongest effect on reticulocyte \((243\% \text{ of baseline at day 12, } p \leq 0.05, \text{ vs PBS}), similar to those of rhEPO therapy \((5.4 \text{ μg/kg, 3 times a week})\) (Fig. 3D).

The partial renal ablation also resulted in high levels of BUN in the rats and neither rhEPO nor rhEPO-Fc treatment affected BUN (Fig. 3E). Although administration of rhEPO-Fc did not improve impaired kidney function, it effectively corrected anemia induced by partial renal ablation.

rhEPO-Fc attenuates anemia induced by cyclophosphamide in rhesus monkeys

The in vivo erythropoietic effect of rhEPO-Fc was further investigated in rhesus monkeys after anemia induction with cyclophosphamide. As shown in Fig. 4A, the mean RBC count of PBS-treated monkeys decreased to the lowest level \((3.6 \times 10^{12}/L, 61\% \text{ of the baseline})\) on day 7 after cyclophosphamide administration and then recovered slowly to the baseline level. rhEPO-Fc dose-dependently alleviated reduction of RBC counts. On day 7, rhEPO-Fc treatment \((5.0, 10.0 \text{ μg/kg weekly})\) increased RBC counts to \(5.4 \times 10^{12}/L, 5.6 \times 10^{12}/L\) and then restored RBC counts to the baseline level. The effect of rhEPO-Fc \((10.0 \text{ μg/kg weekly})\) was similar to that of rhEPO \((2.5 \text{ μg/kg, 3 times a week})\). Similar effects were also observed on hemoglobin and hematocrit upon rhEPO-Fc treatment (Fig. 4B and 4C).

The reticulocyte count reduced to zero on day 3 and increased rapidly to the highest \((3.4\%)\) on day 9 and then dropped to baseline level on day 21 in PBS-treated anemic rhesus monkeys. Upon rhEPO-Fc treatment, there was some increase of reticulocytes \((4.27\% \text{ for } 5.0 \text{ μg/kg on day 7, 4.27\% for } 10.0 \text{ μg/kg on day 12})\), but no significant difference was observed among the monkeys treated with rhEPO, rhEPO-Fc or PBS (Fig. 4D).
Cyclophosphamide also reduced blood platelet and leukocyte counts, which gradually recovered to the baseline levels in PBS-treated rhesus monkeys. Interestingly, rhEPO-Fc (5.0, 10.0 μg/kg weekly) showed some stimulating effects on platelet and leukocyte recovery. However, no significant difference was observed in blood platelet and leukocyte counts in animals treated with rhEPO, rhEPO-Fc or PBS as shown in Fig. 4E and 4F.

Discussion

The novel fusion protein, rhEPO-Fc, reported in the present study has its unique advantages. First, no mutation was introduced into the EPO molecule itself. Although the mutation on a disulfide bond of EPO structure has been reported to improve its pharmacokinetics and hematopoietic effects [25], some mutations on EPO could change its erythropoietic activity. For instance, EPO mutant S100E induces a significantly lower hematocrit increase than natural EPO while provides similar protection from progressive photoreceptor degeneration [26,27]. To maintain its erythropoietic property and to avoid unpredicted risk of immunogenicity in clinical application, no mutation was introduced into EPO in our rhEPO-Fc fusion protein.

Second, the Fc region of modified IgG2 with diminished CDC and ADCC function was linked to the EPO molecule. Fusion proteins consisting of the Fc fragment of human IgG have been shown to have significantly longer in vivo half-lives while retaining their biological and therapeutic properties [20,28]. Several fusion proteins comprising an Fc fragment have been successfully developed for clinical application and approved by FDA for treatment of rheumatoid arthritis and chronic plaque psoriasis [29,30]. In this study, the Fc region of IgG2 was selected to link to the EPO molecule for the following reasons. The IgG2 molecule does not bind to FcγR, which can activate antibody dependent

Figure 3. rhEPO-Fc attenuated anemia induced by partial renal ablation in rats. Blood samples were collected at the indicated time points (0–4 week, and additional two weeks after the treatment ended). RBC (Fig. 3A), hemoglobin (Fig. 3B), hematocrit (Fig. 3C), reticulocyte (Fig. 3D) and BUN (Fig. 3E) were shown. Data were presented as Mean±SD. doi:10.1371/journal.pone.0072673.g003
cellular cytotoxicity (ADCC). In addition, IgG2 is particularly resistant to proteases, which might reduce the clearance of the fusion protein mediated by autoantibody [31]. Furthermore, a site mutation (Pro331Ser mutation) had been made near the carboxyl-terminus of the CH2 domain of human IgG that appears to be important for both FcγR and C1q binding. So the Fc variant should have less complement-activating activity than the natural Fc fragment while remain as a non-binder to FcγR [19]. Thus the novel rhEPO-Fc molecule was expected to have a extended half-life for in vivo applications.

In this study, the half-life of rhEPO-Fc were 29.5 to 38.9 h at doses of 8, 25, 80 μg/kg in rhesus monkeys and 35.5 to 43.5 h at doses of 16, 50, 160 μg/kg in rats. Compared with the reported pharmacokinetics data, the half-life of the EPO-Fc fusion protein was obviously extended [14]. Administration of rhEPO-Fc once a week exerted similar hematopoietic effects to that of rhEPO given 3 times a week in anemia animal models induced by irradiation, partial renal ablation and cyclophosphamide, which verified the possibly extended half-life of rhEPO-Fc. When repeated subcutaneous injections were performed in rhesus monkeys, no EPO protein was detected before the 2nd, 3rd and 4th injections. This indicated that no rhEPO-Fc accumulation in long-term administration. It also supports safe long-term use of rhEPO-Fc as has been reported previously [32].

**Figure 4.** rhEPO-Fc attenuated anemia induced by cyclophosphamide in rhesus monkeys. Blood samples were collected at 0–49 days during treatments and additional 2 times weekly after the treatment ended. RBC (Fig. 4A), hemoglobin (Fig. 4B), hematocrit (Fig. 4C), reticulocyte (Fig. 4D), leukocyte (Fig. 4E) and blood platelet (Fig. 4F) were determined. Data were presented as Mean±SD.

doi:10.1371/journal.pone.0072673.g004
Reticulocyte is the newly-produced red blood cell and the count is used to assess bone marrow response to an anemic state. Reticulocyte production increases in response to loss of red blood cells. It increases within 2–3 days of a major acute hemorrhage and reaches its peak in 6–10 days [33,34]. In this study, similar responses of reticulocyte in different anemia models were also observed, and the rhEPO-Fc administration clearly enhanced production of reticulocytes, supporting rapid and efficient recovery of anemia induced by irradiation, cyclophosphamide and partial renal ablation.

In summary, the low administration frequency of rhEPO-Fc (once a week) was shown to have similar erythropoietic effects in a variety of rodent and primate anemia models when compared with rhEPO administrated three times a week. The pharmacokinetics and pharmacodynamics profiles of rhEPO-Fc strongly indicate that rhEPO-Fc could be potentially used as clinical treatment for anemia associated with chronic renal failure or chemotherapy, and future clinical trials are warranted.

Acknowledgments

The authors thank Dr. Qiang Li of PharmMab for critical reading of the manuscript and thank National Chengdu Center for Safety Evaluation of Drugs for technical assistance. The authors also thank the lyediting for the English editing and thank two reviewers for their valuable suggestions.

Author Contributions

Conceived and performed the experiments: QT DY BNCS MY DJ. Performed the experiments: XS JY HZ JL HH LY MF GH YL. Analyzed the data: LHKS CRYL MY PZ. Wrote the paper: XS PZ DJ.

References

1. MacDougall IC (2012) New anemia therapies: translating novel strategies from bench to bedside. Am J Kidney Dis 59: 444–451.
2. Fukuda S, Yamaguchi T, Hashimoto S, Nakai S, Ieki K, et al. (2012) Erythropoiesis-stimulating agent responsiveness and mortality in hemodialysis patients: results from a cohort study from the dialysis registry in Japan. Am J Kidney Dis 59: 108–116.
3. Silva M, Grillo D, Benito A, Richard G, Nunez G, et al. (1996) Erythropoietin can promote erythroid progenitor survival by repressing apoptosis through Bel-2 and Bel-2. Blood 88: 1576–1582.
4. Eschbach JW, Egrie JC, Downing MR, Browne JK, Adamson JW (1987) Correction of the anemia of end-stage renal disease with recombinant human erythropoietin. Results of a combined phase I and II clinical trial. N Engl J Med 316: 73–78.
5. Abels RI (1992) Use of recombinant human erythropoietin in the treatment of anemia in patients who have cancer. Semin Oncol 19 (3 Suppl 8): 29–35.
6. Henry DH, Beall GN, Benson CA, Carey J, Cone LA, et al. (1992) Recombinant human erythropoietin in the treatment of anemia associated with human immunodeficiency virus (HIV) infection and zidovudine therapy. Overview of four clinical trials. Ann Intern Med 117: 739–749.
7. Sharples EJ, Thiemenmann C, Yaqoob MM (2006) Novel applications of recombinant erythropoietin. Curr Opin Pharmacol 6: 184–189.
8. Joyoux- Faure M (2007) Cellular protection by erythropoietin: new therapeutic implications? J Pharmacol Exp Ther 325: 759–762.
9. MacDougall IC (2001) An overview of the efficacy and safety of novel erythropoietin stimulating protein (NESP). Nephrol Dial Transplant (Suppl 3): 4–21.
10. Bunn MS (2002) Darbepoetin-alfa: a novel erythropoiesis-stimulating protein. Ann Pharmacother 36: 1183–1192.
11. Elliott S, Lorenzini T, Asher S, Aski K, Branowski D, et al. (2003) Enhancement of therapeutic protein in vivo activities through glycoengineering. Nat Biotechnol 21: 1413–1419.
12. Elliott S, Egrie J, Browne J, Lorenzini T, Bush E, et al. (2004) Control of rHuEPO biological activity: the role of carbohydrate. Exp Hematol 32: 1146–1153.
13. Jolling K, Ruixo JJ, Hemeryck A, Piotrovskij V, Greeway T (1996) Erythropoietin enhances the in vivo potency of recombinant human non-glycosylated erythropoietin: a comparison with glycosylated erythropoietin. J Pharm Pharmacol 48: 3027–3038.
14. Wang YJ, Hao SJ, Liu YD, Hu T, Zhang GF, et al. (2010) Pegylation markedly enhances the in vivo potency of recombinant human non-glycosylated erythropoietin: a comparison with glycosylated erythropoietin. J Control Release 145: 306–313.
15. Dalle B, Henri A, Rouyer-Fessard P, Bettan M, Scherman D, et al. (2001) Dimeric erythropoietin fusion protein with enhanced erythropoietic activity in vitro and in vivo. Blood 97: 3754–3762.
16. Kochendoerfer GG, Chen SY, Mao F, Cressman S, Travaglia S, et al. (2003) Design and chemical synthesis of a homogenous polymer-modified erythropoietin protein. Science 299: 1044–1047.
17. Syrkowski AJ, Lunn ED, Davis KL, Feldman L, Sickman S (1998) Human erythropoietin dimers with markedly enhanced in vivo activity. Proc Natl Acad Sci U S A 95: 1184–1188.
18. Syrkowski AJ, Lunn ED, Risinger MA, Davis KL (1999) An erythropoietin fusion protein comprised of identical repeating domains exhibits enhanced biological properties. J Biol Chem 274: 24773–24779.
19. Sun Lee-Hwei K, Sun Bill NC, and Sun Cecily RY, Fc fusion proteins of human erythropoietin with increased biological activities. Unite states patent. No 609029B2.
20. Yang J, Zhang QD, Zhou J, Gong Z, Tao Q, et al. (2007) High Expression, Purification, Quality Control of rhEPO-Fc Fusion Protein,China Biotechnol 27: 6–9.
21. Stußen G, Pottgen C, Kruhnmann K, Schmidt K, Stuschke M, et al. (2003) Erythropoietin restores the anemia-induced reduction in radiosensitivity of experimental human tumors in nude mice. Int J Radiat Oncol Biol Phys 55: 1350–1362.
22. Amann K, Tornig J, Buzello M, Kuhmann A, Gross ML, et al. (2002) Effect of antioxidant therapy with dl-alpha-tocopherol on cardiovascular structure in experimental renal failure. Kidney Int 62: 877–884.
23. Shanafiel TD, Lin T, Geyer SM, Zent CS, Leung N, et al. (2007) Pentostatin, cyclophosphamide, and rituximab regimen in older patients with chronic lymphocytic leukemia. Cancer 109: 2291–2296.
24. Nehrus T, Klatte T, de Riese W, Haynes A, Filleur S. (2010) Clinical outcome of patients with docetaxel-resistant hormone-refractory prostate cancer treated with second-line cyclophosphamide-based metronomic chemotherapy. Med Oncol 27: 363–367.
25. Way JC, Lauder S, Brunhorst B, Kong SM, Qi A, et al. (2005) Improvement of Fe-erythropoietin structure and pharmacokinetics by modification at a disulide bond. Protein Eng Des Sel 18: 111–118.
26. Colella P, Iodice C, Di Vincio U, Annunziata I, Surace EM, et al. (2011) Non-erythropoietic erythropoietin derivatives protect from light-induced and genetic photoreceptor degeneration. Hum Mol Genet 20: 2251–2262.
27. Sullivan TA, Greent EE, Templeton JP, Rex TN (2012) Dose-dependent treatment of optic nerve crush by exogenous systemic mutant erythropoietin. Exp Eye Res 96: 36–41.
28. Xu W, Jones M, Liu B, Zhu X, Johnson CB, et al. (2013) Efficacy and Mechanism-of-Action of a Novel Superagonist Interleukin-15: Interleukin-15 Receptor a/alpha/Fc Fusion Complex in Syngeneic Murine Models of Multiple Myeloma. Cancer Res 73: 3075–3086.
29. Goldenberg MM (1999) Etaicopept, a novel drug for the treatment of patients with severe, acute rheumatoid arthritis. Clin Ther 21: 75–87.
30. Wong VK, Leebaldo M (2003) The use of alfacetap in the treatment of priapism. Skin Ther Lett 8: 1–2, 7.
31. Brezski RJ, Oberholzer A, Strake B, Jordan RE (2011) The in vitro resistance of IgG2 to proteolytic attack concurs with a comparable paucity of autoantibodies against peptide analogs of the IgG2 hinge. MAbs 3: 558–567.
32. Zhang X, Liu B, Wang L, Cen XB (2007) Long-term toxic effect and immunogenicity of recombinant EPO-Fc in rhesus monkeys. Chinese J New Drugs 16: 196–199.
33. Adamson JW, Longo DL (2001) Anemia and polycythemia. in: Braunwald E, Faucci AS, Kasper DL, Hauser SL, Longo DL, Jameson JL, ed. New York, pp. 349–354.
34. Holfbrand AV, Moss PAH, Pettit JE (2001) Essential Haematology. Fourth Ed., Blackwell Science, Oxford.