

# Pharmacokinetics and Pharmacodynamics of an EPO-Mimetic Fusion Protein in a Model of Chronic Renal Insufficiency Anemia

Connie M. Kliwinski\*, Dorie Makropoulos, Debora Kwok, Amy L. Volk, Kim Foster, Thomas Nesspor, Chichi Huang and Peter J. Bugelski

Biotechnology Toxicology, RC-1 Centocor R&D 145 King of Prussia Road, Radnor, PA 19087, USA

**Abstract:** Renal insufficiency is commonly associated with an erythropoietin (EPO) dependent anemia. CNTO 530 is an EPO-mimetic antibody fusion protein with EPO-receptor agonist activity. The pharmacokinetics and pharmacodynamic activity of CNTO 530 were evaluated in a rat model of chronic renal insufficiency (CRI) induced anemia. Following 5/6 nephrectomy and 13 -17 weeks of stabilization, rats received a single subcutaneous (SC) dose of saline or CNTO 530 (0.03, 0.1, or 0.3 mg/kg). CNTO 530 caused dose-dependent reticulocytosis and long-lived increases in red blood cells, hemoglobin, and hematocrit. After receiving a dose of 0.1 mg/kg, red cell indices increased and remained within normal range for 44 days. Pharmacokinetic analysis showed that clearance of CNTO 530 increased 1.4 fold and terminal  $t_{1/2}$  and AUC decreased 1.6 fold in CRI rats compared to controls. CNTO 530 is active in a CRI rat model of anemia and widely spaced treatments with CNTO 530 may be sufficient to combat anemia.

**Keywords:** Partial nephrectomy, recombinant proteins, erythropoietin, rats.

## INTRODUCTION

Erythropoiesis, the formation of red blood cells from multipotent stem cells in the bone marrow, is an exquisitely regulated process in which the glycoprotein hormone erythropoietin (EPO) plays a central role [1]. The kidney is an important source of EPO [2], and renal insufficiency in addition to causing azotemia, is also commonly associated with EPO dependent anemia. Anemia associated with renal insufficiency, as well as that caused by chemotherapy or AIDS treatments, is commonly treated with recombinant human erythropoietin (rHuEPO) [3, 4]. However, due to its short half-life, rHuEPO must be administered relatively frequently, typically 3 times a week either intravenously or subcutaneously [5]. This dosing regime is sub-optimal with regards to patient compliance. A molecule which has EPO-R agonist activity, longer lasting effects, and requires less frequent dosing, is therefore desirable.

CNTO 530 is a 58 kD glycoprotein MIMETIBODY™ construct EPO-mimetic antibody fusion protein that has been shown to produce long-lived stimulation of erythropoiesis in mice and rats [6, 7]. CNTO 530 has no sequence homology with EPO; instead, it includes 2 EMP1 sequences, a 20-amino acid peptide that binds EPO-R and expresses EPO-like bioactivity on a human IgG4 Fc framework [8, 9].

The purpose of these experiments was to study the pharmacokinetics and pharmacodynamic activity of CNTO 530 in a rat model of chronic renal insufficiency (CRI) induced anemia.

## MATERIALS AND METHODS

Male Sprague-Dawley CD rats obtained from Charles River Laboratories (Kingston, NY, USA) weighed approximately 300 grams at study start and were pair housed in filter-top shoebox cages. Food and water were available *ad libitum*. Rats were maintained on a 12-hour light/dark cycle in the pathogen-free vivarium at Centocor R&D, Inc., Radnor, Pa, USA. The Institutional Animal Care and Use Committee approved all procedures. To establish CRI, a total of 70 rats underwent 5/6 nephrectomy. CRI was induced by a modification of the method originally described by Platt *et al.* which involves two surgeries with a recovery period in between [10]. Rather than wait 10-14 days following surgery for resection of the poles of 1 kidney before performing a total nephrectomy of the intact kidney, both procedures were accomplished in a single surgery under isoflurane inhalation anesthesia. Buprenex®, (Reckitt Benckiser Healthcare, UK) 0.03-0.06 mg/kg SC was administered for analgesia at the conclusion of all surgical procedures and repeated if the animals exhibited any signs of post-operative pain. At 2, 9, and 13-17 weeks following 5/6 nephrectomy, rats were anesthetized with CO<sub>2</sub>/O<sub>2</sub> and blood drawn from the retro-orbital sinus for analysis of hematology parameters, blood urea nitrogen (BUN), and creatinine. Blood was collected into micro-centrifuge tubes containing EDTA and immediately mixed. Whole blood was used for analysis of hematology parameters with an ADVIA® 120 hematology analyzer (Siemens Medical Solutions Diagnostics, Tarrytown NY, USA) and plasma was used for analysis of BUN and creatinine with a VetACE™ Clinical Chemistry System (Alfa Wasserman, Inc., West Caldwell, NJ, USA).

Rats that developed anemia between weeks 13-17 post nephrectomy were selected for the CNTO 530 treatment phase of the study. To ensure a similar distribution of anemia

\*Address Correspondence to this author at the Biotechnology Toxicology, RC-1 Centocor R&D 145 King of Prussia Road, Radnor, PA 19087, USA; Tel: 610-240-8492; Fax: 610-651-6152; E-mail: Ckliwins@its.jnj.com

across the groups, rats were assigned to treatment based on hemoglobin (Hgb) values. On the day of treatment, rats (4-5 rats/group), received a single SC dose of CNTO 530 at 0.03, 0.1, or 0.3 mg/kg. Blood samples were collected from anesthetized rats *via* the retro-orbital sinus into EDTA coated microcentrifuge tubes on days 5, 12, 23, 29, 36 and 44 after treatment for analysis of hematology parameters. Two control groups were included in the study; an untreated non-surgical group and an untreated 5/6 nephrectomy group which underwent the same bleeding regime and euthanasia procedure as the treatment groups to obtain control values for comparative purposes.

To evaluate the pharmacokinetics of CNTO 530, three normal, non-nephrectomized rats, and six nephrectomized rats received a single intravenous dose 0.3 mg/kg of CNTO 530. Retro-orbital blood samples were collected pre-dose and at various time points up to 21 days post-dose. These samples were centrifuged at 2500 rpm for 15 minutes to separate the serum which was stored at  $-80^{\circ}\text{C}$  until analyzed as described previously [11].

Data are expressed as mean  $\pm$  standard deviation (SD). Statistical differences were determined by *t*-test or an analysis of variance (ANOVA) with Bonferroni's correction for multiple comparisons. Where the data were not normally distributed, or if there was an unequal variance among the groups, a Mann Whitney Rank Sum or Kruskal-Wallis ANOVA on Ranks with Dunn's correction for multiple comparisons was used.  $P < 0.05$  was accepted as significant.

## RESULTS

Starting on post treatment day 12, a number of renal insufficient rats across the surgical control and CNTO 530 treatment groups became emaciated and lethargic. An additional rat was found dead on post treatment day 60. Thus, only data to post treatment day 44 are shown; expressed as the group mean  $\pm$  SD and plotted as a function of days after dosing. As expected, 5/6 nephrectomy caused a statistically significant increase in BUN and creatinine that was essentially stable from day 1 through 44 (Figs. 1a,b) (Mann Whitney Ranks Sum test,  $P < 0.001$ ). Compared to non-surgical control rats, reticulocyte counts in the surgical control group were increased throughout the course of the study (Mann

Whitney Ranks Sum test,  $P < 0.001$ ). In contrast, compared to the non-surgical control group, red blood cell counts (RBC) (*t*-test,  $P < 0.001$ ) and Hgb (Mann Whitney Ranks Sum test,  $P < 0.001$ ) in the surgical control group decreased and remained so throughout the course of the study (Figs. 2b,c).

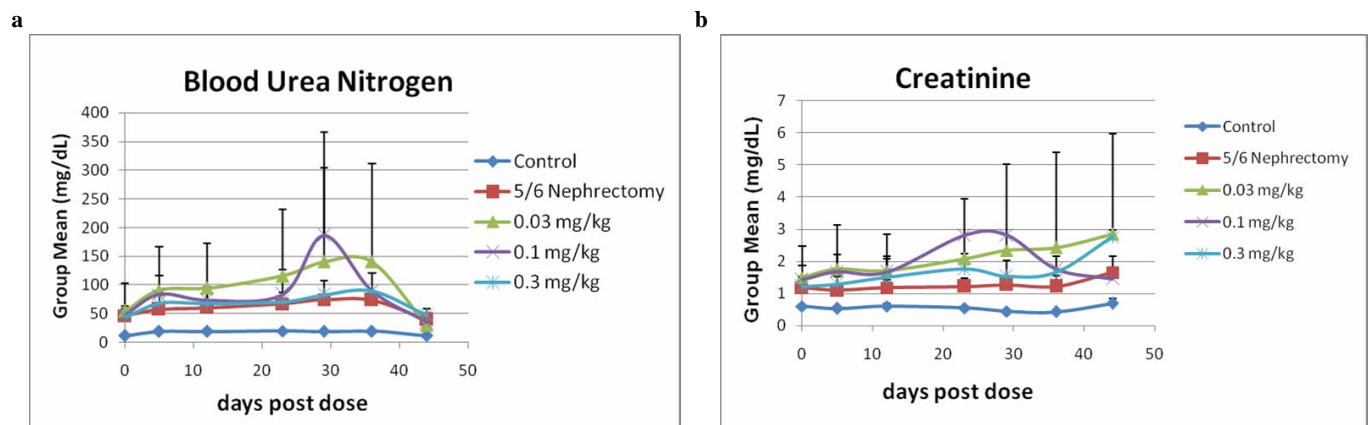
A single SC dose of CNTO 530 had no statistically significant effect on creatinine or BUN levels (Figs. 1a,b). CNTO 530 induced a dose-responsive increase (magnitude as well as duration) in reticulocyte counts (Fig. 2a). All doses of CNTO 530 were effective in increasing reticulocyte numbers but the increase was statistically significant only at the two higher doses (ANOVA on Ranks with Dunn's Correction,  $P < 0.05$ ). Reticulocyte counts peaked between day 1 and 5 and then fell to or below baseline values for all CNTO 530 treated groups by day 23. CNTO 530 also caused a dose-responsive increase in RBC and Hgb levels (Figs. 2b,c) (ANOVA with Bonferroni's correction  $P < 0.05$ ). Doses of 0.03 and 0.1 mg/kg were sufficient to increase Hgb to the non-surgical control mean for up to 44 days and a dose of 0.3 mg/kg resulted in a mean increase in Hgb of 3.6 g/dL.

The mean serum concentration of CNTO 530 as a function of time in normal and CRI rats is shown in Fig. (3). Pharmacokinetic parameters (mean  $\pm$  SD) are summarized in Table 1. Compared to normal rats, CRI rats showed increased clearance of CNTO 530 as reflected in decreased AUCs and a statistically significant decrease in terminal half-life (*t*-test,  $P < 0.05$ ).

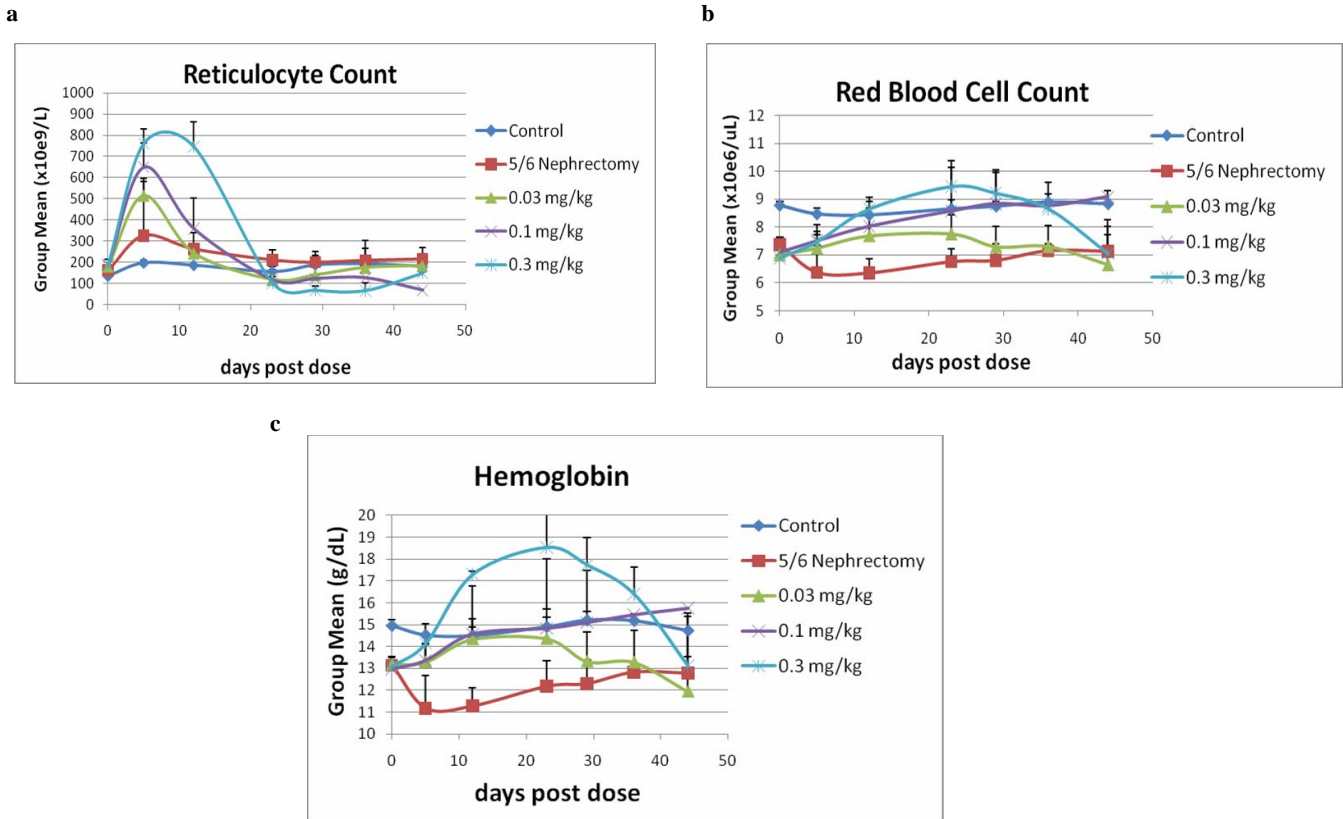
## DISCUSSION

Anemia is a well documented co-morbidity associated with CRI and deficiency of EPO is considered the primary cause of anemia associated with CRI [12]. In this study, it has been shown that an EPO-mimetic fusion protein, CNTO 530, was effective in treating anemia of CRI without exacerbating the condition, and importantly, the clearance of CNTO 530 was increased in CRI animals.

Partial nephrectomy (also referred to as 5/6 nephrectomy or the remnant kidney model) is a well studied model of anemia of CRI [13, 14] and a number of agents effective in human anemia of CRI have been shown to treat anemia associated with CRI in rats [15-17]. However, to sustain the increase in Hgb achieved following a single dose of CNTO



**Fig. (1a).** BUN values in control, CRI, and CRI rats treated with a single SC dose of CNTO 530. **(1b).** Creatinine values in control, CRI and CRI rats treated with a single SC dose of CNTO 530.



**Fig. (2a).** Reticulocyte counts in control, CRI and CRI rats treated with a single SC dose of CNTO 530. **(2b).** Red blood cell counts in control, CRI and CRI rats treated with a single SC dose of CNTO 530. **(2c).** Hemoglobin values in control, CRI and CRI rats treated with a single SC dose of CNTO 530.

530 observed in this study, the other agents required more frequent dosing; for example, treatment three times per week for rHuEPO or weekly for rHuEPO conjugated to polyethylene glycol [18].

In this study, as evidenced by increases in BUN and creatinine, CRI progressed rapidly between ~15 and 20 weeks after nephrectomy in all groups. Although there was a trend toward increased severity of CRI in the 0.3 mg/kg CNTO 530 treated group, there were no statistically significant drug effects. It has been reported previously that rHuEPO can exacerbate CRI in rats [18, 19]. Hypertension, mediated by endothelin-1 and the renin-angiotensin system

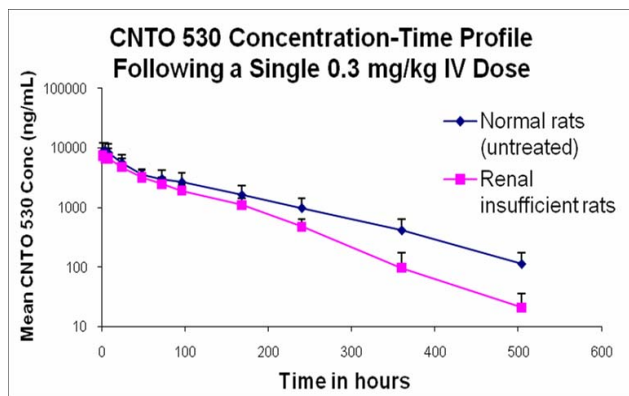
is believed to play a role in exacerbation of CRI in rats receiving rHuEPO [20, 21].

CRI is also associated with changes in the clearance of many drugs. For drugs that are cleared *via* renal excretion, CRI usually results in decreased clearance. In the case of EPO-R agonists, however, CRI has been somewhat paradoxically associated with increased clearance [22, 23]. Similarly, in this study it was found that clearance of CNTO 530 was increased in rats with CRI. Taken together with the previous work, our findings suggest that renal clearance is not an important route of elimination for CNTO 530 and that CNTO 530 behaves similar to other EPO-R agonists.

Current treatments for anemia of CRI are less than ideal, require frequent dosing, and sometimes result in poor compliance. A more efficacious treatment with less stringent dosing requirements could provide a better standard of care. By testing the drug in a rat model of CRI, we demonstrated its effectiveness in a physiologic state similar to that found in patients with renal impairment. Importantly, we were able to show that renal function, which would be compromised in target patients, is not problematic with regard to drug retention. The prolonged activity demonstrated by CNTO 530 could translate into less frequent dosing making it a more convenient treatment with enhanced compliance as compared to the current standard of care.

**CONCLUSION**

The rat 5/6 nephrectomy model successfully produces anemia associated with CRI, and as such is an acceptable



**Fig. (3).** Serum concentration of CNTO 530 in control and CRI rats following a single SC dose of 0.3 mg/kg CNTO 530.

**Table 1. Pharmacokinetic Parameters Following a Single (0.3 mg/kg) IV dose of CNTO 530**

Parameter	Units	Normal Rats	Renal Insufficient Rats	% Change
C <sub>max</sub>	µg/mL	9.1 ± 2.9	7.8 ± 4.4	-15
AUC <sub>t</sub>	µg·day/mL	33.4 ± 11.9	23.6 ± 7.6	-29
AUC	µg·day/mL	34.0 ± 12.3	23.7 ± 7.6	-30
CL	mL/day/kg	9.9 ± 4.4	13.5 ± 3.3	37
V <sub>ss</sub>	mL/kg	47.4 ± 14.3	50.5 ± 16.0	7
t <sub>1/2</sub>	Days	3.6 ± 0.3	2.2 ± 0.5*	-39

\* Statistically different from normal rats (t-test, P= 0.005).

model used to mimic the human condition. Following a single administration of CNTO 530, an EPO mimetic antibody fusion protein, we were able to effectively treat CRI associated anemia in rats as evidenced by a sustained increase in hemoglobin. These results warrant further investigation of CNTO 530 as a clinically relevant agent for the treatment of erythropoietin dependent anemia.

## DISCLOSURE

All authors are employees of Centocor R&D Inc. and hold an equity stake in Johnson & Johnson that markets epoetin-α.

## ACKNOWLEDGEMENTS

This work was supported by Centocor R&D, Inc.

## REFERENCES

- Jelkmann W. Erythropoietin after a century of research: younger than ever. *Eur J Haem* 2007; 78: 183-205.
- Jacobson LO, Goldwasser LF, Fried W, Plzak L. Role of the kidney in Erythropoiesis. *Nature* 1957; 179: 633-4.
- Nemoto T, Yokota N, Keane WF, Rabb H. Recombinant erythropoietin rapidly treats anemia in ischemic acute renal failure. *Kidney Int* 2001; 59: 246-51.
- Nurko S. Anemia in chronic kidney disease: Causes, diagnosis, treatment. *Clev Clin J Med* 2006; 73, 3: 280-97.
- Pinevich AJ. Erythropoietin therapy in patients with chronic renal failure. *West J Med* 1992; 157: 154-7.
- Bugelski PJ, Capocasale RJ, Makropoulos D, *et al.* CNTO 530: molecular pharmacology in human UT-7EPO cells and pharmacokinetics and pharmacodynamics in mice. *J Biotechnol* 2008; 134: 171-80.
- Sathyanarayana P, Houde E, Marshall D, *et al.* CNTO 530 functions as a potent EPO mimetic *via* unique sustained effects on bone marrow proerythroblast pools. *Blood* 2009; 113: 4955-62.
- Johnson DL, Farrell FX, Barbone FP, *et al.* Identification of a 13 amino acid peptide mimetic of erythropoietin and description of amino acids critical for the mimetic activity of EMP1. *Biochemistry* 1998; 37: 3699-710.
- Livnah O, Johnson DL, Stura EA, *et al.* An antagonist peptide-EPO receptor complex suggests that receptor dimerization is not sufficient for activation. *Nat Struct Biol* 1998; 5: 993-1004.
- Platt R, Roscoe MH, Smith FW. Experimental renal failure. *Clin Sci* 1952; 11: 217-31.
- Picha K, Huang C, Bugelski P, O'Neil K. Engineering peptide therapeutics using MIMETIBODY™ technology. *Methods Mol Biol (In Press)* 2011.
- Eschbach J. The anemia of chronic renal failure: Pathophysiology and the effects of recombinant erythropoietin. *Kidney Int* 1989; 35: 134-48.
- Anagnostou A, Vercellotti G, Barone J, Fried W. Factors which affect erythropoiesis in partially nephrectomized and sham-operated rats. *Blood* 1976; 48: 425-33.
- Mason C, Thomas TH. A model for erythropoiesis in experimental chronic renal failure. *Br J Haematol* 1984; 58: 729-40.
- Egrie JC, Strickland TW, Lane J, *et al.* Characterization and biological effects of recombinant human erythropoietin. *Immunobiology* 1986; 172: 213-24.
- Kawamura A, Higuchi M, Imai N, Kawaguchi T, Ogura Y. Effect of purified recombinant human erythropoietin on anemia in rats with experimental renal failure induced by five-sixth nephrectomy. *Biotherapy* 1990; 2: 77-85.
- Tillmann HC, Kuhn B, Kränzlin B, *et al.* Efficacy and immunogenicity of novel erythropoietic agents and conventional rhEPO in rats with renal insufficiency. *Kidney Int* 2006; 69: 60-7.
- Kinoshita H, Ohishi N, Tokura S, Okazaki A. Pharmacokinetics and distribution of recombinant human erythropoietin in rats with renal dysfunction. *Arzneimittel-Forschung* 1992; 42: 682-6.
- Torralbo A, Blanco J, Fontanellas A, *et al.* Long-term erythropoietin in rats with reduced renal mass. *Nephron* 1996; 73: 280-5.
- Lacasse S, Kingma I, Lariviere R, *et al.* Uremia Enhances the blood pressure response to erythropoietin. *Clin Exp Hypertens* 1997; 19: 389-401.
- Lebel M, Rodrigue ME, Agharazii M, Lariviere R. Antihypertensive and renal protective effects of renin-angiotensin system blockade in uremic rats treated with erythropoietin. *Am J Hypertens* 2006; 19: 1286-92.
- Bellizzi V, Sabbatini M, Fuiano G, *et al.* The impact of early normalization of haematocrit by erythropoietin on renal damage in the remnant kidney model. *Nephrol Dial Transplant* 1998; 13: 2210-5.
- Yoon WH, Park SJ, Kim IC, Lee MG. Pharmacokinetics of recombinant human erythropoietin in rabbits and 3/4 nephrectomized rats. *Res Commun Mol Pathol Pharmacol* 1997; 96: 227-40.

Received: September 20, 2010

Revised: October 12, 2010

Accepted: October 14, 2010

© Kliwinski *et al.*; Licensee *Bentham Open*.

This is an open access article licensed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted, non-commercial use, distribution and reproduction in any medium, provided the work is properly cited.