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

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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.

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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 R *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_2/O_2 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

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a

b

c

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.

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

CONCLUSION

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

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

Parameter	Units	Normal Rats	Renal Insufficient Rats	% Change
Cmax	µg/mL	9.1 ± 2.9	7.8 ± 4.4	-15
AUCt	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
Vss	mL/kg	47.4 ± 14.3	50.5 ± 16.0	7
t1/2	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.

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