Chronic preclinical safety evaluation of Hematide™, a pegylated peptidic erythropoiesis stimulating agent in monkeys

Kathryn W. Woodburn,[1] Susan D. Wilson,[2] Kei-lai Fong,[3] Peter J. Schatz,[1] Thomas Ferrell,[4] Charles B. Spainhour,[4] and Daniel Norton[4]

[1]Affymax, Inc., Palo Alto, CA; [2]Aclairo Pharmaceutical Development Group, Inc, Vienna, VA; [3]Pennsylvania Biolab Inc., Radnor, PA, and [4]Calvert Laboratories, Olyphant, PA, USA

ABSTRACT

Hematide is a synthetic peptide-based, pegylated erythropoiesis stimulating agent in clinical development for treatment of anemia. To support chronic clinical dosing requirements, a 9-month repeat dose IV monkey safety study was undertaken. Animals received 0, 0.2, 2 or 20 mg/kg hematide IV every three weeks for nine months followed by a 14-week recovery. Hematide administration was associated with time and dose-dependent polycythemia. Histological findings were related to exaggerated pharmacology that was secondary to the administration of an erythropoiesis stimulating agent to a normocythemic animal. In conclusion, these results support the use of repeated administration of hematide for the correction of anemia.

Key words: erythropoiesis, hematopoiesis, pharmacology, safety, pharmacokinetics.

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Introduction

Anemia is prevalent in patients with chronic renal failure (CRF),[1] and is associated with increased morbidity.[2] Hematide is a synthetic peptide-based, PEGylated ESA that was designed to specifically bind and activate the erythropoietin (EPO) receptor resulting in production of red blood cells.[3,4] Hematide's amino acid sequence is unrelated to EPO and, as a result is not likely to induce a cross-reactive immune response against either recombinant or endogenous EPO. In the clinical setting, recombinant EPO ESAs have been associated with the development of anti-EPO antibody-mediated pure red cell aplasia (PRCA).[5] By virtue of this lack of immunological cross-reactivity, hematide corrects anemia in rats with PRCA,[5] and restores Hgb to the target range in PRCA patients while eliminating the need for red blood cell transfusions.[6]

This is the first study in which an exogenous ESA has been evaluated following long-term administration to normocythemic adult animals without data being confounded by extramedullary hematopoiesis, as occurs in rodents,[4,7,8] and immunogenic interference, as is the case for human EPO variants.[8,9] Although transgenic mice expressing the human EPO gene are able to tolerate chronic polycythemia, due to developmental conditioning (increased plasma nitric oxide levels and blood viscosity regulation),[7,10] they are not subject to pulsatile reticulocyte and associated changes caused by repeat injections administered to animals that underwent normal development. The anemia in chronic renal failure (CRF) patients requires long term therapeutic support. Therefore a comprehensive non-clinical safety program was undertaken including a 9-month IV safety study in monkeys.

Design and Methods

All animals received care in compliance with the Guide for the Care and Use of Laboratory Animals (*NIH Publication, 1996*) and the study was conducted under the auspices of an Internal Animal Care and Use Committee. Ninety-six experimentally naïve Cynomolgus monkeys (Covance, Denver, PA, USA; 2 years old), received 0, 0.2, 2, and 20 mg/kg IV every three weeks (Q3W) for up to nine months followed by a 14-week recovery. Four animals/sex in the 0 and 20 mg/kg/dose groups were sacrificed on Day 90. Four animals/sex in all dose groups were sacrificed on Day 195 and on Day 279. The remaining four animals/sex in the 0 and 20 mg/kg/dose groups (only three females in Group 4 due to an early death)

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Correspondence: Kathryn W. Woodburn, PhD, Affymax, Inc, 4001 Miranda Avenue, Palo Alto, CA, 94304 USA. E-mail: kathryn_woodburn@affymax.com

were euthanized on Day 372. Clinical observations including ophthalmological examinations, body weight, and food consumption were recorded. Clinical pathological evaluations (hematology, blood chemistry, and urinalysis), organ weight, histopathological, and electrocardiographic examinations were performed. Determination of plasma drug levels and detection of anti-hematide antibodies were performed by enzyme-linked immunosorbent assays (ELISAs) as previously described.[5,11]

Results and Discussion

Clinical observations

There were generally no test article-related effects on body weight, mean food consumption, or electrocardiographic measurements including QTc interval.

Erythropoietic pharmacology

Hematologic changes occurred that were consistent with the known erythropoietic pharmacological action of an ESA (Figure 1). Changes in reticulocytes were synchronous with dosing for the 0.2 and 2 mg/kg groups and were elevated throughout the dosing period for the 20 mg/kg group. Hematide significantly increased hemoglobin (Hgb) production. At Day 279, Hgb levels for 0.2. 2.0, and 20 mg/kg males were 2.2, 8.4, and 9.5 g/dL over concurrent controls respectively. Following cessation of dosing, Hgb values steadily decreased towards control values.

Changes in platelets showed a synchronicity with dosing (Figure 1) for the 0.2 and 2 mg/kg animals. High dose animals showed platelet increases through the first three doses followed by decreased levels. Immediate increases, followed by decreases, in WBCs were observed. The changes were not considered toxicologically relevant and are consistent with data from other ESAs. Mechanisms that may account for these findings include non-specific stimulation of hematopoietic progenitor cells, followed by compensatory production, functional iron deficiency and feedback control of accelerated erythropoiesis in normocythemic animals.[8,12,13]

RBC indices through Day 104 are shown in Figure 2. The increases in MCV (mean corpuscular volume) and MCH (mean corpuscular hemoglobin) followed by subsequent decreases and then decreases in MCHC (mean corpuscular hemoglobin content) are consistent with anticipated RBC rheological changes following accelerated erythropoiesis and subsequent iron-restricted erythropoiesis. The early increase in MCH followed by significant decreases (high dose group) in MCH correlates with decreased serum iron levels measured on Day 90 for the high dose animals.

Due to the pronounced polycythemia, recovery of serum from blood samples collected on Days 90, 195 and 279 was significantly reduced or absent from several animals in the 2.0 and 20 mg/kg dose groups.

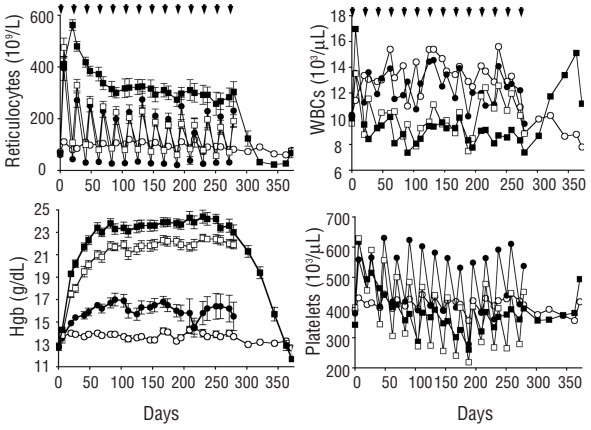

Figure 1. Dose and time-dependent increases in mean hematologic parameters (± SE) following IV Hematide Q3W dosing in male monkeys at 0 (○), 0.2 (●), 2 (□) and 20 (■) mg/kg. Arrow denotes administration day. For the 0 and 20 mg/kg groups; n=16 (Day 1 through Day 90), n=12 (Day 104 through 195), n=8 (Day 209 through 279), n=4 (Day 302 through 372. For the 0.2 and 2 mg/kg groups; n=8 (Day 1 through Day 195) and n=4 (Day 209 to 279). Similar results were observed in the female monkeys.

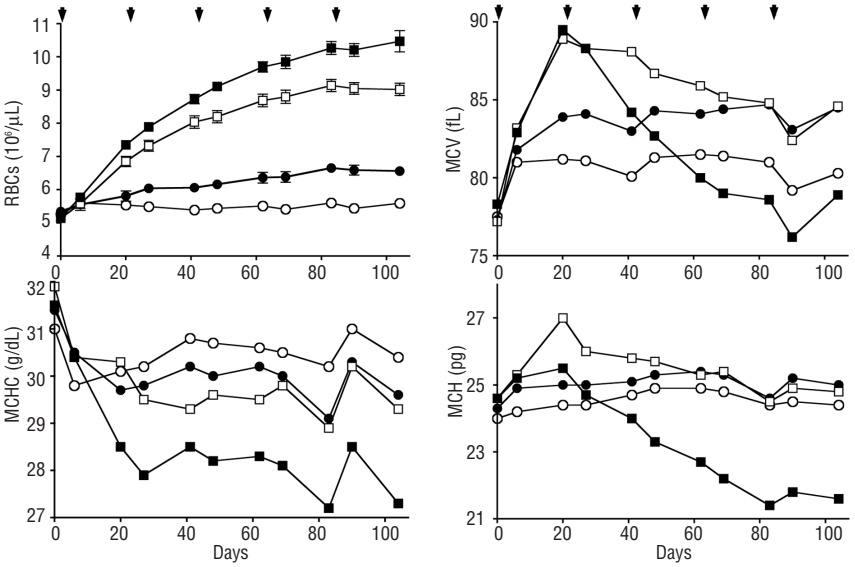

Figure 2. Red blood cell (RBC) and secondary hematologic indices (MCV [mean corpuscular volume], MCHC [mean corpuscular hemoglobin content] and MCH [mean corpuscular hemoglobin]) profiles for female monkeys following IV Hematide at 0 (○), 0.2 (●), 2 (□) and 20 (■) mg/kg every three weeks for 5 administrations. Data are expressed as mean with arrows depicting administration days. For the 0 and 20 mg/kg groups; N=16 (Day 1 through Day 90), N=10-12 (Day 104) and for the 0.2 and 2 mg/kg groups; N=8. Similar results were generally observed in the male monkeys.

Commonly affected serum chemistry parameters included increased aspartate aminotransferase, total bilirubin and potassium and decreased iron. AST, total bilirubin, and potassium can be increased following increased erythrocyte destruction and/or hemolysis.[8] No differences from controls in serum chemistry parameters were observed following the fourteen-week recovery period.

Immunogenicity

Upon initial screening, five of the 32 monkeys in the 20 mg hematide/kg group tested positive for anti-hematide antibodies. A specificity retest demonstrated that one animal contained hematide-specific antibodies. Samples from two others had insufficient volume for re-testing and so were classified as likely positives. The remaining two animals were classified as likely negatives based on antibody binding that was not specific for hematide. No animals contained antibodies reactive with EPO at any time point tested. The presence of antibodies did not alter pharmacology, pharmacokinetics or toxicology.

Pharmacokinetics

Hematide induced sustained plasma levels following IV administration (Table 1). Increases in exposure (AUC values) were greater than dose proportional. Apparent half-life (t1/2) at steady state (Day 106) ranged from approximately 30 hours at the low dose to approximately 90 hours at the high dose. There were no gender-related differences. There were no changes in pharmacokinetics after every third week dosing at 0.2 and 2 mg/kg for nine months. At a 20 mg/kg dose, a 1.5 fold increase in plasma AUC was noted after 6-month dosing, which may have been related, in part, to the reduced plasma volume.

Unscheduled deaths

Unscheduled deaths included 3 high-dose monkeys (20 mg/kg) on Days 79, 104 and 207. No adverse clinical signs preceded these deaths. Based on increased erythron, (Hgb levels of 22-25 g/dL) hypertension may have been present. Histologically, congestion (accumulation of blood in the microvasculature), in the sternal bone marrow, kidney, liver, lungs, spleen and gastrointestinal tract occurred with the early deaths. Congestion in the choroid plexus of brains from two of the monkeys and inflammation and myocardial vacuolation of the heart with accompanying inflammation of the third monkey likely contributed to their early death.

Histopathology

No drug-induced alterations were observed in the 0.2 mg/kg groups. Sternal bone marrow hypercellularity and microvasculature congestion were observed in brain, kidneys, liver, lung, spleen, and gastro-intestinal tract in the 20 mg/kg monkeys from Day 90 and from the 2 and 20 mg/kg monkeys from Days 195 and 279. Incidences increased with dose. In general, 14 weeks following cessation of dosing (Day 372), the changes had reversed or were trending to normalcy. Unlike rodents and patients with polycythemia vera,[7] extramedullary hematopoiesis and splenomegaly were not observed in these monkeys. Splenectomy of transgenic mice overexpressing EPO resulted in a 30% reduction in hematocrit.[7] This further enforces the clinical value of studying chronic polycythemia in monkeys, a hematopoietic model that is more reflective of the human condition.

Table 1. Pharmacokinetic parameters of hematide following every three week (Q3W) IV administration in monkeys.

Day	Doses	Dose (mg/kg)	$t_{1/2}$ (h)		AUC(0-inf) (μg•h/mL)		Cl (mL.kg/h)	
			M	F	M	F	M	F
1	1	0.2	35.9	27.2	0.22	0.28	0.96	0.71
		2.0	58.8	75.1	5.21	7.04	0.39	0.28
		20	129.2	112.2	83.42	73.67	0.24	0.27
106	6	0.2	31.3	27.2	0.27	0.25	0.86	0.80
		2.0	45.6	49.8	4.13	3.82	0.49	0.53
		20	92.0	80.9	86.30	71.78	0.23	0.28
190	10	0.2	26.1	26.0	0.23	0.22	0.87	0.93
		2.0	37.2	35.1	5.07	5.66	0.40	0.36
		20	72.5	63.5	110.89	99.62	0.18	0.20
274	14	0.2	25.6	22.9	0.24	0.20	0.85	1.01
		2.0	37.5	37.1	3.10	3.30	0.65	0.61
		20	72.8	50.7	66.72	54.19	0.30	0.37

The toxicological findings associated with chronic dosing of hematide in monkeys are attributable to an exaggerated pharmacological response and changes were generally reversed following the 14-week recovery period. This is the first reported study in which an ESA was able to be administered chronically to animals without findings being confounded by neutralizing antibodies and extramedullary hematopoiesis[4,7-9] In the clinical setting, hematide will be administered to anemic CRF patients at lower doses, and less frequently,[14] than used in this study and, therefore, the sequelae to exaggerated pharmacology is unlikely to occur. In conclusion, hematide is a potent erythropoiesis stimulating agent exhibiting sustained pharmacological activity, minimal immunogenicity and toxicity.

Authorship and Disclosures

KWW developed the hypothesis for this study, designed the study, interpreted the data and wrote the manuscript; SDW participated in the design of the study, interpreted the data and drafted the manuscript; K-LF participated in the design of the study, analyzed and interpreted the data, and participated in drafting the manuscript; PJS interpreted the data and participated in drafting the manuscript; TF participated in the design and conduct of the study and drafted the manuscript; CBS designed the study, interpreted the data and participated in drafting the manuscript; DN designed and conducted the study, interpreted the data and drafted the manuscript. KWW and PJS are employees of Affymax. Affymax is developing Hematide™ for the treatment of anemia. Other authors reported no potential conflicts of interest.

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