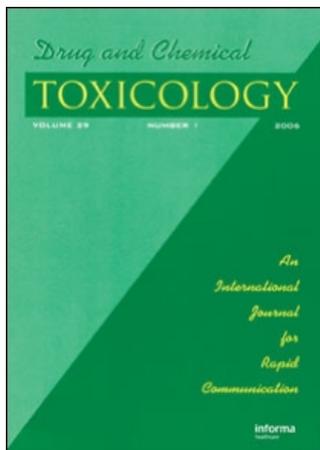


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Preclinical Safety and Pharmacology of Hematide™, a Peptidic Erythropoiesis Stimulating Agent (ESA), in Rats and Monkeys

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The pharmacology, toxicokinetics, and safety of Hematide™, a synthetic peptidic erythropoiesis-stimulating agent (ESA), were characterized. Hematide was given intravenously (0, 0.5, 5, and 50 mg/kg) weekly for five weeks with a 6- (rat) and 12-week (monkey) recovery period. The pharmacological action of Hematide resulted in polycythemia. Histopathology consistent with drug-induced exaggerated pharmacology was observed primarily in rats. Secondary sequelae resulting from pronounced polycythemia was considered the cause of deaths in rats and a single high-dose monkey. Toxicokinetic analysis indicated prolonged exposure. In conclusion, Hematide is a potent ESA and the safety and efficacy profile support clinical development.

Keywords Erythropoiesis stimulating agents, Anemia, Pharmacology, Safety, Toxicology.

INTRODUCTION

Recombinant human erythropoietin (rHuEPO) and a hyperglycosylated variant, darbepoetin alfa, are currently used clinically to correct anemia in patients with chronic kidney disease and cancer due to insufficient erythropoietin production (Nissenson et al., 2003; Weiss and Goodnough, 2005). Hematide, a synthetic, PEGylated, erythropoietic peptide was developed to overcome the

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limitations of currently approved erythropoiesis-stimulating agents (ESAs), namely, the frequent administration of temperature-sensitive recombinant proteins. Hematide potently stimulates erythropoiesis by binding to the erythropoietin (EPO) receptor on red blood cell (RBC) precursors supporting their survival, differentiation, and proliferation (Fan et al., 2006).

Hematide was discovered by screening phage display peptide libraries against the human EPO receptor, followed by extensive chemical analoging (Fan et al., 2006; Wrighton et al., 1996). The primary amino acid sequence of Hematide is unrelated to human EPO and, therefore, is not expected to induce a cross-reactive immune response against either recombinant or endogenous erythropoietin. Four-week repeat-dose rat and monkey studies, which are described here, were conducted as part of a comprehensive safety program designed to support the initiation of clinical trials.

MATERIALS AND METHODS

Animals

All animals received care in compliance with the *Guide for the Care and Use of Laboratory Animals* (NIH Publication, 1996). Sprague-Dawley rats (87 males and 87 females), approximately seven weeks old and weighing 161–300 g, were obtained from Harlan Sprague Dawley, Inc. (Frederick, Maryland, USA). Thirty-six *Cynomolgus* monkeys (Covance, Denver, Pennsylvania, USA; 18 males and 18 females), were approximately two years old and weighed 1.7–2.1 kg at study start. A subset of rats was utilized for toxicokinetic evaluation (nine per gender for Hematide-dosed groups). Hematide is a dimeric peptide, weighing approximately 5000 amu, synthesized via traditional solid-phase synthesis followed by conjugation to polyethylene glycol to increase circulatory persistence of the peptide (Woodburn et al., 2007; Wrighton et al., 1996). Hematide was formulated in 10 mM of acetate-buffered saline, at a pH of 5.5 (Fan et al., 2006).

Sprague-Dawley rats (15 per gender per group) were dosed with 0, 0.5, 5, and 50 mg of Hematide/kg on Days 1, 8, 15, 22, and 29, followed by a six-week recovery period. *Cynomolgus* monkeys were dosed with 0, 0.5, 5, and 50 mg of Hematide/kg on Days 1, 8, 15, 22, and 29, followed by a 12-week recovery period. Vehicle-control and 50 mg/kg cohorts had six per gender per group, and the 0.5- and 5 mg/kg groups had three per gender per group. Doses were given as a single intravenous (IV) bolus 5 mL/kg injection via the tail vein for rats and via the saphenous vein for monkeys. Mortality and clinical observations were evaluated daily. Body weights and food consumption were recorded weekly. Ophthalmology examinations were performed prior to treatment initiation and subsequent to the final dose but prior to the terminal sacrifice. An additional ophthalmic examination was performed during the recovery period for the monkeys. Electrocardiogram tracings (monkeys only) were obtained

prior to treatment initiation, after the first dose on Day 1 and after the final dose on Day 29.

In rats, hematology was evaluated from blood collected on Days 20, 33, 50, and 71, and serum chemistry was evaluated from blood collected on Days 33, 50, and 71. For monkeys, blood was collected for the evaluation of hematology prior to dosing and on Days 6, 13, 20, 27, 33, 50, 64, 78, 92, 106, and 113 and for serum chemistry prior to dosing and on Days 33 and 113. Coagulation parameters and urinalyses were determined on blood and urine samples, respectively, collected on Days 33 (rats and monkeys), 71 (rats), and 113 (monkeys). In addition, the generation of the anti-Hematide antibody was evaluated in blood samples collected pre-test (monkeys) and on Days 33 (rats and monkeys), 71 (rats), and 113 (monkeys).

Blood for toxicokinetic analysis was collected from rats in the toxicokinetic subgroups and monkeys at predose and at 0.25, 1, 4, 8, 24, 48, 72, and 90–96 (monkeys) h postdose on Days 1 and 29. Determination of plasma drug levels and toxicokinetic analysis and detection of anti-Hematide antibodies were performed by enzyme-linked immunosorbent assays (ELISAs), as described previously (Fan et al., 2006; Stead et al., 2006). Specificity of the monkey antibodies for the peptide portion of Hematide was demonstrated, in a similar ELISA format, through binding to wells coated with only the peptide portion of Hematide that was competed with the peptide. Cross-reactivity with rHuEPO was evaluated by using the assay described by Tacey and coworkers (Tacey et al., 2003). *In vitro* neutralizing activity of antibodies was tested by evaluating their ability to inhibit binding of a biotinylated analog of Hematide to microplate wells coated with an EPO receptor fusion to the constant region of IgG (R&D Systems, Minneapolis, MN, USA).

All surviving animals in the toxicology groups were euthanized either at terminal sacrifice on Day 33 or following a 6- (Day 71 for the rat) or 12-week (Day 113 for the monkey) recovery period. A complete necropsy was performed, tissues were harvested, and organs were weighed. Microscopic evaluation was performed on animals that survived until scheduled necropsy and for premature decedents.

Statistical Analyses

The evaluation of the equality of means was made by a one-way analysis of variance. If statistically significant differences between the means were found, a Dunnett's test was used to determine the degree of significance from the control means. A p-value < 0.05 was considered statistically significant.

RESULTS

Erythropoiesis

The primary pharmacological action of Hematide resulted in polycythemia (i.e., significantly increased RBC, hematocrit [Hct], and hemoglobin [Hgb]

values) following a weekly IV administration to normocythemic rats and monkeys. Erythrogenic responses to Hematide were similar in both the rat and the monkey, with no significant gender-related differences observed. The effect of Hematide on reticulocytes and Hgb concentrations in the rat and monkey are displayed in Figures 1 and 2, respectively. In the rat, following cessation of dosing, reticulocyte levels fell sharply to levels below concurrent vehicle-control levels (Day 50) before recovering back to control levels (Day 70). In contrast, the sustained production and circulatory persistence of reticulocytes resulted in elevated reticulocytes in the monkey (50 mg/kg; Figure 2) for up to 50 days following cessation of dosing, before falling below control levels.

Hematide induced significant Hgb production in both species (Figures 1 and 2). After the six-week recovery period in the rat, the Hgb values had returned to, or were below, concurrent control values (Figure 1). In the monkey (Figure 2), Hgb peaked in the high-dose animals by Day 78 and were 8.7 and 6.9 g/dL above concurrent control levels for males and females, respectively. Values were returning toward baseline after 12 weeks, but were still elevated compared to concurrent controls by 5.8 and 3.8 g/dL for males and females, respectively.

Concurrent with the observed Hematide-induced reticulocytosis and subsequent increases in Hgb levels were increases in RBCs and Hct levels (Figure 3). Similar responses were observed between genders of both species. In rats,

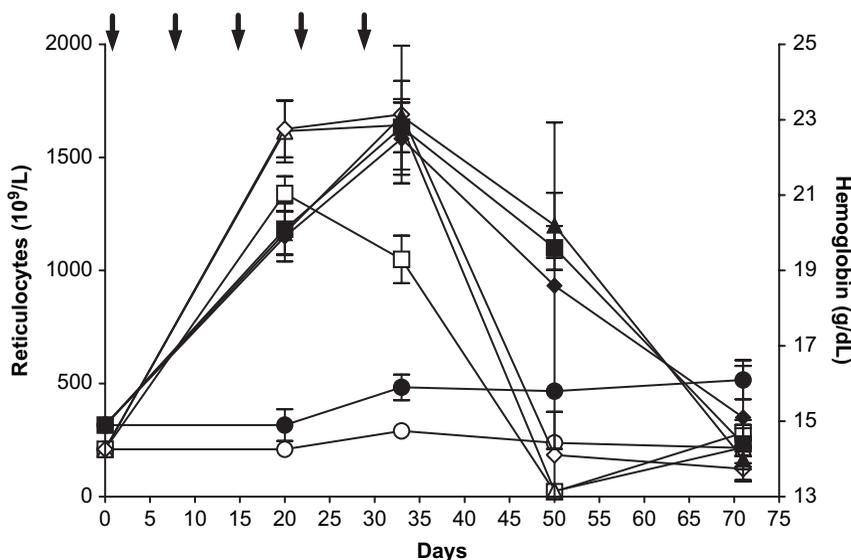


Figure 1: Temporal profile of reticulocytes (open symbols) and hemoglobin (Hgb, closed symbols) concentration, expressed as mean \pm standard deviation, in normocythemic male Sprague-Dawley rats following repeat administration. Rats received an intravenous administration of vehicle (circles), 0.5 (squares), 5 (triangle), and 50 (diamonds) mg of Hematide/kg, respectively, every week for five weeks. Arrows denote days of dosing. Days 20 and 32 represent the mean of 9 to 15 animals, while days 50 and 71 represent the mean of three to five animals.

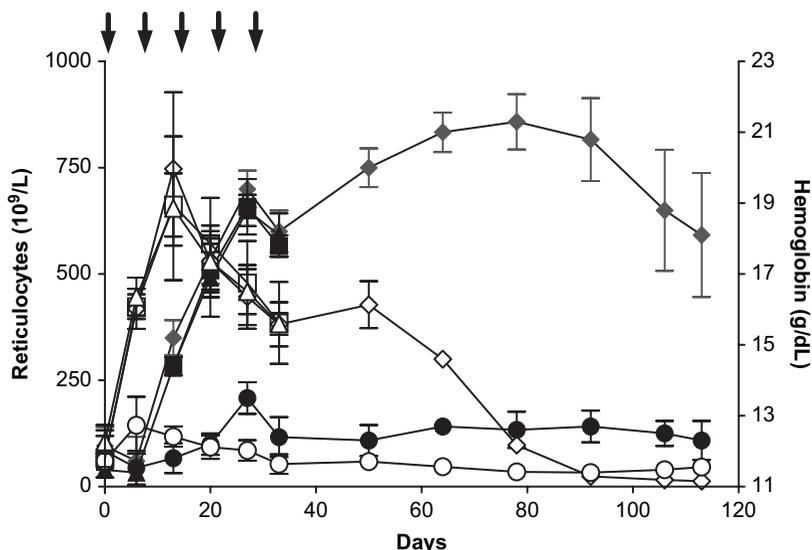


Figure 2: Erythropoietic activity of Hematide given once-weekly, for five weeks, to male Cynomolgus monkeys. Data are expressed as mean \pm standard deviation. Monkeys were intravenously administered vehicle (circles), 0.5 (squares), 5 (triangle), and 50 (diamonds) mg of Hematide/kg, respectively. Open symbols denote percent reticulocyte generation, while closed symbols represent hemoglobin (Hgb) concentration. Arrows denote days of dosing. Through day 33, the vehicle-control and 50 mg/kg groups represent the mean of six animals, while the 0.5 and 50 mg/kg groups represent the mean of three animals. Thereafter, each time point represents the mean of three animals (vehicle-control and 50 mg Hematide/kg groups).

RBC and Hct values peaked in all Hematide-treated groups on Day 33 and were similar to the controls by the end of the six-week recovery. In monkeys on Day 33, after five weekly dose administrations, the mean RBCs counts in all Hematide-treated groups ranged from 6.72 to $7.05 \times 10^6/\mu\text{L}$ compared to $4.96 \times 10^6/\mu\text{L}$ for the concurrent vehicle-controls. Hct levels ranged from 59.4 to 61.4% compared to 37.6% for the respective controls. Mean values peaked in the 50 mg/kg dose group on Day 78 at 72.5% for Hct and $9.18 \times 10^6/\mu\text{L}$ for RBCs compared to 39.6% and $5.08 \times 10^6/\mu\text{L}$ for concurrent controls. A trend toward recovery in RBC counts and Hct was observed on Days 92, 106, and 113. All values, however, remained significantly elevated through Day 113, corresponding to 84 days following the last Hematide administration. Mean RBC counts and Hct were $2.98 \times 10^6/\mu\text{L}$ and 20.5%, respectively, greater than control values.

Hematide, at the doses tested, induced significant changes in secondary hematologic indices (Figures 4 and 5). There were no gender-related differences in the response. Increases in mean corpuscular volume (MCV), which reflects the generation of large sized cells, were observed in all Hematide-treated groups of monkeys, with levels returning to within normal ranges following cessation of dosing. In contrast, MCV was initially increased in rats in

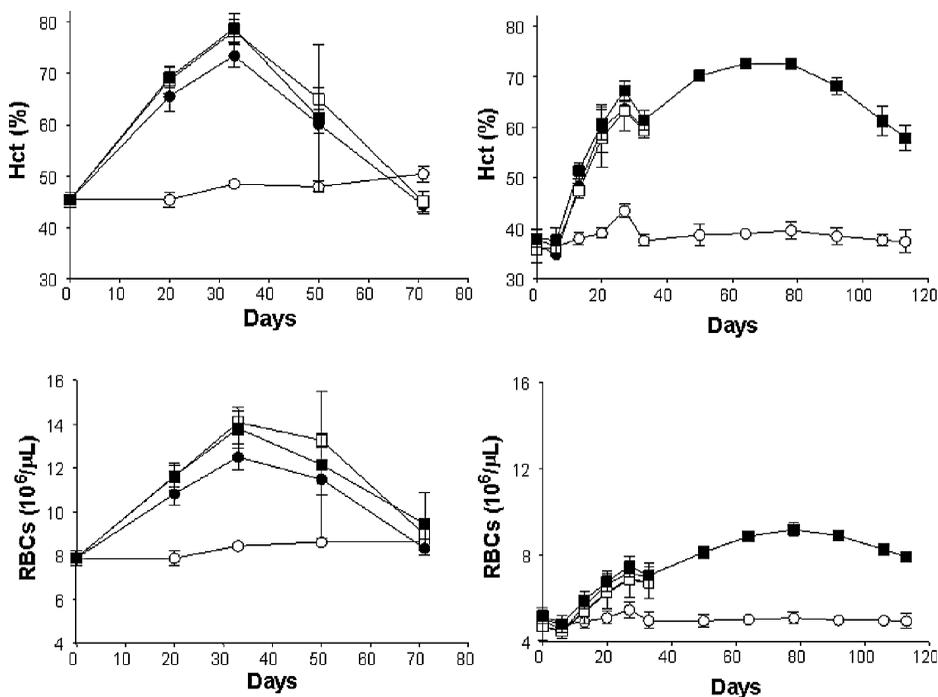


Figure 3: Erythropoietic activity of male rats (left) and monkeys (right), as expressed by hematocrit (Hct) and red blood cell (RBC) levels, following an intravenous Hematide administration once-weekly for five weeks. Animals were IV administered vehicle (open circles), 0.5 (closed circles), 5 (open squares), and 50 (closed squares) mg Hematide/kg, respectively. Data are expressed as mean \pm standard deviation. For rats, Days 20 and 32 represent the mean of 9 to 15 animals, while Days 50 and 71 represent the mean of three to five animals. For monkeys, through Day 33, the vehicle-control and 50 mg/kg groups represent the mean of six animals, while the 0.5- and 50 mg/kg groups represent the mean of three animals. Thereafter, each time point represents the mean of three animals (vehicle-control and 50 mg Hematide/kg groups).

all treatment groups by Day 20, decreased by Day 50, and was significantly lower than control values by Day 71. Mean corpuscular hemoglobin content (MCHC), which measures the mean RBC Hgb concentration, was decreased at all sampling points in the rat and monkey. Mean corpuscular hemoglobin (MCH) levels were decreased in rat, while in the monkey increases were observed during the dosing phase with decreases, to below concurrent control levels, following cessation of dosing.

In rats, decreased mean platelet values were observed in all Hematide-treated groups, with the exception of the high-dose (50 mg/kg) females on Day 33. In contrast to the rat, platelet increases, which were generally not statistically significant, were observed in monkeys on Days 6 through 33 in groups treated with Hematide (Figure 6, upper panel). Platelet changes were not of a magnitude considered to be toxicologically adverse in either species, and effects were reversible.

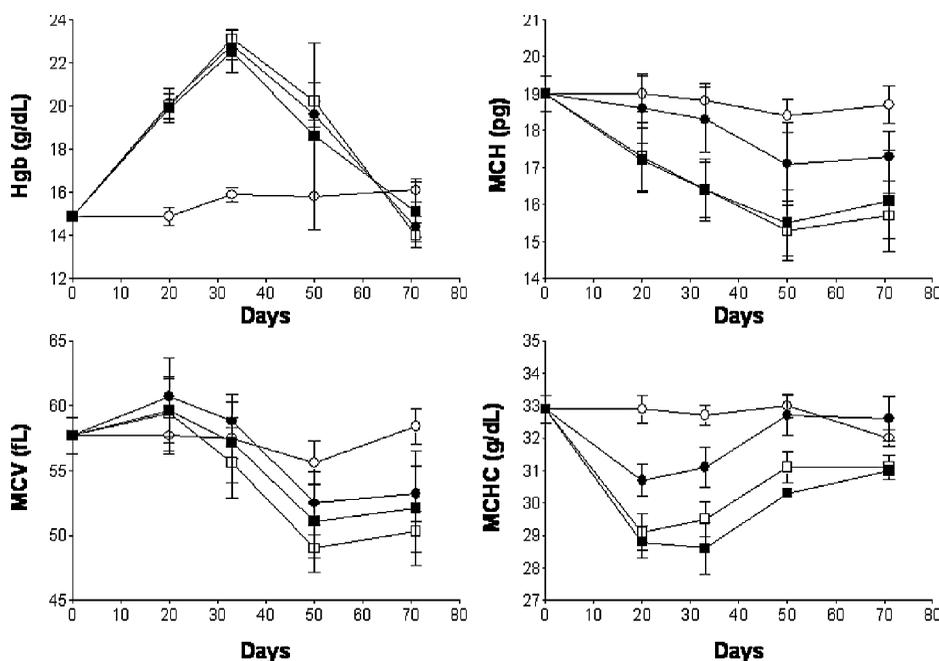


Figure 4: Hemoglobin and secondary hematological indices profile for male rats administered five weekly doses of Hematide. Rats were intravenously administered vehicle (open circles), 0.5 (closed circles), 5 (open squares), and 50 (closed squares) mg Hematide/kg, respectively. Data are expressed as mean \pm standard deviation. Days 20 and 32 represent 9 to 15 animals, while Days 50 and 71 denotes three to five animals.

Safety

The Hematide-induced exaggerated pharmacology and secondary sequelae were considered the cause of the premature deaths observed in two and nine rats in the 5- and 50 mg/kg dose groups, respectively, on Days 20 through 35, and in a single female monkey at 50 mg/kg on Day 61. The findings were consistent with the premature mortality observed with approved ESAs (U.S. Food and Drug Administration [FDA] toxicology review for darbepoetin). The most common gross findings in the rats that died prematurely were enlarged spleen, dark liver, dark kidneys, and/or gastric effects (i.e., ulcerated or dark foci that increased in frequency with dose). As a result of the early mortality in the rats, there were no high-dose females available for the recovery period. Histopathological lesions in the monkey that was sacrificed prematurely included infarcts in the heart and kidneys as well as nephropathy (hemorrhage).

In the animals of both species that survived until scheduled necropsy, there were generally no Hematide-related effects on clinical observations, body weight, and food consumption. Activated partial thromboplastin time

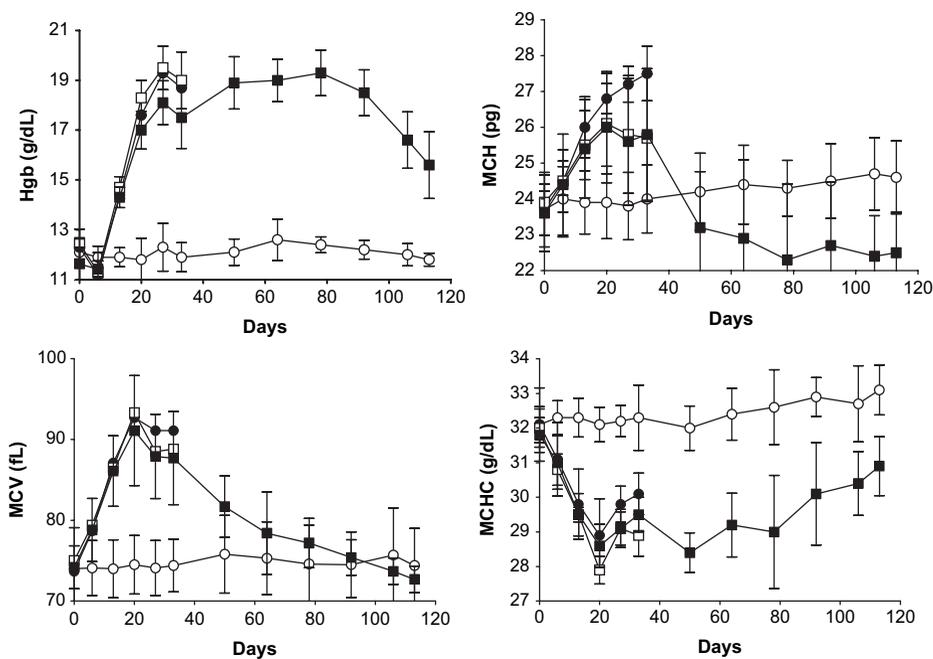


Figure 5: Hemoglobin and secondary hematological indices profile for female monkeys administered five weekly doses of Hematide. Monkeys were intravenously administered vehicle (open circles), 0.5 (closed circles), 5 (open squares), and 50 (closed squares) mg Hematide/kg, respectively. Data are expressed as mean \pm standard deviation. Through Day 33, the 0- and 50 mg/kg groups represent six animals, while the 0.5- and 50 mg/kg groups represent three animals. Thereafter, each time point represents three animals for 0 mg/kg and following Day 50 represents two animals.

and/or prothrombin time were increased in both rat and monkey; however, following the 6-week rat and 12-week monkey recovery periods, no differences in coagulation parameters, compared to control values, were observed.

Hematide-related serum chemistry changes identified on Day 33 included a substantial increase in bilirubin (rat and monkey), decreased total serum iron (rat and monkey), decreased glucose (rat), increased aspartate aminotransferase (rat and monkey), and increased serum potassium (monkey). Values for the altered parameters had returned toward control values following the recovery period, with the exception of changes in bilirubin and serum iron. On Day 33, iron decreases were 56 and 65 $\mu\text{g/dL}$ for male and female monkeys, compared to 79 and 278 $\mu\text{g/dL}$ for male and female rats, respectively. At Day 50, total serum iron increased significantly in the rat. Serum iron levels in the rat and monkey following the recovery period remained elevated.

As noted earlier, histopathologic changes were generally observed in the rat, whereas microscopic evaluation of tissues from monkeys at the terminal and recovery sacrifices revealed no drug-induced alterations. In rats, microscopic findings consistent with exaggerated erythrogenic pharmacology included

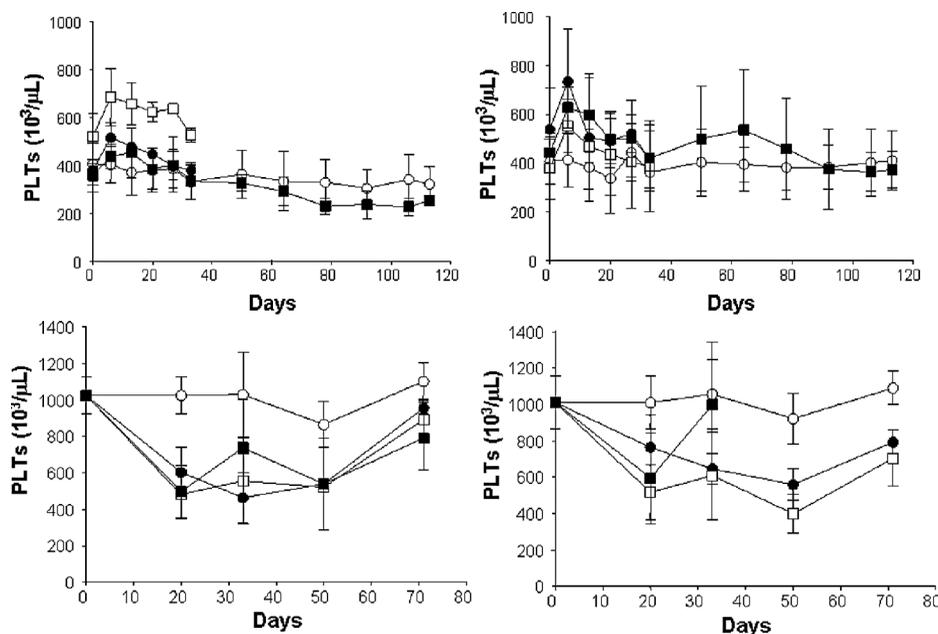


Figure 6: Platelet profile in male (left) and female monkeys (top) and rats (bottom) following an intravenous (IV) Hematide administration once-weekly for five weeks. Animals were IV administered vehicle (open circles), 0.5 (closed circles), 5 (open squares), and 50 (closed squares) mg Hematide/kg, respectively. Data are expressed as mean \pm standard deviation. For monkeys through day 33, the 0- and 50 mg/kg groups represent six animals, while the 0.5- and 50 mg/kg groups represent three animals. Thereafter, each time point represents three animals for the 0 mg/kg male 50 mg/kg groups and following Day 50 represents two animals for the 50 mg/kg female group. For rats, Days 20 and 32 represent 9 to 15 animals, while days 50 and 71 represent three to five animals.

increased extramedullary hematopoiesis in the spleen and liver, hypercellularity in the bone marrow, and organ congestion. Minimal to mild tubular epithelial changes in the kidney were observed in all dose groups, including the control group. In the Hematide-treated animals, the renal changes were often associated with Hgb crystals, which may have been nephrotoxic. Additional findings in all dose groups included gastric erosion/ulceration and hemorrhage. At dose levels ≥ 5.0 mg/kg, findings included minimal to moderate peritrabecular fibroplasia or hyperostosis, minimal to moderate inflammation, and thrombotic deposits associated with the atrium or heart valves. At 50 mg/kg, minimal depletion of ovarian corpora lutea, minimal uterine and cervical atrophy, and minimal vaginal epithelial atrophy with mucosal mucification were noted.

Toxicokinetics

Toxicokinetic parameters for Hematide were evaluated in both rats and monkeys on Day 1 and following the weekly administration of Hematide for a total of five injections on Day 29 (Tables 1 and 2; Figure 7). Hematide

Table 1: Hematide rat toxicokinetic parameter following weekly administration.

Day	Dose mg/kg	Doses	Males			Females				
			AUC ^(0-∞) mg•h/mL	t _{1/2} h	CL mL/h•kg	V _{ss} mL/kg	AUC ^(0-∞) mg•h/mL	t _{1/2} h	CL mL/h•kg	V _{ss} mL/kg
1	0.5	1	0.21	21.7	2.36	65.6	0.20	23.7	2.55	79.3
	5		4.41	31.6	1.13	50.2	3.12	32.4	1.60	71.6
29	50	5	64.0	38.6	0.78	42.0	59.4	35.0	0.84	41.6
	0.5		0.24	13.9	2.23	41.8	0.14	15.6	3.48	71.9
	5		4.95	22.2	1.01	27.9	4.37	23.8	1.14	33.3
	50		62.9	30.7	0.80	34.6	57.4	27.3	0.87	31.4

Note: Data are expressed as the mean. AUC, area under the curve; CL, plasma clearance, t_{1/2}, half-life; V_{ss}, volume of distribution at steady state.

Table 2: Toxicokinetic parameters in monkeys following weekly administration of Hematide.

Day	Dose mg/kg	Doses	Males			Females				
			AUC ^(0-∞) mg•h/mL	t _{1/2} h	CL mL/h•kg	V _{ss} mL/kg	AUC ^(0-∞) mg•h/mL	t _{1/2} h	CL mL/h•kg	V _{ss} mL/kg
1	0.5	1	0.41	36.4	1.22	61.2	0.52	37.3	0.97	52.4
	5		ND	61.4	ND	ND	6.75	34.9	2.11	39.5
29	50	5	ND	92.0	ND	ND	150.1	144.4	0.43	87.4
	0.5		0.39	22.5	1.31	41.7	0.43	17.5	1.2	30.4
	5		10.3	50.3	0.49	33.3	10.2	48.8	0.49	32.2
	50		172.8	64.7	0.30	18.9	180.2	55.2	0.29	23.2

Note: Data are expressed as the mean. AUC, area under the curve; CL, plasma clearance, t_{1/2}, half-life; V_{ss}, volume of distribution at steady state; ND, not determined. Due to the long t_{1/2} and limited data from the terminal phase, the pharmacokinetic parameters including CL, V_{ss}, and terminal t_{1/2}, represent estimates.

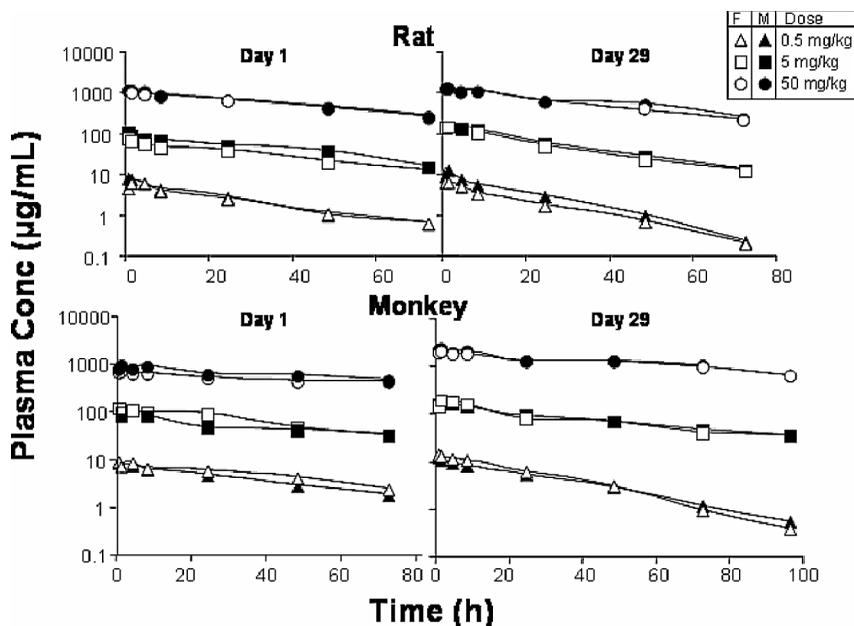


Figure 7: Mean plasma concentration time profiles for Hematide following an intravenous administration in rats and monkeys. Data represent three animals per gender per group, with the exception of 50 mg/kg for the monkey, which represents six animals per gender.

administration resulted in sustained plasma levels in the rat and monkey, with no clear gender related differences observed. At all dose levels and at both sampling intervals, maximal concentration (C_{max}) and area under the curve (AUC) values increased with dose. AUC increase on Day 29 of dosing was generally greater than dose proportional. The apparent half-life at steady state (Day 29) in rats (males and females) ranged from an average of 14.8 h at the low dose to 29.0 h at the high dose. In monkeys the average half-life at steady state varied from 20 h at the low dose to 60 h at the high dose. There were no remarkable changes of systemic exposure upon multiple weekly dosing in rats at all doses and in the monkey following administration at the low dose. Systemic exposure to Hematide was increased by 1.5- to 3-fold following five weekly administrations to the monkeys at the mid- and high dose.

Immunogenicity

Hematide-reactive antibodies were not detected in any rats. Two monkeys (one in the 0.5 mg/kg group and one in the 50 mg/kg group) out of 24 dosed animals were positive for Hematide-specific antibodies. The antibodies were specific for the peptide dimer and were able to neutralize binding of the peptide portion to HuEPOr *in vitro*. The Hematide-specific antibodies did not cross-react with rHuEPO.

DISCUSSION

Hematide is a PEGylated synthetic peptide that binds specifically to the EPO receptor and initiates a signaling cascade similar to that generated by endogenous EPO (Fan *et al.*, 2006). The non-PEGylated dimer of Hematide binds to an EPO-competitive site on HuEPO_r with an affinity equivalent to that of the natural ligand. PEGylation of the peptide functions to increase solubility of the molecule and increases the hydrodynamic volume, thereby decreasing plasma clearance rates and suppressing immunogenicity and antigenicity (Calcieti and Veronese, 2003). Potential indications for Hematide include the treatment of anemia associated with chronic kidney disease (CKD) and cancer, treatment of anemia associated with chemotherapy in cancer patients, as well as the treatment of anemia in patients with erythropoietin antibody-mediated pure red cell aplasia.

The pharmacology of Hematide is similar to that of other ESAs that have been approved for marketing in Europe and/or the United States, such as the epoetins and darbepoetin alfa. Hematide can be administered less frequently than currently approved ESAs (Egrie *et al.*, 2003). Hematide induces the stimulation of reticulocytes in rats and monkeys with subsequent maturation into RBCs and increases in Hgb and Hct. The pharmacology (RBC generation) induced by Hematide is reversed following discontinuation of dosing with a predictable time to recovery, which is a function, in part, of the RBC lifespan.

The toxicological effects observed here with Hematide are considered to be secondary to the exaggerated pharmacology that occurs with administration at relatively high doses or dosing frequency to a normocythemic animal. The toxicology of Hematide is similar to that of other ESAs that have been approved, with generally no novel toxicities observed. However, unlike other rHuEPO-based ESAs, Hematide is nonimmunogenic in the rat and minimally immunogenic in the monkey. Antibodies that were produced in two monkeys following dosing with Hematide did not neutralize or reduce the pharmacologic activity of the drug. It is possible that these antibodies were produced sufficiently late during the erythropoietic response or were of sufficiently low titer that they were unable to interfere with pharmacological activity. Importantly, Hematide-specific antibodies did not cross-react with rHuEPO and, therefore, would not be expected to interfere with erythropoiesis driven either by endogenous EPO or rHuEPO-based ESAs. For rHuEPO-based ESAs, the generation of neutralizing antibodies in animal studies has been a confounding factor (FDA toxicology review for darbepoetin), resulting in much shorter durations of polycythemia and presumably altered toxicological properties.

The hematologic response following administration of Hematide to rats and monkeys included an increase in RBC parameters (e.g., RBC count, Hgb, Hct), altered RBC morphology, increased serum bilirubin, short-term decreases and long-term increases in serum iron levels, splenomegaly, organ congestion,

thromboses, extramedullary hematopoiesis, and bone marrow hypercellularity. Perturbations in hemodynamics, including chronic blood hyperviscosity, increased peripheral resistance, and hypertension, have been reported with this class of drugs and are considered to be secondary to the exaggerated pharmacology associated with erythropoiesis-stimulating agents (FDA toxicology review for darbepoetin, Cowgill et al., 1998).

Although the pharmacological response was similar between the two species with respect to the changes in the RBC indices, the rat was more sensitive to the effects of exaggerated pharmacology. The persistent polycythemia in the monkeys, compared to the rats, likely reflects a mean RBC lifespan in the monkey of 85 days (range, 52–128) (Moore, 2000a) versus 45–68 days in the rat (Moore, 2000b). Reticulocytosis, following accelerated erythropoiesis, induces changes in RBC rheology, which is temporally influenced by such factors as iron stores and age of the circulating cells (Maeda et al., 1989). Hematide administration induced substantial polycythemia with reticulocytosis and an immediate accompanying increase in MCV. The release of these larger cells is thought to be due to the increased mitotic frequency of late erythroid precursors residing in the bone marrow (Muntzel et al., 1992). The subsequent decrease in MCV (rat only) likely reflects, in part, initial iron depletion, which was more pronounced in the rat. In support of this, reticulocytes in rats fell below concurrent controls at Day 50. This phenomenon has been attributed to functional iron deficiency incurred following massive reticulocytosis (R'zik et al., 2001) and/or erythroid bone marrow exhaustion. A similar response has been observed following intensive rHuEPO administration in the rat (Piron et al., 2001). Erythrocyte precursor cell division continues until a critical level of Hgb is achieved. Under conditions of iron deficiency, Hgb levels are lower and consequently additional cell divisions occur, resulting in smaller cells (Watson and Canfield, 2000). Additionally, RBC production from extramedullary hematopoiesis in the spleen, kidney, adrenal gland, lymph node, and liver of rats during intense erythropoiesis (Greaves, 2000; Greaves, 1992) likely factors into the decrease in MCV and associated iron depletion.

Hematide administration resulted in changes in platelet levels. The changes were not considered to be toxicologically relevant and are consistent with data from other ESAs. rHuEpo has been shown to stimulate platelet production and, with intense chronic dosing, results in thrombocytopenia (Beguín, 1999; Berridge et al., 1988). The increase in platelet numbers is proposed to be related to effects on bone marrow megakaryocytes and their immediate precursors. Decreased platelets counts were noted only in the rat. The decrease in platelets in the rat may be a function of the timing of blood collection with respect to the administration of Hematide. If sampling times occurred soon after dosing, as was done in the monkey, then most likely increases in platelets would have been observed. The decrease in platelets observed in the rat when samples were obtained at later time points after

dosing likely reflects a compensatory response to a drug-induced thrombocytopenia. Alterations in serum chemistry were considered to reflect increased medullary and extramedullary hematopoiesis, RBC destruction, and/or assay interference. The increases in the coagulation assays are likely due to interference from the increased number of RBCs, hemolysis, or elevated plasma citrate (Arkin et al., 2003).

CONCLUSION

In conclusion, Hematide exhibits pronounced, prolonged erythrogenic action in rats and monkeys. Exposure to Hematide is sustained, with a half-life in rats and monkeys of up to approximately 30.7–92 h. The toxicologic effects observed with Hematide are generally considered to be related to the exaggerated pharmacology that is secondary to the administration of an ESA to a normocythemic animal. In the clinical setting, Hematide will be administered to anemic patients with CKD or cancer at lower doses and less frequently than used in these toxicology studies and, therefore, the sequelae to exaggerated pharmacology are considered unlikely to occur. The nonclinical studies indicated that Hematide was minimally immunogenic. The low level of immunogenicity in the nonclinical studies is consistent with the single-dose human trials in which an absence of immunogenicity was observed (Stead et al., 2006). The absence of immunological cross-reactivity between Hematide and rHuEPO (Woodburn et al., 2007) indicates that Hematide does not pose a risk of inducing antibodies reactive with endogenous human EPO. A lack of cross-reactivity was anticipated based on the differences in amino acid sequence of Hematide compared to endogenous EPO. The sustained pharmacodynamic activity of Hematide following a single injection is expected to translate into a dosing interval of every three to four weeks in humans, thus leading to improved convenience and compliance for patients with CKD- and cancer-induced anemia.

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