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Phase II Prospective Open-Label Trial of Recombinant Interleukin-11 in DDAVP-Unresponsive Von Willebrand Disease and Mild or Moderate Hemophilia A

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Summary

Desmopressin (DDAVP) is the treatment of choice in those with mild von Willebrand disease (VWD), yet 20% are unresponsive to DDAVP, and among the 80% who respond, the response is transient, as endothelial stores are depleted after 3 days. We, therefore, conducted a single-center Phase II clinical trial to determine safety and biologic efficacy of recombinant interleukin-11 (rhIL-11, Neumega®) in patients with VWD unresponsive or allergic to DDAVP, or mild or moderate hemophilia A (HA). Increases in VWF:RCo were observed by 48 hours after rhIL-11, with a 1.54-fold increase by day 4, 1.30-fold in VWD and 1.73-fold in HA. Similarly, by 48 hours, increases in VIII:C were observed, with a 1.65-fold increase by day 4, 1.86-fold in VWD and 1.48-fold in HA. Platelet VWFmRNA expression by qPCR increased 0.81-fold but did not correlate with plasma VWF:Ag responses. rhIL-11 was well tolerated, with grade 1 or less fluid retention, flushing, conjunctival erythema, except for transient grade 3 hyponatremia in one subject after excess fluid intake for diabetic hyperglycemia, which resolved with fluid restriction. In summary, rhIL-11 increases VWF levels in 2 of 4 DDAVP-unresponsive or allergic VWD and F.VIII levels in 4 of 5 mild or moderate hemophilia A subjects, suggesting its potential use in treatment of these disorders.

Keywords

clinical trial; recombinant interleukin-11; von Willebrand disease; hemophilia A

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Authorship M. Ragni and T. Nichols designed the research; M. Ragni and E. Novelli performed the clinical trial; A. Murshed performed nursing assessments on study subjects and obtained and managed the clinical data; E. Merricks and M. Kloos performed laboratory assays; M. Ragni and T. Nichols analyzed the results; and M. Ragni wrote the paper with contributions from T. Nichols.

Introduction

Desmopressin (1-deamino-8-D-arginine vasopressin, DDAVP) is a synthetic analog of vasopressin that raises VWF levels while avoiding side effects of vasopressin, and is currently used in the treatment of mild or moderate hemophilia A (HA) and von Willebrand disease (VWD). Hemophilia A is an X-linked disorder associated with bleeding into joints and muscles and is caused by deficient or defective coagulation factor VIII (F.VIII). VWD is the most common congenital bleeding disorder, an autosomal disorder associated with mucosal bleeding, occurring in 1% of the population,[1] and is caused by a quantitative or qualitative deficiency of von Willebrand factor (VWF), a multimeric glycoprotein which mediates platelet adhesion to damaged vascular endothelium.[2-5] DDAVP acts by stimulating the release of VWF and F.VIII from tissue stores in Weibel-Palade bodies in the vascular endothelium, which increases circulating levels of VWF and F.VIII.[6] As many as 20% of those with VWD are unresponsive to DDAVP, and among the 80% who do respond, response is short-lived, up to three days;[6] thus, DDAVP may be inadequate during surgery or after trauma. Alternatives to DDAVP, including plasma-derived VWF concentrate or recombinant factor VIII, are limited by higher cost and potential viral transmission risk. Thus, safer, more effective products are goals for improving treatment of VWD and hemophilia A.[7] Recombinant human interleukin-11 (rhIL-11), a gp-130 signaling cytokine with hematopoietic and anti-inflammatory activity, currently FDA-approved for chemotherapy treatment of thrombocytopenia,[8] increases VWF and F.VIII levels 1.5 fold when given daily by subcutaneous injection, with levels persisting each day it is given,[9] and reduces menstrual bleeding in women with VWD.[10] The effects of rhIL-11 in individuals with VWD, unresponsive or allergic to DDAVP, or with hemophilia A have not been previously evaluated. We, therefore, conducted a prospective, open-label phase II trial of rhIL-11 in non-bleeding subjects with VWD who are unresponsive or allergic to DDAVP, or mild or moderate hemophilia A.

Materials and Methods

Study subjects

After giving signed informed consent, nine subjects 18 years or older, including five with mild or moderate hemophilia A (HA) and four with mild or moderate von Willebrand disease (VWD) unresponsive or allergic to DDAVP were enrolled in and completed the study between January 2010 and February 2012. The VWD subjects included two with type 1 VWD and two with type 2 VWD, including one with type 2B, with increased platelet aggregation at low strength ristocetin and absent high molecular weight multimers, and one with type 2M disease, with reduced VWF:RCoF/ VWF:Ag ratio <0.50, per NHLBI criteria (Table 2).[5] All subjects had positive past bleeding histories. Pregnancy, lactation, heart disease, uncontrolled hypertension, arrhythmia, thrombosis, recent surgery or receipt of blood products were exclusions. The study was approved by the Institutional Review Board and Clinical Translational Research Center Advisory Board, University of Pittsburgh.

Study design

This was a phase II prospective biologic effects trial of rhIL-11 25μg/kg/day by subcutaneous injection for four consecutive days (Days 1-4) in the non-bleeding state. The dose was selected based on previous safety and efficacy trials in patients with VWD.[9, 10] On Day 4, beginning 30 minutes after rhIL-11, DDAVP 0.3 mcg/kg was given by intravenous infusion, except in the two subjects in whom it was contraindicated. Safety was assessed by medical history, physical examination, vital signs, height, weight, cardiac, chest, and fundoscopic exams at baseline and days 1-4 (Table 2). Coagulation tests were measured at baseline and days 1-4, and platelet VWF mRNA at baseline and Day 4. Coagulation tests

included VWF:RCo, VWF:Ag, FVIII:C, FVIII:Ag, VWF multimers, epinephrine (CEPI) and adenosine diphosphate (CADP) closure times; and safety tests, hemoglobin, platelet count, electrolytes, and electrocardiogram.

Laboratory assays

VWF activity was measured by ristocetin-induced platelet agglutination (Chronolog Corp., Havertown, PA) using a Chronolog aggregometer[11-14] and VWF:Ag by "sandwich" ELISA, using anti-VWF antibodies (DakoA082, Carpintera CA). Results were expressed in percent, with normal human plasma pool designated 100%, and severe type 3 VWD plasma used as the negative control (George King Bio-Medical, Overland Park, Kansas). The limit of detection of the VWF:RCo assay is 12.5%. VWF multimers were analyzed by SDS gel electrophoresis using 1.5% and 0.65% agarose gels. FVIII:C was determined by chromogenic substrate assay (Coamatic, DiaPharma Group, Westchester OH); FVIII:Ag by immunoassay (Asserachrom, Diagnostica Stago, Gennevilliers France); closure times by PFA-100 analyzer;[9] and platelet VWF mRNA by reverse transcription of platelet-rich plasma and amplification by quantitative PCR, normalized against platelet GP1Bb mRNA.[9]

Endpoints and statistical methods

The sample size of nine evaluable subjects was considered adequate to provide sufficient power to evaluate biologic efficacy in this phase II trial,[15] with 95% confidence to show an increase of 0.2 VWF U/ml, with σ=0.1 VWF U/ml, at α=.05 and β=.90 (1-sided). Biologic efficacy was based on fold-increase in VWF and F.VIII after rhIL-11 injection. Safety was based on fluid retention, flushing, and injection site bruising, rated by NCI Common Terminology Criteria.[16] Data were analyzed by descriptive statistics. Pre- and post-rhIL-11 VWF and FVIII were compared by student's t test; VWF assays were compared with fold-increase in platelet VWFmRNA by Spearman rank correlation.

Results

VWF and F.VIII levels after rhIL-11

A total of nine subjects were enrolled and completed study, including five with hemophilia A and four with VWD. The median age was 26 years, range 22-51 years (Table 1). There was an increase in VWF:RCo, VWF:Ag, FVIII:C, and FVIII:Ag beginning at 48 hours after rhIL-11 and persisting the four days the drug was given (Table 1). There was a 1.54-fold increase in VWF:RCo by day 4 after rhIL-11 over baseline, including 1.30-fold in VWD and 1.73-fold in hemophilia patients; and in VWF:Ag, overall 1.69-fold over baseline, including 1.69-fold each in VWD and hemophilia A subjects. Similarly, increases in FVIII:C were observed, with a 1.65-fold increase by day 4 over baseline, 1.86-fold in VWD and 1.48-fold in hemophilia A; and in FVIII:Ag, overall 1.58-fold, and 1.47-fold in VWD and 1.66-fold in hemophilia A subjects.

These increases in VWF and F.VIII were further boosted after DDAVP on day 4, overall with a 1.88-fold further boost in VWF:RCo, 1.98-fold in VWF:Ag, 3.91-fold in FVIII:C and 2.45-fold in FVIII:Ag. Among those with VWD, the DDAVP-boosted increases were 2.33-fold, 3.25-fold, 6.17-fold, and 3.32-fold, respectively; and among those with hemophilia, these DDAVP-boosted increases were 1.71-fold, 1.47-fold, 3.00-fold, and 2.10-fold, respectively (Table 1).

VWF multimers after rhIL-11

Baseline VWF multimer patterns, performed in 1.5% and 0.65% agarose gels, were normal in all hemophilia A patients and all but one of those with VWD (Pt # 2), the latter of whom had increased high molecular weight bands compared to baseline (not shown). No change in

VWF multimer pattern was observed after rhIL-11 infusion in any subject, but, following DDAVP, there was an increase in high molecular weight VWF multimers, in parallel with the increase in plasma VWF antigen levels (not shown).

Platelet VWFmRNA by qPCR

The increases in VWF:RCo and VWF:Ag were accompanied by a 0.81-fold increase in platelet VWF mRNA (-0.07-fold to 2.65-fold), but the latter did not correlate with absolute or fold-increase in VWF:RCo, r=0.297 and r=0.344, respectively, or with absolute or fold-increase in VWF:Ag, r=0.469 and r=0.041, respectively, all $p>0.05$.

Adverse effects and safety of rhIL-11

rhIL-11 was well tolerated. Adverse events were generally mild (Table 3). There was grade 1 or less conjunctival injection in six subjects, dyspepsia in three subjects, and fluid retention, flu-like symptoms, headache, and injection site erythema in two subjects each. Transient grade 3 hyponatremia, Na 129 mEq/L, developed in one subject with type 2 diabetes mellitus (Pt #6), after excess fluid intake for symptomatic hyperglycemia, which resolved with fluid restriction. Hypertension, defined as a diastolic blood pressure of 90 mm Hg or higher, was not observed in any subject. Dilutional anemia occurred in all subjects, as expected, with an average 2.6 gm% decrease in hemoglobin, concomitant with mild fluid retention with a mean 0.4 kg increase in body weight. A dilutional decrease in platelet count, mean decrease of 41,000/μl, occurred in all but one subject who had type 2B VWD and baseline thrombocytopenia (Pt #2). Transient thrombocytopenia (132,000/μl) occurred after rhIL-11 in only one subject (Pt #5). The anemia and fluid retention resolved by day 10, or six days following cessation of the drug, accompanied by a reactive mean increase in platelets of 63,000/μl (Table 4).

Discussion

This study demonstrates that subcutaneous rhIL-11 administered for four consecutive days results in a gradual increase in VWF and F.VIII beginning day 2 and continuing for the four days the drug is given. The responses to rhIL-11 among VWD patients unresponsive or allergic to DDAVP, as well as in those with mild or moderate hemophilia A, were between 1.5 and 2-fold increased over baseline, similar to responses previously observed in mild VWD.[9, 10] In contrast to DDAVP, the increases in VWF and F.VIII observed after rhIL-11 were sustained and not refractory to repeated injections.[6,17] While the increases in VWF and F.VIII levels possible with rhIL-11 were modest as compared with DDAVP, they appear to be clinically effective in reducing blood loss in women with VWD and menorrhagia[10] and in VWD patients undergoing surgery (unpublished). Further, although not assessed in this study, the increase in F.VIII would be anticipated to improve hemostasis or prevent bleeding in individuals with mild or moderate hemophilia A. It is worth noting that elevated VWF and F.VIII levels have been associated with cardiovascular risk:[18, 19] thus, modest increases in VWF and F.VIII might be preferred. It is possible that the mild increases in VWF and F.VIII may represent natural variation from one day to the next, e.g. after inflammation, physical stress. Such increase in patients, with ability to synthesize VWF, may have influenced our results.

DDAVP administration during the rhIL-11 trial resulted in a boost in VWF and F.VIII responses, suggesting that rhIL-11 acts by a mechanism different than DDAVP, that is, by an effect other than release of VWF and F.VIII from endothelial stores.[17] The further increase in VWF high molecular weight multimers by DDAVP also implies that a DDAVP-releasable VWF pool persists even after rhIL-11 treatment and, moreover, that rhIL-11 has little if any effect on VWF trafficking to endothelial Weibel Palade bodies, consistent with

previous findings in dogs.[20] These findings suggest that rhIL-11 might possibly be used in combination or sequentially in the treatment of mild or moderate VWD or hemophilia A, especially when tachyphylaxis prevents further use of DDAVP, or in individuals who are partially responsive, unresponsive, or allergic to DDAVP and prolonged higher levels of VWF or F.VIII are desired, e.g. postoperatively. Further studies will be needed to investigate the clinical efficacy of rhIL-11 in such patients. Concomitant with the increases in VWF:RCo and VWF:Ag, there was a modest increase in VWF mRNA following rhIL-11, providing additional evidence that the mechanism of rhIL-11 increase in VWF is via upregulation of VWF mRNA, as previously suggested in studies of humans[9, 10] and dogs[20] with VWD, but not mice.[21] It is still not known whether rhIL-11 induces an increase in the number of Weibel Palade bodies or prevents loss of DDAVP responsiveness.

The side effects of rhIL-11 are generally mild and reversible on cessation of the drug, as shown in previous studies of mild VWD.[9, 10] Further, rhIL-11 is effective in doses lower than used in cancer chemotherapy patients to boost platelet counts,[22, 23] thereby avoiding potential complications including arrhythmias and fluid overload. Another advantage of rhIL-11 is the lack of transmissible agent risk, thereby allowing for safer hemostasis than with current plasma-derived VWF concentrates.

Although the current data regarding rhIL-11 are from small phase II studies, if proven safe and effective in future clinical trials, rhIL-11 might be a useful adjunct to the current agents available for management of mild or moderate hemophilia A or VWD, in particular those in whom DDAVP response or duration is limited or in whom DDAVP is contraindicated. A randomized controlled clinical trial has been proposed to evaluate the safety and efficacy of rhIL-11, as compared with the antifibrinolytic agent, tranexamic acid, in reducing bleeding in women with VWD and menorragia.[24]

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1. What is known on this topic

- Up to 20% of those with VWD are unresponsive or allergic to desmopressin (DDAVP).
- There is a lack of alternative treatment acting as endogenous VWF and FVIII release, respectively, for individuals with VWD type 1 or 2 and with hemophilia A who are unresponsive or allergic to DDAVP.

2. What this paper adds

- In this pilot Phase II trial, recombinant interleukin-11 (rhIL-11) increased VWF and F.VIII levels 1.3 to 2-fold in individuals with VWD unresponsive or allergic to DDAVP and in mild and moderate hemophilia A.
- Recombinant interleukin-11 was well tolerated, with grade 1 or less toxicity, except for transient grade 3 hyponatremia in one subject caused by excessive fluid intake for diabetic hyperglycemia.
- There is a progressive daily increase in VWF, which is believed to be related to a progressive increase in new VWF mRNA translation.
- rhIL-11 effects on VWF and F.VIII indicate it may be an alternative agent for individuals with VWD who are allergic or unresponsive to DDAVP, and mild or moderate hemophilia A.

Table 1

Clinical and Laboratory Findings in Study Subjects

Pt	Age/ Sex	Dx	VWF: RCo (%)	VWF: Ag (%l)	FVIII: C (%)	DDAVP Response (Historic)	OCP Use	Pre	VWF:RCo (%) Day 4	DDAVP	Pre	VWF:Ag (%) Day 4	DDAVP	Pre	FVIII:C (%) Day 4	DDAVP	Pre	FVIII:Ag (%) Day 4	DDAVP	VWF mRNA Fold-increase Pre - Day 4
VWD Patients																				
# 1	25/F	1 VWD	12.0	11.0	32.0	Incomplete	−	≤12.5±0.0	22.0±9.5	45.7±26.2	12.0±5.4	25.4±6.2	48.8±15.8	18.0±1.0	48.0±7.0‡	124.7±35.6†	24.6±5.3	25.7±1.0	66.3±22.0	0.55-fold
# 2	22/F	2B VWD	20.0	6.0	39.0	Not indicated	−	≤12.5±0.0	≤12.5±0.0	NA	24.7±2.6	35.4±18.0	NA	37.4±12.3	85.7±13.2‡	NA	29.7±1.3	50.9±5.4‡	NA	NS
# 3	25/F	1 VWD	10.0	6.0	16.0	Incomplete	+	≤12.5±0.0	≤12.5±0.0	32.2±19.7	4.5±0.1	5.7±0.4	26.2±14.9	6.9±0.0	5.5±0.1‡	53.6±33.8	8.9±0.6	11.3±0.3‡	46.0±22.8	−0.29-fold
# 4	26/F	2M VWD	10.0	48.0	99.0	Allergic	+	64.0±4.0	92.0±2.0‡	NA	36.7±1.3	71.7±18.3	NA	88.5±6.3	149.9±14.8‡	NA	46.1±0.3	86.6±19.1*	NA	−0.47-fold
Fold Increase									1.30-fold	2.33-fold		1.69-fold	3.25-fold		1.86-fold	6.17-fold		1.47-fold	3.32-fold	0.07-fold
Hemophilia A Patients																				
# 5	25/M	Mild HA	58.0	65.0	8.0	Incomplete	−	78.0±5.0	121.0±25.0	227.0±11.0‡	80.8±7.1	138.9±2.7‡	207.1±5.9‡	10.2±0.3	19.2±1.2‡	78.2±12.4‡	5.8±0.6	12.7±0.7‡	36.1±6.2‡	2.07-fold
# 6	51/M	Mild HA	128.0	92.0	12.0	Incomplete	−	73.5±3.5	151.0±43.0‡	191.0±25.0	80.2±5.7	171.3±8.9‡	222.0±19.7†	24.8±0.7	37.1±1.4‡	74.0±3.7‡	12.5±0.4	25.5±0.3‡	43.3±3.5‡	0.40-fold
# 7	31/M	Mild HA	135.0	141.0	11.0	Responsive	−	112.0±24.0	208.0±0.0‡	324.0±60.0	135.4±2.9	235.3±13.0‡	273.4±43.8	7.4±0.2	7.8±0.0*	23.8±7.9*	11.4±2.6	19.1±0.8‡	46.5±17.6	1.15-fold
# 8	28/M	Mild HA	NA	NA	12.0	Responsive	−	92.0±10.0	140.0±4.0	290.0±10.0	122.1±11.1	141.9±30.0	277.9±7.0‡	26.9±0.4	32.6±0.1‡	103.1±11.0‡	15.0±0.3	21.2±1.1‡	53.3±4.3‡	0.40-fold
# 9	34/M	Mod HA	NA	NA	4.0	Incomplete	−	58.0±8.0	97.0±3.0‡	171.0±9.0‡	80.3±6.6	135.7±16.1†	198.1±11.8†	1.7±0.2	1.9±0.2	5.2±1.3†	6.9±0.0	6.9±0.0	6.9±0.0	2.65-fold
Fold increase									1.73-fold	1.71-fold		1.69-fold	1.47-fold		1.48-fold	3.00-fold		1.66-fold	2.10-fold	1.33-fold

Dx is diagnosis; VWD is von Willebrand disease; HA is hemophilia A; VWF:RCo is von Willebrandristocetin cofactor activity; VWF:Ag is von Willebrand factor antigen; FVIII:C is factor VIII activity; DDAVP is desmopressin; OCP is oral contraceptive use during study; VWF mRNA is VWF messenger RNA; NA is not applicable; NS is not sufficient. Results are expressed as mean ± standard error of the mean (SEM).

*indicates $p<0.05$;

†is $p<0.01$;

‡is $p<0.001$. Fold-increase in VWF and F.VIII values by Day 4 post rhIL-11 are comparedwith pre-rhIL-11, and Day 4 post DDAVP with Day 4 pre-DDAVP (post rhIL-11) values.For the “Pre” values, we averaged two baseline levels, one at screening and one before rhIL-11 injection on Day 1. For the “Day 4” values, we averaged the day 3 and day 4 values. For the “DDAVP” values, we averaged values obtained at 30 minutes and 4 hours after DDAVP.

Table 2

Study Design and Schema

ASSESSMENT	Fluid Status (Height, Weight, Hx, Exam)				Pregnancy Test (Urine)	Safety Tests (Na, K)	Coagulation Tests (VWF,F.VIII, mRNA)	Hematology Tests (H/H, Platelets)	Diary
PRE-TRIAL In-Hospital Assessment Day 1-4	X	X	X	X	X	X	X	X	-
DURING TRIAL In-Hospital Assessment Day 1-4	X	X	X	X	X	X	X	X	-
POST-TRIAL Outpatient Assessment Day 10 after rhIL-11	X	X	X	X	-	X	X	X	X

Fluid status, including height, weight, physical exam for congestive heart failure and papilledema, and clinical assessment for finger orankle swelling, were performed in-hospital. Urine pregnancy tests were performed in females daily in-hospital before taking rhIL-11. Safety Testsincluded Na+ and K+; Coagulation Tests included VWF assays, VIII assays, closure times, and VWF mRNA; and Heme Tests included hemoglobin, hematocrit (H/H) and platelets. All assessments were repeated day 10 post rhIL-11 in the outpatient clinic. All symptoms and concomitant medications were collected by study subjects and reviewed with nurses at the day 10 outpatient visit.

Table 3

Adverse Events

Adverse Event	No. of Events	Percent
Conjunctival injection	6	66.7%
Dyspepsia	3	33.3%
Fluid retention	2	22.25
Flu-like symptoms	2	22.2%
Headache	2	22.2%
Injection site erythema	2	22.2%
Lightheadedness	1	11.1%
Flushing	1	11.1%
Insomnia	1	11.1%
Drowsiness	1	11.1%
Hyponatremia *	1	11.1%
Thrombocytopenia †	2	22.2%

* Hyponatremia occurred in a diabetic patient after excess fluid intake for symptomatic hyperglycemia, which resolved with fluid restriction.

† One of the two patients with thrombocytopenia was the 2B VWD patient, whose baseline platelet count was low and remained unchanged after rhIL-11. All adverse events were grade 1 or lower, except for hyponatremia which was grade 3 (129 mEq/L).

Table 4

Platelet Counts Before and After rhIL-11

Study Subject			*Platelet Count*(No./μl)		
Pt	**Age/Sex**	**Diagnosis**	**Pre**	**Day 4**	**Day 10**
			VWD Patients		
#1	25/F	1 VWD	227,000	180,000	298,000
#2	22/F	2B VWD	NA (clumped)*	22,000	NA (clumped)*
#3	25/F	1 VWD	217,000	215,000	201,000
#7	26F	2M VWD	266,000	200,000	333,000
	Fold Increase			0.84-fold	1.17-fold
			Hemophilia A Patients		
#4	25/M	Mild HA	307,000	250,000	399,000
#5	51/M	Mild HA	181,000	132,000	202,000
#6	31/M	Mild HA	221,000	184,000	270,000
#8	26/M	Mild HA	250,000	231,000	377,000
#9	34/M	Mod HA	274,000	224,000	364,000
	Fold Increase			0.83-fold	1.31-fold

VWD is von Willebrand disease; Dx is diagnosis; HA is hemophilia A; rhIL-11 is recombinant interleukin-11; NA is not available. The day 4 platelet count was obtained at 30 minutes following the fourth of four consecutive daily doses of rhIL-11, while the Day 10 platelet count was obtained 10 days after the first dose (or 6 days after the final dose of rhIL-11). It should be noted that while plateletsclumped in the single patient with 2B VWD, basal platelet count are in the 20,000/μlrange, most recently 26,000/μl, indicating no increaseoccurred after rhIL-11.