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Phase II prospective open-label trial of recombinant interleukin-11 in women with mild von Willebrand disease and refractory menorrhagia*

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Summary

Lack of effective treatment for menorrhagia is the greatest unmet healthcare need in women with von Willebrand disease (VWD). We conducted a single-centre phase II clinical trial to determine efficacy and safety of recombinant IL-11 (rhIL-11, Neumega®) given subcutaneously for up to seven days during six consecutive menstrual cycles each in seven women with mild VWD and menorrhagia refractory to haemostatic or hormonal agents. rhIL-11 reduced menstrual bleeding severity as measured by pictorial blood assessment chart (PBAC) ≥50% (to <100) in 71% of subjects, cycle severity ≥50% in 71%, and bleeding duration ≥2 days in 85%, all p≤0.01. After rhIL-11, plasma VWF:RCo increased 1.1-fold, but did not correlate with PBAC, r=0.116, bleeding duration, r=0.318, or cycle severity, r=−0.295, or hsCRP, r=−0.003, all p>0.05. Platelet VWF mRNA expression by quantitative PCR increased mean four-fold (1.0–13.5). rhIL-11 was well tolerated with grade 1 or less fluid retention, flushing, conjunctival erythema, and local bruising. In summary, rhIL-11 reduces menorrhagia safely and warrants further study.

Keywords

Clinical trial; interleukin-11; menorrhagia; von Willebrand disease; women

Introduction

Among menstruating women with von Willebrand disease (VWD), up to 80% have menorrhagia (1), with significant morbidity, iron deficiency anaemia, and poor quality of life (1–5). Yet, ineffective therapy for menorrhagia is a major unmet healthcare need in women with VWD (1, 2). While 80% respond to intravenous or intranasal 1-deamino-8-D-arginine vasopressin (DDAVP) (1, 4, 6–8), only 31% use it for menorrhagia (9), as the effect

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Conflicts of interest

None declared.

is short-lived and though convenient, the intranasal route may be less predictable (6). Among 70% who seek hormonal treatment for menorrhagia at haemophilia centres, only 35% use it (9), with side effects of headache and hypertension (4). While tranexamic acid (TA) is effective in reducing menses by pictorial blood assessment chart (PBAC) in 33%, its use is limited by headache (10). Thus, safe, effective drugs are needed for women with menorrhagia (5, 11, 12). Recombinant human IL-11 (Neumega®, rhIL-11) is a gp-130 cytokine (13, 14) which increases von Willebrand factor (VWF) over a range of doses, 10–50 μg/kg, with good tolerance (15). We therefore initiated a phase II trial to test the novel hypothesis that rhIL-11 would be effective and safe in reducing menorrhagia in women with VWD.

Materials and methods

Study subjects

Women age 18–45 years with mild VWD, defined as VWF:RCo <50 IU/dl with normal VWF multimers present, and menorrhagia, unresponsive to or intolerant of haemostatic or hormonal agents were recruited between January 2007 and March 2010. Pregnancy, lactation, heart disease, hypertension, arrhythmia, thrombosis, recent surgery or use of blood products were exclusions. Four subjects had type 1 VWD, defined by VWF:RCo/VWF:Ag ratio ≥0.50 (Patients. 2, 3, 5, 6; ▶ Table 1), and three had type 2M VWD, defined by VWF:RCo/VWF:Ag ratio <0.50 (Patients 1, 4, 7) (11). All subjects gave written informed consent. 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 open-label trial of rhIL-11 25 μg/kg/day by subcutaneous self- injection for up to seven days during each of six consecutive menstrual cycles. The dose was selected based on safety and biologic efficacy in a previous trial in patients with VWD (15). To assess safety and biologic efficacy prior to home use, rhIL-11 was given days 1–4 pre-trial in the non-bleeding state (see ▶ Table 1). Safety measures included pre-dose orange juice to avoid hypokalaemia, and urine pregnancy testing. VWD tests were measured at baseline and days 1–4, and platelet VWF mRNA at baseline and day 4. Safety was assessed by medical history, physical examination, vital signs, height, weight, cardiac, chest, and fundoscopic exams at baseline and days 1–4. Menstrual severity was assessed by pictorial blood assessment chart (PBAC) (16), bleeding duration, and cycle severity, compared with two pre-trial cycles. Cycle severity was subjectively rated 0=mild bleeding, much less than usual, 1=moderate bleeding, less than usual, 2=moderately severe bleeding, not as bad as the worst, 3=severe bleeding, as bad as the worst cycle. Coagulation tests included VWF:RCo, VWF:Ag, FVIII:C, FVIII:Ag, VWF multimers, epinephrine (CEPI) and adenosine diphosphate (CADP) closure times; and safety tests, haemoglobin, platelet count, electrolytes, and electrocardiogram. Study diaries completed by the patients included all symptoms, dates of study drug administration, concomitant medications, pre-dose orange juice, and urine pregnancy testing. Safety monitoring and drug compliance were by diary records, the former cross-checked by subject interview, the latter cross-checked with returned drug vials. Safety monitoring was by daily history, examination, fluid assessment (as above) pre-trial, and by daily subjective fluid assessment during menstrual cycles.

Laboratory assays

VWF activity was measured by ristocetin-induced platelet agglutination (Chronolog Corp., Havertown, PA, USA) using a Chronolog aggregometer (17–20); and VWF:Ag by “sandwich” ELISA, using anti-VWF antibodies (DakoA082, Carpintera, CA, USA). Results were expressed in U/ml, with normal human plasma pool designated 1.00 U/ml, and severe

type 3 VWD plasma, the negative control, VWF:RCo 15 U/ml and VWF:Ag 1 U/ml (George King Bio-Medical, Overland Park, KS, USA). VWF multimers were analysed by SDS gel electrophoresis using 1.5% or 0.65% agarose gels. FVIII:C was determined by chromogenic substrate assay (Coamatic, DiaPharma Group, Westchester, OH, USA); FVIII:Ag by immunoassay (Asserochrom, Diagnostica Stago, Gennevilliers, France); closure times by PFA-100 analyser (15); and platelet VWF mRNA by reverse transcription of platelet-rich plasma and amplification by quantitative PCR, normalised against GP1Bb mRNA (15). Highly sensitive C-reactive protein (hsCRP) was performed by "sandwich" ELISA, using anti-CRP antibodies (Zymutest CRP, Aniara, Mason, OH, USA).

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 (21), with 95% confidence intervals (CI) to show an increase of 0.2 VWF U/ml, with σ=0.1 VWF U/ml, at α=0.05 and β=0.90 (one-sided). Accrual was slow, and, in the absence of significant toxicity in the first seven subjects, the study was closed early, as two additional subjects added no additional power to assessment of adverse event rates, upper bound 35% versus 28% for seven and nine subjects, respectively. The seven who participated represented 6.1% of the 115 potentially eligible women age 18–45 years with mild VWD followed at the Hemophilia Center of Western Pennsylvania: of the 108, 42 (36.5%) had no menorrhagia due to hormone treatment, hysterectomy, or pregnancy; 21 (18.3%) had moved out of town due to college, job, family; 17 (14.8%) were unable to participate due to family, childcare, work; 14 (12.2%) had medical exclusions including hypertension, heart disease, second coagulation disorder, upcoming surgery or chronic illness); and 14 (12.2%) were lost to follow-up.

Efficacy was based on menstrual severity by PBAC, and safety was based on fluid retention, flushing, and injection site bruising, rated by NCI Common Terminology Criteria (22). Data were analysed by descriptive statistics. Pre- and post-rhIL-11 VWF were compared by Student's t-test; VWF assays were compared with PBAC, bleeding duration, cycle severity, hsCRP, and fold-increase in platelet VWFmRNA and VWF:Ag by Spearman rank correlation.

Results

Following rhIL-11, there was a significant reduction in PBAC, cycle severity, and bleeding duration, as compared with cycles before rhIL-11 (▶Table 2). PBAC decreased ≥50% in 71% of subjects after six months, cycle severity by ≥50% in 71%, and bleeding duration by ≥2 days in 85%, all $p \leq 0.01$, persisting each month rhIL-11 was given. The drug was well tolerated in all subjects with grade 1 or less toxicity, including conjunctival erythema (n=7), mild fluid retention (n=6), injection site bruising (n=6), flushing (n=3), thrombocytosis, 484,000/μl (n=1), hypokalaemia, 3.4 mEq/l (n=1), and diastolic hypertension, 93 mmHg (n=1), all anticipated side effects, the latter two associated with rhIL-11-induced fluid retention. All adverse effects resolved with cessation of rhIL-11, except diastolic hypertension in a single subject which resolved after day 1 despite three additional days of rhIL-11.

By day 4 following rhIL-11, there was a 1.1-fold increase in VWF:RCo, 1.6-fold in VWF:Ag, 1.3-fold in FVIII:C, and 1.7-fold in FVIII:Ag, compared with baseline, beginning on day 2 and returning to baseline by day 10. VWF:RCo did not correlate with PBAC ($r=0.116$), bleeding duration ($r=0.318$), or cycle severity ($r=-0.295$) (all $p>0.05$). CEPI and CADP, not prolonged at baseline, decreased by 1.1– to 1.2-fold ($p<0.05$). Multimer distribution remained unchanged. There was a four-fold increase in platelet VWF mRNA (1.0– to 13.5-fold), but it did not correlate with fold-increase in VWF:Ag ($r=-0.103$,

p>0.05). Highly sensitive CRP levels increased minimally (1.2-fold) after hIL-11, median 7.0 to 11.1 μg/ml, p>0.50, (normal range, 0.2–10.0 μg/ml), and there was no correlation with VWF:RCo (r=−0.003, p>0.50).

Discussion

The results of this trial indicate rhIL-11 safely reduces menstrual bleeding (PBAC), cycle severity, and bleeding duration in women with VWD. The reduction in PBAC ≥50%, in 71%, is similar to that with TA, 40% (10), which falls within 95% CI around 71%. The increase in platelet VWF mRNA expression after rhIL-11 suggests the VWF increase occurs via increased synthesis (15). The lower magnitude increase in VWF:Ag than platelet VWF mRNA may be due to a progressive increase in new platelet VWF mRNA translation, and, further, as the four-day VWF:Ag increases observed in this study were less than those observed during the seven-day increases observed in our previous study (15), it is possible that longer rhIL-11 treatment, e.g. seven days or more, is necessary to achieve greater increase in VWF levels, as we found in our previous seven-day biologic effects trial (15). More time points will be needed for clarification in future prospective studies (23).

It is also possible that rhIL-11 had no effect on menstrual cycles and that the improvement in subsequent menstrual cycles may reflect regression to the mean, as women often seek treatment when their symptoms are worst. The lack of controls in this study, however, makes it difficult to address this question. Alternatively, if rhIL-11 does have an effect on menstrual cycles, it is possible it is mediated not through VWF but possibly through a local uterine mechanism. While studies of uterine fluid for markers of increased coagulation and fibrinolysis have been detected during menses, these do not appear to correlate with menstrual blood loss (24). Finally, it is also possible that rhIL-11-induced inflammation contributes to the increase in VWF and factor VIII levels observed in this study (25, 26). However, the lack of significant increase in hsCRP following rhIL-11 suggests that the observed changes are not likely due to inflammation. Future prospective studies of more patients with more time points and inclusion of controls will be needed to help interpret the findings of this study. In that regard, an ongoing phase II biologic effects study of rhIL-11 in type 2 and 3 VWD and mild haemophilia A patients, including those refractory to DDAVP, is enrolling patients, and a phase III randomised trial comparing rhIL-11 versus TA to reduce menorrhagia in women with VWD is planned (27).

There are a number of limitations of this study. First, the small sample size and lack of controls as well as blinding of subjective endpoints (e.g. PBAC) limit interpretation of the findings. Secondly, the lack of correlation between VWF levels and PBAC, and the higher VWF levels during the in-hospital study than at diagnosis, both of which were also observed in our previous study (15), will require further study of larger numbers of subjects to clarify the mechanism of the rhIL-11 effect. Finally, the stress of blood draws, injections, or hospitalisation may increase VWF, and although it was also observed in our previous trial (15), has not been noted before in clinical trials and will require further study.

In conclusion, we present the findings of a novel therapy for a condition in which there is little investigation into therapeutic options and for which lack of therapeutic agents is a major unmet health need. Specifically, we report that low-dose subcutaneous rhIL-11 safely reduces menorrhagia in women with mild VWD. We caution, however, that these findings are experimental and will require further study before any recommendations can be made about its use in clinical practice for the treatment of menorrhagia.

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What is known about this topic?

- Menorrhagia is the most common symptom among menstruating women with von Willebrand disease (VWD).
- The single greatest unmet need among women with VWD is the lack of safe, effective agents to treat menorrhagia.

What does this paper add?

- In this pilot phase II study, we found that recombinant interleu-kin-11 (rhIL-11) reduces the severity of menorrhagia by reducing pictorial blood assessment chart (PBAC), an objective, validated measure of menstrual blood loss, cycle severity, and duration of menses.
- rhIL-11 was well tolerated, with grade 1 or less toxicity, and could be given by self subcutaneous injection by all the women participating in the study.
- There was a progressive daily increase in von Willebrand factor (VWF), which we believe is due to a progressive increase in new VWF mRNA translation, as four-day VWF:Ag increases were less than seven-day increases reported in our previous trial (15).

Table 1

Study design and schema.

Assessment	Fluid status				Pregnancy test (Urine)	Safety tests (Na, K)	Coagulation tests (VWF, VIII, mRNA)	Heme tests (H/H, platelets)	Diary
	Ht	Wt	Exam	Swelling					
PRE-TRIAL		X	X	X	X		X	X	-
In-hospital assessment	X					X			
Day 1–4									
DURING TRIAL	–	–	–	X	X	-	-	-	X
Home assessment									
Month 1–6									
POST-TRIAL	X	X	X	X	-	X	X	X	X
Outpatient assessment									
Day 10 after rhIL-11									

Fluid status, including height, weight, physical exam for congestive heart failure and papilledema, and assessment for finger or ankle swelling, were performed in hospital, and the latter also at home. Urine pregnancy tests were performed daily in-hospital and monthly at home before taking rhIL-11. Safety tests, including Na+ and K+, were performed in-hospital. Coagulation tests, including VWF assays, VIII assays, closure times, and VWF mRNA, were performed in-hospital. Heme tests, including haemoglobin, haematocrit (H/H) and platelets, were performed in-hospital. All assessments were repeated day 10 post rhIL-11, month 6, in clinic. Diaries, including all symptoms, concomitant medications, time and date of orange juice daily before rhIL-11, and time and date of rhIL-11 injections, were maintained during study by subjects and reviewed with nurses at monthly outpatient visits, at which time all used supplies were returned and new supplies were provided.

Table 2

Clinical and laboratory findings in study subjects.

No./age (years)	BS at Dx/OCP Use (−/+)	VWF:RCo U/ml	VWF:Ag U/ml	VIII:C U/ml	RCo/Ag ratio/MULT	PBAC			Bleeding duration – Day			Cycle severity (0 – 3)			VWF:RCo U/ml		VWF:Ag U/ml		VIII:C U/ml		VIII:Ag U/ml		VWF mRNA fold- increase Pre to Day 4
						Pre	Mo 2	Mo 6	Pre	Mo 2	Mo 6	Pre	Mo 2	Mo 6	Pre	Day 4	Pre	Day 4	Pre	Day 4	Pre	Day 4	
#1/28	5 (−) c,e,m,p	0.34	1.23	1.32	0.28/Nl	1400 ± 0	17 ± 2 *	281 ± 345 *	28.5 ± 16.5	7.0 ± 0.0	12.6 ± 2.8	2.5 ± 0.5	0.0 ± 0.0 *	0.8 ± 0.5 †	1.61± ± 05	1.26 ± 0.00 *	1.76 ± 0.37	1.82 ± 0.10	1.56 ± 0.22	1.15 ± 0.25 *	1.57 ± 0.05	2.25 ± 0.07 *	1.2 (1.2–1.3)
#2/35	6 (−) c,d,e,m,mu	0.37	0.58	0.46	0.64/Nl	222 ± 18	92 ± 1 *	61 ± 11 *	7.0 ± 0.5	3.5 ± 0.5 *	4.0 ± 0.3 *	2.0 ± 0	1.0 ± 0.0 *	1.3 ± 0.2 *	1.25 ± 0.17	1.56 ± 0.10	0.78 ± 0.03	1.11 ± 0.09 *	0.81 ± 0.01	1.46 ± 0.08 *	0.72 ± 0.05	1.14 ± 0.01 *	13.5 (8.5–21.4)
#3/36	4 (−) c,g,m	0.29	0.44	0.39	0.66/Nl	145 ± 138	19 ± 14	15 ± 4	6.0 ± 1.0	4.0 ± 2.0	4.0 ± 0.6	1.5 ± 0.5	0.5 ± 0.5	0.2 ± 0.2 *	1.06 ± 0.12	1.30 ± 0.02 ‡	0.58 ± 0.29	0.98 ± 0.36	1.09 ± 0.10	1.95 ± 0.66	0.99 ± 0.22	2.19 ± 0.02 *	2.7 (1.7–4.3)
#4/29	6 (+) c,e,m,p	0.22	0.72	0.82	0.30/Nl	1025 ± 0	701 ± 49	557 ± 161 *	6.0 ± 0.0	12.0 ± 4.0	9.0 ± 1.8	2.0 ± 0	2.5 ± 0.5	1.8 ± 0.3	0.87 ± 0.17	1.42 ± 0.20 ‡	0.75 ± 0.13	1.71 ± 0.10 *	0.98 ± 0.11	1.35 ± 0.03 *	0.90 ± 0.02	2.37 ± 0.05 *	1.0 (0.9–1.1)
#5/40	5 (+) c,m,mu,w	0.43	0.54	1.03	0.80/Nl	410 ± 0	140 ± 60 *	94 ± 39 *	6.0 ± 0.0	4.0 ± 0.0 *	3.8 ± 0.2 *	2.0 ± 0	1.0 ± 0.0 *	1.0 ± 0.3 *	0.88 ± 0.05	1.37 ± 0.17 *	0.60 ± 0.01	0.69 ± 0.07	0.80 ± 0.18	0.84 ± 0.11	0.67 ± 0.21	0.80 ± 0.34	5.1 (4.5–5.8)
#6/42	4 (−) c,e,m	0.10	0.15	0.24	0.67/Nl	561 ± 4	46 ± 0.5 *	56 ± 24 *	6.5 ± 0.5	4.5 ± 0.5 *	4.2 ± 0.3 *	3.0 ± 0	0.0 ± 0.0 *	0.7 ± 0.3 *	0.12 ± 0.00	0.12 ± 0.00	0.12 ± 0.02	0.17 ± 0.01 *	0.31 ± 0.01	0.60 ± 0.12 †	0.25 ± 0.01	0.36 ± 0.01 *	1.6 (1.4–1.7)
#7/21	2 (+) m	0.10	0.25	0.36	0.40/Nl	299 ± 78	177 ± 59	171 ± 32	9.5 ± 1.5	6.5 ± 1.5	5.4 ± 0.7 *	3.0 ± 0	1.5 ± 0.5 *	1.4 ± 0.2 *	0.12 ± 0.00	0.12 ± 0.00	0.06 ± 0.02	0.12 ± 0.01 *	0.38 ± 0.02	0.29 ± 0.11	0.21 ± 0.01	0.31 ± 0.01 *	ns

BS is bleeding score; Dx is diagnosis; OCP is oral contraceptive use during study; VWF:RCo, von Willebrand ristocetin cofactor; Ag; antigen; FVIII:C, factor VIII activity; MULT is multimers; Nl is normal multimers; OCP is oral contraceptive use during study; c, cutaneous; d, dental; e, epistaxis; g, gastrointestinal; m, menorrhagia; mu, musculoskeletal; o, oral; p, postpartum; s, surgery; w, wound bleeding; PBAC is pictorial blood assessment chart. VWFmRNA is von Willebrand factor mRNA. ns is sample not sufficient. Clinical and laboratory values are expressed as mean ± standard error of the mean (SEM). Student's t test was used to compare pre-treatment clinical parameters from two baseline cycles, with values after 2 cvcles (inclusive) and after 6 cycles (inclusive) post-treatment; and to compare pre-treatment laboratory assays with day 4 values post-treatment. Significance is indicated by

* $p < 0.001$;

† $p < 0.01$;

‡ $p < 0.05$.