A randomised pilot trial of the anti-von Willebrand factor aptamer ARC1779 in patients with type 2b von Willebrand disease

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Summary

Desmopressin aggravates thrombocytopenia in type 2B von Willebrand disease (VWF type 2B) by release of large and hyper-adhesive von Willebrand Factor (VWF) multimers. This pilot study investigated whether the anti-VWF aptamer ARC1779 can prevent desmopressin-induced thrombocytopenia and interferes with the excessive VWF turnover in patients with VWF type 2B. Concentration effect curves of ARC1779 were established for five patients *in vitro* and two patients with VWF type 2B were treated by infusion of ARC1779, desmopressin, or their combination in a randomised, controlled, double-blind design. ARC1779 concentrations in the range of 1–3 μg/ml blocked free A1 domain binding sites by 90% *in vitro*. *In vivo*, desmopressin alone induced a profound (-90%) drop in platelet counts in one of the patients. ARC1779 (4–5 μg/ml) completely inhibited VWF A1 domains and prevented this desmopressin-induced platelet drop. Desmopressin alone increased VWF antigen two- to three-fold, accompanied by concordant changes in VWF Ristocetin cofactor activity (RCo) and coagulation factor VIII activity. ARC1779 substantially enhanced the desmopressin-induced maximal increase in these parameters, and improved multimer patterns. No treatment related adverse events were observed and no bleeding occurred despite marked thrombocytopenia. These data provide first proof of concept in humans and evidence that ARC1779 is a potent inhibitor of VWF. ARC1779 prevented the rapid consumption of VWF multimers together with agglutinated platelets that occurred in response to desmopressin challenge in patients with VWD type 2B.

Keywords

Clinical trials, antiplatelet drugs, von Willebrand factor, thrombocytopenia, desmopressin

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Introduction

Von Willebrand type 2B (VWF type 2B) is a rare disorder which is not caused by a deficiency of von Willebrand factor (VWF), but instead by gain of function mutations of VWF (1). The molecular defects in VWF type 2B enhance the affinity of VWF to glycoprotein Ib and thus binding to platelets (2). The mutated VWF molecules spontaneously bind to normal platelets, and the high-molecular-weight (HMW) multimers are digested by the metalloprotease ADAMTS-13. As a consequence, VWF type 2B patients become deficient in both large VWF multimers and platelets. Thus, the principal clinical manifestations of VWF type 2B are epistaxis, menorrhagia, and bleeding resulting from trauma, surgery, or childbirth.

Transfusion of factor VIII/VWF concentrates is of benefit to most of type 2B VWD patients. Unlike type 1 VWD, the defect in VWF type 2B is not a simple problem of deficiency of VWF and cannot be entirely corrected with desmopressin (3). In fact, infusion of desmopressin induces the release of large VWF and hyperactive multimers from endothelial cells, thus causing a transient thrombocytopenia in patients with VWF type 2B because of formation of platelet agglutinates. Hence, it was previously suggested not to use desmopressin in these patients. More recently, however, it has been discussed that some VWF type 2B patients may also benefit from prophylactic desmopressin treatment in terms of protection from surgical bleeding, despite the transient thrombocytopenic response (4, 5) , and, notably, desmopressin administration is not pro-thrombotic in these patients (6).

The hyperactive multimers present in VWF type 2B constitute a unique *in vivo* model to demonstrate proof of concept for the efficacy of a VWF antagonist in humans.

Aptamers are a new class of oligonucleotide drugs (7) that, similar to antibodies, are able to block many proteins including those with pro- or anticoagulant action. ARC1779 is a synthetically manufactured aptamer which binds to the A1 domain of human VWF with high affinity, preventing interaction with platelet GpIb;

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the crystal structure of its parent compound has recently been characterisd (8). The core aptamer portion of ARC1779 (molecular weight, ~13.7 kDa without pegylation), is a 40-mer modified DNA/RNA oligonucleotide, which is conjugated with a 20-kDa polyethylene glycol moiety to form the active pharmaceutical ingredient, ARC1779 (molecular weight, ~33 kDa) (9). The potent anti-thrombotic activity of ARC1779 was recently demonstrated *in vivo* in a cynomolgus monkey carotid electrical injury thrombosis model (10). As with other VWF antagonists (11) it has little effect on bleeding time and may potentially have a decreased bleeding risk (12).

We hypothesised that ARC1779 inhibits the excessive VWF activity, enhanced clearance of large and hyper-active multimers (3), and desmopressin induced thrombocytopenia in VWF type 2B. To answer this question, we performed a pilot trial with VWF type 2B patients who received desmopressin alone, desmopressin after an infusion of ARC1779 and ARC1779 alone.

Methods

Study design

The Ethics Committee of the Medical University of Vienna and the National Authority approved the study protocol, and the study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all patients before study entry. Thrombocytopenic patients with type 2B VWD were eligible: in 2008 we enrolled two subjects with VWF type 2B into this clinical trial, one male (aged 48 years) and one female (aged 66 years), and treated them by IV infusion of ARC1779, or desmopressin, or their combination in a randomised, double-dummy treatment sequence at the Department of Clinical Pharmacology.

Patients were treated in a three-period, randomised, crossover, double-dummy treatment sequence with wash-out periods of a minimum of one week. Randomisation was performed by a contract research organisation, and concealed by using sealed opaque envelops for each treatment period. These envelops were opened by an independent pharmacist, who performed the blinding of the study medication, so that all other staff members and the patients remained blinded. Patients received a 30-minute (min) infusion (0.23 mg/kg) plus a 4-hour (h) continuous infusion of 0.001 mg/kg/min ARC1779 injection (total of 0.47 mg/kg) or placebo. ARC1779 was started 30 min prior to the infusion of desmopressin-acetate (0.4 μg/kg over 30 min after start of ARC1779) or placebo. Based on a previous *ex vivo* study (13) and a trial in a patient (14) we estimated to be able to fully block free A1 domains with the continuous infusion of 0.001 mg/kg/min, whereas the 0.4 μg/kg dose of desmopressin is well known to be the highest clinically used dose to degranulate Weibel Palade bodies *in vivo*. Blood samples were taken pre-treatment, after 15 and 30 min, after 1, 2, 4, 8, and 12 h, and on the following two days.

Laboratory methods

Samples for analysis were obtained into evacuated tubes containing 129 mM (3.8 %) citrate (Vacuette tubes; Greiner Bio-One, Kremsmuenster, Austria). Platelet counts were analysed in the hospital's clinical laboratory with a cell counter (Sysmex XE 2100, Kyoto, Japan). Blockade of the A1 domains of VWF was measured with a quantitative direct ELISA kit (REAADS VWF Activity ELISA Test Kit, Corgenix, Inc, Broomfield, CO, USA). (9) This ELISA utilises a purified murine anti-VWF monoclonal antibody which recognises a functional epitope on the VWF molecule to assess VWF activity levels. Results are reported in percent (%) of normal, relative to a calibrator that has been standardised against the third International Standard for Factor VIII and von Willebrand Factor in Plasma (91/666). Intra-assay and inter-assay variability are <6% and <8%, respectively. The detection limit was <3%, when this was reached the level of inhibition is set to 97% to provide a conservative estimate.

Before the pilot trial an *ex vivo* study was performed in five VWD type 2B patients (M:F = 2:3, age: 30–71 years). To construct concentration-effect curves for ARC1779, we spiked samples with a minimum of 10 different ARC1779 concentrations ranging from 0 to 5 mg/ml using an iterative algorithm. Spiked samples were incubated for 15 min at 37°C before blockade of the A1 domains of VWF was measured as described above.

ARC1779 plasma concentrations were determined with a validated high performance liquid chromatography assay with ultraviolet detection (linear range – 0.25 to 200 μg/ml) (9, 14). This assay measures all ARC1779 in plasma. VWF antigen concentration was measured with a fully automated simultaneous thermal analyzer using the STA Liatest VWF (Diagnostica Stago, Paris, France). Plasma activity of VWF ristocetin cofactor activity (VWF:RCo; primary endpoint) was assayed by turbidometry using a commercial kit (BC von Willebrand reagent; Behring Marburg, Germany) (15). In contrast to the REAADS assay, VWF:RCo cannot be used to quantify the levels or activity of the aptamer, because the aptamer directly binds to ristocetin. The VWF collagen binding assay was purchased from Corgenix (Peterborough, UK), and 10-fold lower activity was also found in samples spiked with ARC1779. The platelet function analyzer PFA-100, as described in more detail previously (16) was used for assessment of primary haemostasis, because closure times are highly sensitive to deficiency of HMW VWF (17), VWD (18, 19), and desmopressin infusion (20) and are inversely related to inhibition of VWF in the REAADS assay (9), but also predict thrombotic events (21–23). As a limitation the PFA-100 is also influenced by platelet VWF, which is not likely to be affected by ARC1779, unless VWF is released during platelet activation. Normal collagen adenosine diphosphate closure time (CADP-CT) is 68–121s. Factor VIII activity was measured on a Sysmex CA 7000 analyzer using factor VIII-deficient plasma (Hyland Baxter Immuno, Vienna, Austria) and Dade® Actin® -FS (Dade Behring, Marburg, Germany) in a one stage clotting assay (24). Quantification of VWF multimers was performed by sodium dodecyl sulphate-agarose discontinuous gel (1.2%) electrophoresis (25) followed by Western-Blotting and

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Table 1: Demographic and biochemical variables of patients in the *in vitro* study.

Sex	Age	VWF:CB (%)	Free A1* domains (%)	VWF:RCO (%)	VWF:Ag (%)	VWF:RCo/ VWF:Ag quotient	FVIII (%)	Platelets (/nl)	CADP-CT (s)
Normal ranges		60– 180%	60–180%	60-180%	60–180%	>0.4	60–180%	150-350/nl	68–121s
F #	66	**4**	**18**	**14**	91	**0.15**	65	**35**	**300**
M #	48	**9**	**35**	**18**	119	**0.15**	93	**82**	**300**
M	59	**16**	**33**	**15**	**49**	**0.31**	**48**	**64**	**300**
F	71	**11**	**66**	**17**	138	**0.12**	80	**54**	**300**
F	30	**3**	**17**	**18**	**28**	**0.64**	68	**40**	**300**

Abnormal values are bold. * designates the functional REAADS ELISA. # those two patients participated in the *in vivo* trial. CADP-CT, collagen adenosine diphosphate closure time measured with the platelet function analyzer (PFA-100). CB, collagen binding assay; F, female; M, male; RCO, ristocetin cofactor activity.

consequent quantification with a luminescence image analyzer (LAS-3000, Raytest GmbH, Berlin Germany). Final analysis was performed using AIDA Image Analyzer software version 4.11.

Pharmacokinetics/Pharmacodynamics Correlation: ARC1779 plasma concentrations and normalised values for percent blockade of the A1 domains of VWF activity or platelet function were fitted to a Sigmoidal Emax model with the following equation:

$$E=E0+(Emax-E0)*(C/(C+EC50)).$$

Sample size and statistical analysis

Due to the rarity of the disease and the demanding study design, we aimed to include as many patients as possible, but no formal sample size calculation was performed. Both patients enrolled in the interventional trial completed all three treatment periods without any relevant protocol deviations. Results are only presented in a descriptive manner.

Results

Ex vivo experiments prior to clinical trial

We established *ex vivo* concentration effect curves for ARC1779 on VWF activity in five patients with VWF type 2B in order to estimate the necessary plasma levels for a subsequent *in vivo* trial. Baseline data in ▶Table 1 show that all patients were thrombocytopenic and had low VWF:CB, low VWF:RCO and – as expected (26) -low VWF:RCO/VWF-Ag quotients. The 90 % inhibitory concentration (IC 90) of ARC1779 was fairly uniform about 1 µg/ml, leaving only median 3% (range 1–6%) of normal vWF activity, i.e. free residual A1 domains in the plasma (▶Fig. 1). Unfortunately, three of these patients were not eligible for the *in vivo* experiment: one did not consent, one could not be contacted and one patient could not participate due to high-grade carotid stenosis. The remaining two patients, who entered the clinical trial, are the first two characterised in Table 1. Both of them had a remarkable history of severe to life-threatening bleeding, particularly after operations, were treated with plasma derived VWF concentrates, and had acquired hepatitis C infection. Thus, for the interpretation of data it may be necessary to remember that hepatitis C increases VWF:Ag levels (27) but chronic liver disease impairs platelet production (28).

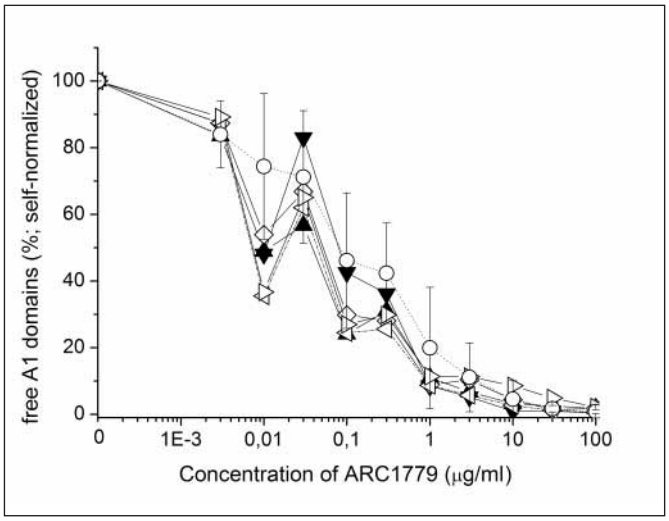

Figure 1: Concentration effect curves of the anti-von Willebrand factor (VWF) aptamer ARC1779 blocking free VWF A1 domains *in vitro*. Plasma of five different patients suffering from type 2B VWD was spiked with ARC1779, and 90% inhibition was seen at concentrations in the range of 1–3 µg/ml. For ease of presentation the first data point on the logarithmic concentration scale of the x-axis is described as zero. The dip at 0.01 µg/ml may be due to chance, but indicates some effects already at very low concentrations. A group of 40 concomitantly assessed control subjects (open circles, dashed line) (35) is presented to demonstrate that ARC1779 has similar inhibitory effects on normal and type 2B mutant VWF.

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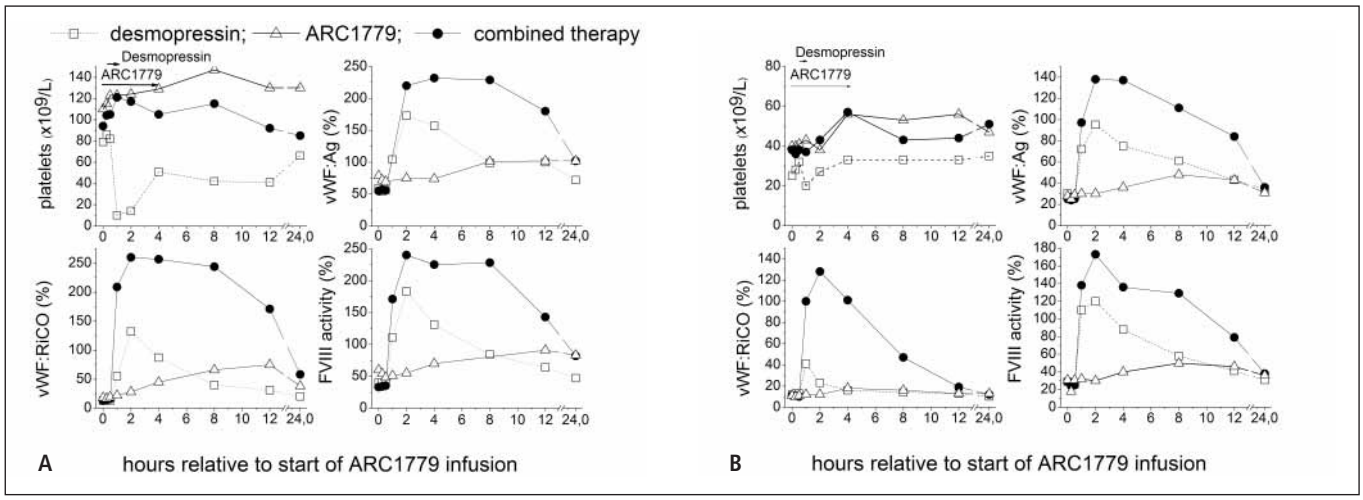

Figure 2: The anti-von Willebrand factor (VWF) aptamer ARC1779 blocks the desmopressin induced aggravation of thrombocytopenia and enhances the effects of desmopressin on VWF/factor VIII. Two patients (male: top 4 layers, female: bottom 4 layers) received ARC1779 (triangles, bolus primed continuous infusion of 0.001 mg/kg/min over 4 h), desmopressin (squares, 0.3 μg/kg) or their combination (circles). Pre-treatment with ARC1779 enhanced VWF/ FVIII levels as compared to desmopressin alone, and prolonged this effect considerably. Desmopressin rapidly aggravated thrombocytopenia in the male patient (top layers) and ARC1779 prevented this effect.

VWF antigen, VWF:RCO, F VIII activity and aPTT

The infusion of desmopressin alone increased VWF antigen concentrations two- to three-fold with peak levels seen at 1 h after end of infusion. This was accompanied by concordant changes in VWF:RCo and factor VIII activity (▶Fig. 2, ▶Table 2). ARC1779 alone slowly but continuously increased all these parameters over 8–12 h, and even normalised VWF:RCo in the first patient. The infusion of ARC1779 together with desmopressin apparently enhanced the desmopressin-induced maximal increase in all parameters. In addition these increases persisted for at least 12 h.

Desmopressin infusion normalised aPTT very rapidly, due to the increase in FVIII activity. (▶Fig. 3, ▶Table 3) The combined treatment of ARC1779 with desmopressin normalised aPTT with the same speed, but the effect lasted longer (Fig. 3, Table 3). The shortening of aPTT between predose and infusion was 10.0 seconds (s) and 11.8 s in both patients. In contrast, ARC1779 slightly prolonged the aPTT during the bolus administration.

HMW multimers of VWF

Consistent with previous reports (3, 4), desmopressin rapidly but transiently increased the amount of HMW VWF multimers (▶Fig. 4). The combination of ARC1779 and desmopressin prolonged the duration of this effect and normalised the VWF multimer pattern for a period of 12 h. ARC1779 alone gradually

Period		0	15'	30'	1 h	2 h	4 h	8 h	12 h	24 h
Platelet count (/nl)	DDAVP	52	57	57	15	21	42	38	37	51
Platelet count (/nl)	ARC1779/DDAVP	66	70	72	79	80	81	79	68	68
Platelet count (/nl)	ARC1779	75	78	82	83	81	93	100	93	89
VWF:Ag (%)	DDAVP	45	41	42	88	134	116	80	72	53
VWF:Ag (%)	ARC1779/DDAVP	40	41	41	97	179	185	170	132	69
VWF:Ag (%)	ARC1779	54	49	50	30	53	55	75	73	67
VWF:RCo (%)	DDAVP	13	14	13	48	78	52	27	22	15
VWF:RCo (%)	ARC1779/DDAVP	12	13	13	155	194	179	146	95	35
VWF:RCo (%)	ARC1779	15	14	15	17	20	32	41	44	26
FVIII activity (%)	DDAVP	35	34	33	111	152	110	71	53	39
FVIII activity (%)	ARC1779/DDAVP	31	29	30	155	207	181	179	111	60
FVIII activity (%)	ARC1779	46	36	40	42	43	55	50	69	60

See figure 2 for graphical presentation of individual values. DDAVP, desmopressin.

Table 2: Median platelet count, von Willebrand factor antigen (vWF:Ag), ristocetin cofactor activity (VWF:RCo) and FVIII coagulation factor levels before and after treatment with ARC1779 and desmopressin alone or in combination.

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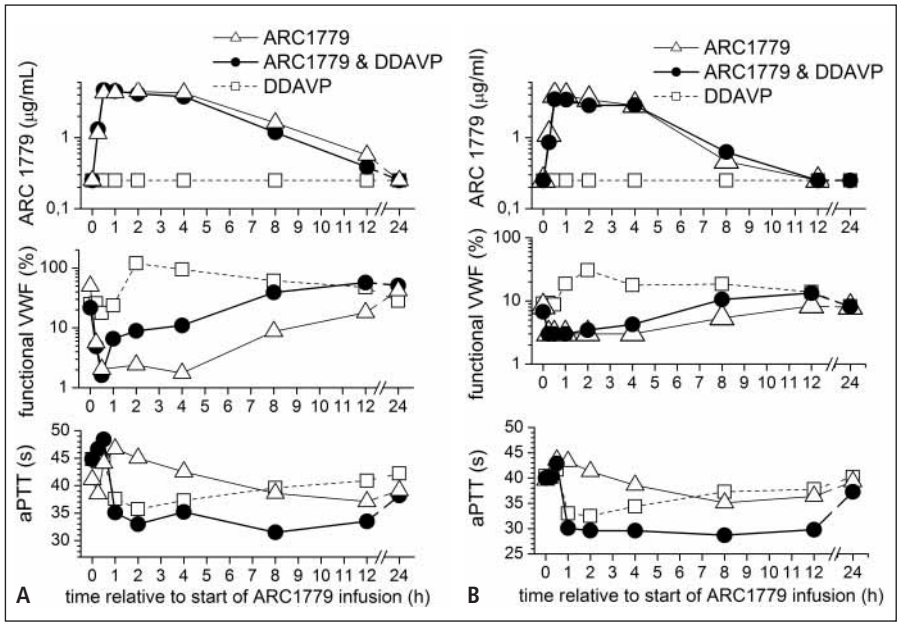

Figure 3: Pharmacokinetics and pharmacodynamics of the anti-von Willebrand factor aptamer ARC1779 in VWF type 2B. Two patients received ARC1779 (triangles, bolus primed continuous infusion of 0.001 mg/kg/min over 4 h), desmopressin (squares, 0.3 µg/kg) or their combination (circles). Plasma levels of 4–5 µg/ml (top layer) ARC1779 inhibited VWF by >97% (middle layer). Functional VWF designates the free A1 domains as measured by the REAADS enzyme immunoassay, desmopressin (squares) normalised activated partial thromboplastin time (aPTT; bottom layer), and this effect was prolonged by combination therapy (circles).

raised the amount of high multimers with peak levels occurring between 8 and 12 h.

Platelet counts

Desmopressin alone induced a profound (-87%) drop in platelet counts from 79/nl to 10/nl within 30 min after infusion in the first VWF type 2B patient, and platelet counts recovered completely within 24 h (Fig. 2). In the second patient, however, desmopressin did not substantially aggravate thrombocytopenia when given alone, perhaps due to the already very low baseline platelet count in this patient (25/nl). Thus, in this latter patient the protective effect of ARC1779 vs. desmopressin-induced thrombocytopenia could not be established. ARC1779, when given before desmopressin, completely prevented the desmopressin-induced platelet drop in the first patient (Fig. 2). Combined treatment slightly and transiently increased platelet counts in both patients. Nevertheless, ARC1779 alone appeared to increase platelet counts by 20–30% over 4–8 h in both patients.

Platelet function analyzer PFA-100

As expected (29) , basal collagen adenosine diphosphate-induced closure times were >300 s in both patients. In the first patient PFA-100 closure times decreased to 224 s at 1 h after desmopressin infusion, and to 183 s at 8 h after combined treatment. The PFA-100 results of the second patient after infusion were technically uninterpretable due to the patient's profound thrombocytopenia (CADP-CT>300 s already at baseline).

Table 3: Median drug concentration, free A1 domains as measured by the REAADS assay, and activated partial thromboplastin time (aPTT) before and after treatment with ARC1779 and desmopressin alone or in combination.

Period		0	15'	30'	1 h	2 h	4 h	8 h	12 h	24 h
ARC1997 (µg/ml)	DDAVP	<LLQ	<LLQ	<LLQ	<LLQ	<LLQ	<LLQ	<LLQ	<LLQ	<LLQ
ARC1997 (µg/ml)	ARC1779/DDAVP	<LLQ	1.2	4.3	4.2	3.8	3.3	0.8	<LLQ	<LLQ
ARC1997 (µg/ml)	ARC1779	<LLQ	1.0	3.9	3.9	3.7	3.6	1.1	0.4	<LLQ
Free A1 domains (%)	DDAVP	17.0	17.5	13.3	21.2	75.2	56.1	39.9	30.7	18.2
Free A1 domains (%)	ARC1779/DDAVP	14.8	4.0	2.3	4.8	6.0	7.0	22.2	32.8	29.5
Free A1 domains (%)	ARC1779	28.3	4.3	2.5	3.0	2.9	3.0	9.6	15.7	25.0
aPTT (s)	DDAVP	42.6	42.3	43.0	35.3	34.1	35.9	38.5	39.4	41.2
aPTT (s)	ARC1779/DDAVP	42.2	43.7	46.0	39.2	37.2	36.9	33.3	35.0	38.8
aPTT (s)	ARC1779	40.6	39.4	43.5	38.4	37.3	36.1	33.7	33.5	38.2

See Figure 3 for graphical presentation of individual values. DDAVP, desmopressin; LLG, lower limit of quantification.

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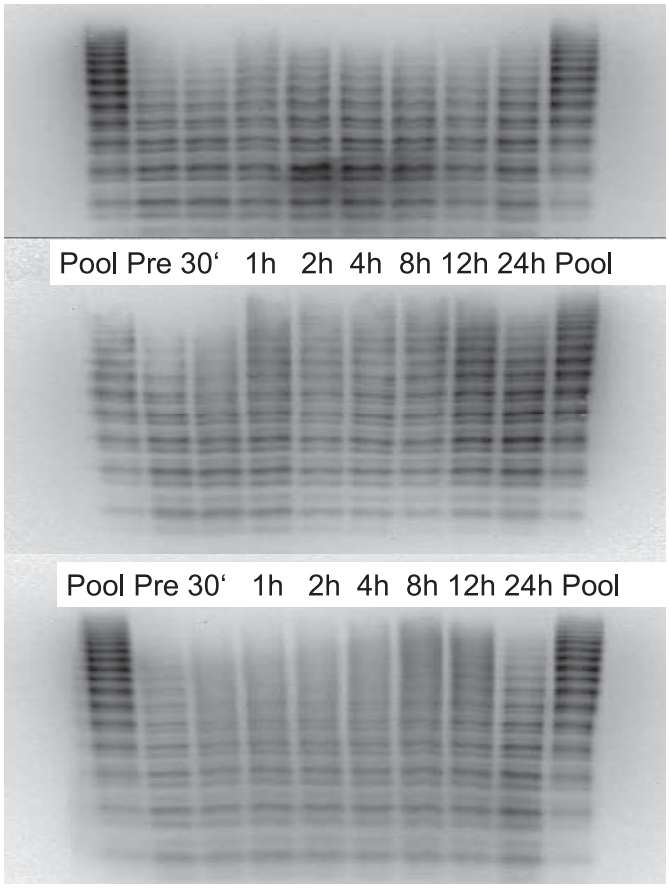

Figure 4: Effects of desmopressin, ARC1779 or their combination on the multimer pattern in a patient with vWD 2B. Desmopressin infusion (from 30'-1 h) improved multimer patterns immediately after infusion (1 h; top layer), but the effect was transient. Pre-infusion of ARC1779 (from Pre-4 h) prolonged the duration of the effect up to 12 h (middle layer). ARC1779 alone slowly increased high-molecular-weight multimers (maximum at 8 h). Multimer patterns were similar in the second VWF type 2B patient (data not shown).

Pharmacokinetics of ARC1779 and blockade of the A1 domains of VWF (REAADS assay)

Based on our *ex vivo* study, our target concentration for ARC1779 was > 3 μg/ml. Steady-state plasma concentrations of ARC1779 were 4 –5 μg/ml after infusion of ARC1779 alone or in combination with desmopressin (Fig. 3, Table 3). This equals 300–350 nM or a five- to 10-fold molar excess as compared to VWF when a molecular weight of 225 kD for mature VWD (30) is assumed. The REAADS assay was used to estimate the blockade of the A1 domains of VWF by ARC1779 (9): infusion of ARC1779 inhibited VWF completely (to less than 3% of the normal range). After desmopressin alone, free A1 domains increased (Fig. 3) due to VWF release (Fig. 2). This VWF release by desmopressin (Fig. 2) slightly antagonised the effect of ARC1779, because blockade of the A1 domains of VWF was slightly less under combined treatment as compared to ARC1779 alone (Fig. 3). This was reflected by a 10-fold increase in the IC_{90} from 1.2 to 11.4 μg/ml.

Safety

No treatment related adverse events were observed. In particular, no signs of bleeding occurred despite marked baseline and/or desmopressin-induced thrombocytopenia. No signs of thrombosis or compromised organ function due to enhanced platelet agglutination were observed.

Discussion

This is the first "paradoxical" approach to target a bleeding condition with an anti-platelet drug. Yet, this therapeutic strategy is rational because ARC1779 blocks the binding of hyperfunctional VWF to GPIb: ARC1779 plasma concentrations of 4–5 μg/ml fully blocked A1 domains in the REAADS assay (Fig. 3, Table 3). However, our data indicate that some shift in the concentration effect relationship (Fig. 3, Table 3) may occur when higher amounts of VWF multimers are released (Fig. 4) by well known stimuli such as desmopressin (31) or inflammation (32, 33). This may have implications for dose finding in another, related target indication of ARC1779, i.e. thrombotic thrombocytopenic purpura, where multimers are not appropriately cleared due to ADAMTS-13 deficiency (34, 35).

The different behaviour of the VWF:RCO and REAADS assays (Figs. 2 and 3, Tables 2 and 3) needs some explanation. As recently reported (13), free A1 domains and VWF:RCO levels correlate reasonably well in patients with acute myocardial infarction, who are known to have increased levels of multimers (36). The correlation coefficient even reached r=0.89 in patients with ST-elevation myocardial infarction. However, there is one important difference between assays. Whereas the REAADS assay is sensitive to inhibition by ARC1779 (Figs. 1 and 3, Table 3), the VWF:RCo assay is not, perhaps partly because ARC1779 directly binds to ristocetin. Hence increasing concentrations of ARC1779 lower VWF activity in the REAADS assay, but not VWF:RCo. Quite on the contrary VWF:RCo increases *in vivo*, because consumption of hyperactive large VWF multimers is prevented by ARC1779.

Hence, ARC1779 enhanced and prolonged the desmopressin-induced release of VWF/FVIII, and blocked the desmopressin-induced thrombocytopenia (Fig. 1). These data suggest that ARC1779 decelerates the rapid clearance of VWF and particularly of high weight multimers (Fig. 4) together with agglutinated platelets. This supports the concept that ARC1779 effectively inhibits the exaggerated aggregation of platelets and their subsequent, although transient, removal from the circulating blood. Future experiments employing longer infusion periods will be necessary to determine whether ARC1779 alone can fully normalize platelet counts in patients with VWD type 2B.

Blockade of VWF by ARC1779 does not affect TRAP-, ADP-, or arachidonic acid-induced aggregation (13). Thus, when platelet counts increase these platelets are haemostatically competent. One could theoretically envisage two potential therapeutic options. First, autologous platelets can be collected before surgery after treatment with ARC1779, which could be temporarily stored and

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What is known about this topic?

- Desmopressin aggravates thrombocytopenia in type 2B von Willebrand disease (VWF type 2B) by release of large and hyper-adhesive von Willebrand factor (VWF) multimers.
- ARC1779 is a novel VWF inhibitor that blocks thrombus formation in animals.

What does this paper add?

- ARC1779 (4–5 μg/ml) completely inhibited VWF A1 domains and prevented the desmopressin-induced platelet drop.
- ARC1779 substantially enhanced the desmopressin-induced increase in VWF antigen, VWF Ristocetin cofactor activity (VWF:RCo) and coagulation factor VIII activity these parameters, and improved multimer patterns.
- This provides first *in vivo* proof of concept that ARC1779 inhibits VWF in humans, and is a drug candidate for VWF driven disease processes such as those found in thrombotic thrombocytopenic purpura.

used intraoperatively after washing, when ARC1779 has been discontinued.

When VWF A1 domains are completely blocked by ARC1779 we would expect a normalisation of VWF antigen levels and, secondarily, of factor VIII activity, accompanied by an increase of platelet counts. Whether this could reduce the bleeding risk in patients with VWD type 2B is unclear. However, the expected increase in platelet counts could at least allow the continuation of interferon treatment of hepatitis C, which could not be started or had to be discontinued previously due to aggravation of thrombocytopenia. Proper dose finding studies are needed to clarify whether a partial blockade of VWF activity by lower concentrations of ARC1779 could be sufficient to improve thrombocytopenia and factor VIII activity to some extent.

ARC1779 slightly prolonged the aPTT during the bolus administration. This phenomenon was not previously observed after infusion of ARC1779 to healthy volunteers, but has been observed after infusion of other therapeutic oligonucleotides (37, 38) and may therefore be an unspecific aptamer effect and not a true coagulation disturbance, as FVIII levels (Fig. 2) did not decrease during this phase. Due to variability in responses between patients, the dose will possibly have to be selected on an individual basis.

Safety was excellent with no evidence for bleeding despite thrombocytopenia. This is in good agreement with the experience in patients with thrombotic thrombocytopenic purpura with very low platelet counts (12, 14).

Although the small sample size is an obvious limitation, we believe that our data are seminal and may stimulate more interest to further investigate ARC1779 in that condition. Although the demographic characteristics of our patients (Table 1) are typical of VWD type 2B, and our results could be externally valid, some variability in response may be seen between different patients, as is seen under treatment with desmopressin. The recommended approach for desmopressin is to administer a test dose, and the same may apply for ARC1779 if further studies prove the potential clinical utility of ARC1779.

In conclusion, these data provide evidence that ARC1779 is a potent inhibitor of the enhanced VWF function in VWF type 2B patients. Our data further indicate that ARC1779 inhibits the otherwise rapid turnover of VWF multimers and preserves platelet counts. These data provide proof of concept that ARC1779 is a potent inhibitor of VWF in humans and potential drug candidate for other diseases including thrombotic thrombocytopenic purpura.

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Conflicts of Interest

Archemix Corp. is the employer of J. Gilbert and R. Hutabarat. Bernd Jilma and Paul Knöbl have acted as consultants and members of an advisory board for Archemix Corp.

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