A dose ranging phase I/II trial of the von Willebrand factor inhibiting aptamer ARC1779 in patients with congenital thrombotic thrombocytopenic purpura

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Summary
Congenital thrombotic thrombocytopenic purpura (TTP) is a very rare but potentially life-threatening disorder. This phase I/II trial compared the pharmacokinetics and pharmacodynamics and safety of three different administration modes of the anti-von Willebrand factor (VWF) aptamer ARC1779. This was a prospective clinical trial with a partial cross-over design: three periods comprised subcutaneous injections of 50 mg of ARC1779 on seven subsequent days, a low-dose infusion of ARC1779 (0.002 mg/kg/min) for 24–72 hours and a high-dose infusion (0.004–0.006 mg/kg/min) up to 72 hours. ARC1779 concentrations were determined with high performance liquid chromatography, VWF inhibition was measured with enzyme immunoassay and platelet function was determined with the platelet function analyser (PFA-100) and impedance aggregometry. ARC1779 was well tolerated without any bleeding at concentrations spanning over three orders of magnitude.

The daily s.c. injection yielded plasma levels (0.5 μg/ml) of the drug that were too low to sufficiently suppress VWF. The low-dose i.v. infusion increased platelet counts in one patient, whereas the high i.v. dose increased plasma concentrations up to 69 μg/ml, completely blocked free A1 domains, VWF-dependent platelet plug formation and enhanced platelet counts in 2/3 patients. In conclusion, infusion of ARC1779 dose-dependently inhibits VWF-dependent platelet function and during infusion ARC1779 increases or stabilises platelet counts in congenital TTP. However, the tested doses, particularly the daily s.c. injections, did not correct all clinical or laboratory features of TTP.

Keywords
Clinical trial, ARC1779, aptamer, von Willebrand factor, congenital thrombotic thrombocytopenic purpura, Upshaw-Schulman syndrome

Introduction
Congenital thrombotic thrombocytopenic purpura (TTP) (Upshaw-Schulman Syndrome) (1, 2) is an extremely rare, but potentially life-threatening disease. It is caused by an inherited deficiency of the metalloprotease ADAMTS-13 (3, 4), which cleaves von Willebrand factor (VWF). This leads to persistence of ultra large-VWF multimers and enhanced platelet aggregation, and subsequently formation of platelet-VWF-rich thrombi. These thrombi induce mechanical haemolytic anaemia, consumptive thrombocytopenia and microangiopathy leading to end organ damage particularly in the brain, and kidneys (5, 6). In patients initially presenting with acute TTP, ADAMTS 13 activity is severely reduced, typically to less than 5%. First symptoms may present at birth, in childhood (7, 8) or in adulthood (usually in pregnancy in females) (4, 9). The subsequent course is characterised by recurrent “microangiopathic” haemolytic anaemia and thrombocytopenic crises. Prophylactic plasma transfusions every 2–3 weeks replace ADAMTS13, avoid relapses and usually control the disease and reduce the frequency of relapses in congenital TTP.

ARC1779 is a synthetically manufactured, modified DNA/RNA aptamer targeting VWF (10). Aptamers are a new class of oligonucleotide drugs (11, 12) that are produced by a selection process to bind to a specific target with high affinity. Similar to antibodies, aptamers are able to block many protein–protein interactions including those with pro- or anticoagulant effects. ARC1779 is a synthetically manufactured aptamer which binds to the A1 domain of human VWF with high affinity, preventing interaction with platelet Gplb and thereby inhibits VWF-mediated thrombus formation (13, 14). The in vivo efficacy of ARC1779 was recently demonstrated in a primate thrombosis model (13). Also, ARC1779 produced dose- and concentration-dependent inhibition of VWF activity and platelet function in the first-in-human evaluation (15). In an ex vivo study ARC1779 effectively inhibited VWF activi-
ity in plasma samples of TTP (16) or patients with myocardial infarction (17). Clinical experience in patients with idiopathic TTP has been reported previously (18, 19), and proof of concept for the potent in vivo action was recently provided in von Willebrand disease type 2B patients (20). Based upon this mechanism of action, we hypothesised that ARC1779 reduces platelet adhesion/aggregation by blocking the ultralarge VWF multimers and thereby improves platelet counts in patients with congenital TTP. The aim of this phase I/II trial was to compare the pharmacokinetics and pharmacodynamics and safety of different administration modes and drug exposures in patients with congenital TTP. In addition, we aimed to further characterise platelet function under high shear stress in these patients.

Methods

Study population

This clinical trial (NCT00632242) was approved by the Ethics Committee of the Medical University of Vienna and conducted according to the principles of the Declaration of Helsinki. The incidence of TTP with severe ADAMTS deficiency is extremely low (1–2/Million) (21), and congenital TTP is only a minor fraction of that. Thus, we tried to enrol every eligible patient of the Austrian TTP Registry with congenital TTP and manifest chronic/recurrent thrombocytopenia requiring regular transfusion of plasma. Three of four patients gave informed consent and were recruited. Exclusion criteria were pregnancy or recent history of trauma or surgery. Congenital ADAMTS13 deficiency has been confirmed before repeatedly by absent plasma ADAMTS13 activity and absent inhibiting and non-inhibiting anti ADAMST13 antibodies. This was a prospective and open trial with a cross over design to assess the pharmacokinetics, pharmacodynamics and safety of three different application modes of ARC1779 in patients with congenital TTP. Wash out periods were >3 weeks in all patients.

The core aptamer portion of ARC1779 (molecular weight, \( \sim 13.7 \) kDa without pegylation), is a 40-mer modified DNA/RNA oligonucleotide, which is conjugated with a 20-kDa polyethylene glycol moiety to form the pharmaceutical product, ARC1779 (molecular weight, \( \sim 33 \) kDa) (15). The crystal structure of its parent oligonucleotide in complex with the A1 domain of VWF has recently been characterised (22). ARC1779 was manufactured at a concentration of 10 mg/ml for clinical use as a sterile isotonic solution for intravenous (i.v.) injection or infusion (13, 15). ARC1779 has received Orphan Drug Designation in the European Union and United States.

Drug doses and routes of administration

The rationale for the doses was as follows: the sc. injections tested an amount of aptamer that could be filled easily into 1 ml solution and would not cause problems with hyperosmolarity. The low dose was derived from previous trials in healthy volunteers (15), in patients with type 11b von Willebrand disease showing >97% VWF inhibition with 4.5 μg/ml, 96% inhibition in a patient with refractory acquired TTP at 10 μg/ml (18), and 90% ex vivo inhibition of VWF by 4.7 μg/ml ARC1779 in plasma from patients with TTP (16). The high dose was introduced after a protocol amendment after we had observed unsatisfactory effectiveness of the low-dose i.v. infusion in one of our congenital TTP patients (F2).

1) Subcutaneous (s.c.) injections: Immediately, before the sc. injections an intravenous loading dose of 0.23 mg/kg ARC1779 was infused as a continuous 30-minute (min) infusion (3 stages of 10 min each). This was done for safety reasons because we had observed one hypersensitivity reaction after a small dose of i.v. ARC1779 previously. Then, ARC1779 (50 mg) was administered once-daily subcutaneously for seven days, then 25 mg on day 8 and 12.5 mg on day 9, the last day of treatment. Tapering was planned in the protocol to avoid rebound thrombocytopenia, if the sc. injections should turn out to effectively increase platelet counts.

2) Low-dose i.v. infusion: ARC1779 was administered as a bolus-primed continuous infusion as described above. The loading dose was 0.46 mg/kg. A continuous maintenance infusion at a dose of 0.002 mg/kg/min was given thereafter for 24 to 72 hours (h).

3) High-dose i.v. infusion: After an i.v. loading dose of 0.46 mg/kg, ARC1779 infusion was continued with 0.004 mg/kg/min. Thereafter the dose could be titrated upward to 0.008 mg/kg/min, if necessary, to achieve an increase in platelet counts. The maximum duration of infusion was three days.

Three units of solvent detergent treated pooled plasma (Octaplas S/D, Octapharma Pharmazeutika, Vienna, Austria) were transfused when patients developed signs of disease aggravation or after the end of the ARC1779 infusion (to avoid rebound platelet aggregation).

Samples for analysis were collected into tubes containing 129 mM (3.8 % citrate; Vacutette tubes; Greiner Bio-One, Kremsmuenster, Austria). All pharmacodynamic analyses were conducted in the laboratory of the Medical University of Vienna. The ELISA for VWF A1 domains and pharmacokinetic analysis were conducted at a contract research organisation (Questpharm, Cambridge, MA, USA) under good laboratory practices with methods developed by Archemix Corp. (15). The inhibition of the VWF-A1 domains was measured with a quantitative direct ELISA kit (REAADS VWF Activity ELISA Test Kit, Corgenix, Inc, Westminster, CO, USA) (15–17, 20). This ELISA utilises a purified murine anti-REAADS VWF monoclonal antibody, which recognises a functional epitope on the REAADS VWF molecule. 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). The detection limit is <3% and the intra- and inter-assay variability is <6% and <8%, respectively. The relationship of ARC1779 plasma concentration with free VWF A1 domains was...
analysed by Emax modelling. Platelet counts were analysed with a cell counter (Sysmex XE 2100, Kyoto, Japan). ARC1779 plasma concentrations were determined with a validated high performance liquid chromatography assay with ultraviolet detection (linear range 0.25 to 200 μg/ml). VWF antigen concentration was measured with a fully automated simultaneous thermal analyser using the STA Liatest VWF (Diagnostica Stago, Paris, France) (23). Plasma activity of VWF ristocetin cofactor activity (VWF:RCO) was assayed by turbidimetry using a commercial kit (BC von Willebrand reagent; Behring Marburg, Germany) (24). ADAMTS13 activity and ADAMTS13 inhibitory antibodies were determined with commercially available assays (Technozone ADAMTS-13 activity ELISA (fluorogenic; lower limit of quantification: 12%), and Technozone ADAMTS-13 INH Elisa; Technoclone, Vienna, Austria (25). A modified collagen binding assay was used as previously described by Reiter et al. (26).

Platelet Function Analyzer (PFA-100)

The effect of ARC1779 on VWF-mediated, shear-dependent platelet function was evaluated with the Platelet Function Analyzer (PFA-100; Dade Behring, Inc, Deerfield, IL, USA) in whole venous blood samples anti-coagulated with 3.8% sodium citrate. The PFA-100 is useful because it is very sensitive to elevations in – as well as inhibition of – VWF (16, 27–29). Blood samples were placed in a test cassette and aspirated through a capillary (200 μm diameter) under constant negative pressure (high shear stress) toward a membrane with a small aperture (150 μm diameter) coated with equine type I collagen to activate platelets and adenosine 5’-diphosphate (CAPD cartridges) to enhance platelet aggregation. CAPD closure time (CT) represents the elapsed time in seconds (up to a maximum of 300 seconds) until aperture occlusion by formation of a platelet plug.

Whole blood impedance aggregometry was determined using Multiple Electrode Aggregometry (MEA) (Multiplate Analyzer, Dynabyte Medical, Munich, Germany). The system detects the electrical impedance change due to the adhesion and aggregation of platelets on two independent electrode-set surfaces in the test cuvette. A 1:2 dilution of whole blood anticoagulated with hirudin (30) and 0.9% NaCl was stirred at 37°C for 3 min in the test cuvettes. thrombin receptor activating peptide (TRAP; 32 μM) was added and the increase in electrical impedance was recorded continuously for 6 min. The mean values of the two independent determinations are expressed in units (U: tenth of area under the curve). The reference range for the TRAP test in our laboratory is 55–135 U (31).

Statistical analysis

Due to the limited sample size, we analysed the results descriptively and looked at consistency as well as dose dependency of effects.

Results

Three patients with congenital chronic relapsing TTP [two females (F1, F2), one male (M)] agreed to participate and were included in the trial; all of them had undetectable ADAMTS-13 levels and no ADAMTS-13 inhibitors at all times. One of the patients with a mild form of congenital TTP did not consent. Natural variations in platelet counts are shown over months in one patient (F2, see Supplementary Fig. S1 available online at www.thrombosis-online.com), and are representative of the disease (1, 32). In brief, the patients had the following history and symptoms: patient F1 was 35 years old, TTP was diagnosed at the age of 19 during pregnancy due to symptoms of hemianopsia and other neurologic symptoms, and she had had recurrent neurologic (cognitive, alertness, difficulties with speech, and half-sided paresthesia in the left arm and left side of the face) symptoms of mild to moderate severity since then despite plasma transfusion (of matched blood group AB Rh neg) every three weeks. Symptoms improved after this clinical trial, when the transfusion interval was shortened to every two weeks, and desogestrol was administered to diminish menstruation-dependent flares. The patient recalls typical recurrent neurological symptoms since the age of 12, even in the presence of normal platelet counts. Her platelet counts show cyclic variations between approximately 180x10⁹/l one week after plasma transfusion, and about 60x10⁹/l when her LDH levels increase and she becomes symptomatic, often triggered pre-menstrually, before the next plasma transfusion. Patient F2 was 31 years of age, diagnosed eight years earlier, and her main symptoms were recurrent petechiae and haematomas during times of thrombocytopenia (see Supplementary Fig. S1 available online at www.thrombosis-online.com) that were managed with plasma transfusions (matched blood group B Rh pos) on demand (recently every 3–4 weeks).

One male patient (M), 18 years of age, had been diagnosed three months earlier with thrombocytopenia (around 40 x10⁹/l) and increased creatinine levels, and was treated with plasma transfusion (matched blood group O Rh pos) every 3–4 weeks since then. His major neurological complaints were headache and dizziness. Two chronically relapsing patients (F1, F2) received all three different administration schemes, the male (M) patient received the low dose of ARC1779 concomitantly with plasma exchange initially until autoantibodies were excluded (data not shown due to superimposed plasma effect) and was treated with the high i.v. dose of ARC1779 two months later. Laboratory parameters before treatment and at the end of treatment or before plasma infusion, whichever came first, are shown in Table 1.

Low bioavailability of ARC1779 after s.c. injection

Thirty minutes after the initial intravenous infusion the ARC1779 concentration peaked at 3.7–4.5 μg/ml and free VWF A1 domains were blocked by up to 97%. During steady state s.c. injections, plasma ARC1779 concentrations decreased to 0.4–0.5 μg/ml. Hence,
there was incomplete suppression of free VWF A1 domains during sc. treatment (patient F1 is depicted in Fig. 1).

As platelet counts decreased in both cases, each patient eventually received 3 units of S/D plasma, which corrected platelet counts to normal within a week (Figs. 2, 3 and Supplementary Fig. S2 available online at www.thrombosis-online.com).

Thus, the daily sc. injection of 50 mg ARC1779 resulted in low plasma levels (0.5 μg/ml), that were insufficient to block free A1 domains.

Low-dose i.v. infusion

Patient F1: The low dose of ARC1779 (0.002 mg/kg/min, given for 24 h) increased ARC1779 plasma concentration to 8.6 μg/ml (Fig. 1) and decreased free A1 domains by 88% 15 min after the bolus infusion (Fig. 1); free A1 domains remained at levels between 12–21% of baseline during the treatment. Platelet counts increased from 67 to 98x10^9/l after 24 h (Table 1, Fig. 2) and dropped again after stopping the ARC1779 infusion. The rise in platelet counts was accompanied by a 10% decrease in LDH. After infusion of plasma on day 4 platelet counts increased again to normal levels (190x10^9/l on day 8).

Patient F2: The low dose ARC1779 (0.002 mg/kg/min) was infused for 72 h which increased ARC1779 plasma concentrations to about 4.5 μg/ml and decreased VWF activity by 90% from 73% to 7%. This drug concentration and level of inhibition seemed to delay the drop in platelet counts as compared to the s.c. injections (Fig. 3). However, this was insufficient, because the platelet counts fell from 63x10^9/l (predose) to 18x10^9/l (end of infusion on day 4) which was accompanied by a rise in LDH from 257 to 481 U/l. Thus, three units of plasma were transfused, which increased platelet counts to 532x10^9/l on day 11.

Hence, a plasma concentration of 4.5 μg/ml ARC1779 was insufficient to increase platelet counts in patient F2, but 8.6 μg/ml ARC1779 increased platelet counts by about 50% after 24 h in patient F1.

Figure 1: Inhibition of free A1 domains of von Willebrand factor by ARC1779. The female (F1) patient received the von Willebrand Factor aptamer ARC1779 either s.c. (50 mg; [A, B]) for seven days or i.v. (0.002 mg/kg) for 24 hours (C, D) or (0.004 mg/kg) for three days (E, F).
Prolonged high-dose i.v. infusion

Patient F1 received the high dose of ARC1779 at 0.004 mg/kg/min up to 48 h and then the dose was increased to 0.006 mg/kg/min until 72 h (end of infusion) because the platelet counts seemed to reach an intermediate plateau at 48 h. Concentrations of ARC1779 peaked at 63.8 μg/ml and the free A1 domains were suppressed from 174% to 3–6% (Fig. 1). Platelet counts increased from 61x10⁹/l (predose) to 142x10⁹/l on day 4 (Fig. 2, Table 1). Because of mild neurological symptoms on day 4 (which were similar to those the patient reported regularly at the end of the 3-week interval between plasma transfusions), the patient received 600 ml plasma, which further enhanced platelet counts to 179x10⁹/l on day 11 (see Supplementary Fig. S3 available online at www.thrombosis-online.com).

Patient F2 received the high-dose infusion (of 0.004 mg/kg/min) ARC1779 for 48 h which increased ARC1779 plasma concentrations to a maximum of 19.6 μg/ml. The free A1 domains decreased from 14% to <3% after bolus infusion and remained below detectable levels until the end of treatment. Platelet counts increased from 38x10⁹/l (predose) to 94x10⁹/l after 48 h (Fig. 3, Table 1). After an accidental interruption of the ARC1779 infusion the platelet count dropped to 42x10⁹/l (Fig. 3) due to the short half-life of ARC1779, which is in contrast to the persisting effect of plasma transfusion (see Supplementary Fig. S2 available online at www.thrombosis-online.com). The drop in platelet counts was also reflected by an increase in LDH to 450 U/l. After an accidental interruption of the ARC1779 infusion the platelet count dropped to 42x10⁹/l (Fig. 3) due to the short half-life of ARC1779, which is in contrast to the persisting effect of plasma transfusion (see Supplementary Fig. S2 available online at www.thrombosis-online.com). The drop in platelet counts was also reflected by an increase in LDH to 450 U/l. Thus, the patient received 600 ml plasma which increased platelet counts to 570x10⁹/l after eight further days (see Supplementary Fig. S3 available online at www.thrombosis-online.com).

Patient M received the high-dose infusion of ARC1779 (0.004 mg/kg/min) until 72 h. This increased the ARC1779 plasma concentrations to a maximum of 68.8 μg/ml. The free A1 domains decreased from 78% to <3% within 24 h. Apart from a transient 15% increase in platelet counts during the first 6 h, platelet counts remained at about 90x10⁹/l during the whole infusion period (see Supplementary Fig. S4 available online at www.thrombosis-online.com). Interestingly, discontinuation of the infusion precipitated a fall from 91 to 70x10⁹/l within 8 h. Hence, the patient was transfused with 600 ml plasma which increased platelet counts to 178x10⁹/l at a follow up visit seven days later (see Supplementary Fig. S3 available online at www.thrombosis-online.com).

Thus, plasma concentrations of ARC1779 ranging from 20–69 μg/ml increased platelet counts to a clinically relevant extent in two out of three patients, and stabilised platelet counts in a third patient.

Table 1: Laboratory parameters of three patients with congenital TTP.

<table>
<thead>
<tr>
<th>Pat</th>
<th>Platelets (10⁹/l)</th>
<th>LDH (U/l)</th>
<th>Crea. (mg/%)</th>
<th>Hb (g/dl)</th>
<th>CADP-CT (s)</th>
<th>TRAP (AU)</th>
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</thead>
<tbody>
<tr>
<td>S.C. injection of 50 mg/day for 7 days, then tapered</td>
<td></td>
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<tr>
<td>F1</td>
<td>predose 164 232</td>
<td>1.21 11.8</td>
<td>48 80</td>
<td></td>
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<tr>
<td>day 7</td>
<td>83 255</td>
<td>1.15 11.3</td>
<td>224 29</td>
<td></td>
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<tr>
<td>F2</td>
<td>predose 87 210</td>
<td>0.9 13.7</td>
<td>97 39</td>
<td></td>
<td></td>
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<tr>
<td>24h</td>
<td>18 319</td>
<td>0.99 12.7</td>
<td>300 0</td>
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<tr>
<td>Low-dose infusion of 0.001 mg/kg/min</td>
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<tr>
<td>F1</td>
<td>predose 67 250</td>
<td>1.17 12.1</td>
<td>100 52</td>
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<tr>
<td>24h</td>
<td>98 225</td>
<td>1.09 11.7</td>
<td>300 48</td>
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<tr>
<td>F2</td>
<td>predose 63 257</td>
<td>0.84 13.7</td>
<td>69 15</td>
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<tr>
<td>72h</td>
<td>18 481</td>
<td>0.84 10.4</td>
<td>300 0</td>
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<tr>
<td>High-dose infusion up to 0.006 mg/kg/min</td>
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<tr>
<td>F1</td>
<td>predose 61 378</td>
<td>1.24 11</td>
<td>85 30</td>
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<tr>
<td>72h</td>
<td>142 223</td>
<td>1.14 9.6</td>
<td>300 83</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>F2</td>
<td>predose 38 348</td>
<td>0.86 11.6</td>
<td>300 10</td>
<td></td>
<td></td>
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<tr>
<td>48h</td>
<td>94 336</td>
<td>1</td>
<td>10.8 300 54</td>
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<tr>
<td>M</td>
<td>predose 90 201</td>
<td>1.35 13.8</td>
<td>94 n.d.</td>
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<tr>
<td>72h</td>
<td>91 323</td>
<td>1.42 13.8</td>
<td>300 25</td>
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Baseline platelet counts (predose) indicate that patients were relapsing already except for F1 during the sc. injection period. The different sampling times resulted from logistic reasons (how long the patients could stay at the research ward), as well as early termination before plasma transfusion in patient F2 during the sc. injection period. CADP-CT, collagen adenosine diphosphate; Crea, creatinine; Hb, haemoglobin; n.d., not done; LDH, lactate dehydrogenase; TRAP, thrombin receptor activating peptide.

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Effects of ARC1779 on shear induced ADP-mediated platelet hyperaggregation

ARC1779 infusion blocked VWF-dependent platelet function, measured by the PFA-100 (see Supplementary Fig. S3 available online at www.thrombosis-online.com). After the end of the s.c. injections, when platelet counts further increased due to plasma transfusion, normal CADP-CT were seen even in the presence of moderate thrombocytopenia (see Supplementary Figs. S2 and S3 available online at www.thrombosis-online.com). In contrast, CADP-CT are shortened to pathologically short values when platelet counts normalised (see Supplementary Fig. S3 available online at www.thrombosis-online.com).

To characterise the specificity of ARC1779, we also measured whether ARC1779 interfered with TRAP-induced platelet aggregation in whole blood. ARC1779 did not inhibit TRAP-induced platelet aggregation, but when platelet counts dropped platelet aggregation decreased, and when ARC1779 elevated platelet counts platelet aggregation increased (Table 1).

Tolerability and safety

There were no serious, severe or any treatment-related adverse events during any of the treatment periods. Local tolerability after s.c. injection of ARC1779 was excellent. There were also no bleeding episodes, even when ARC1779 was given to thrombocytopenic patients. No TTP symptoms were observed during ARC1779 infusion except for mild paresthesia (typical tingling sensation in left side of face) in patient F1.

Discussion

To our knowledge this is the first drug trial in congenital TTP, providing proof of concept that ARC1779 dose-dependently increases platelet counts in some patients with congenital TTP, which could have broader implications for idiopathic TTP as well. For this purpose patients were exposed to different plasma concentrations of
ARC1779 spanning over two orders of magnitude in a cross-over design. The daily s.c. injection of ARC1779 resulted plasma levels (Fig. 1) too low to sufficiently suppress free A1 domains and hence failed to increase platelet counts in both patients during treatment (Figs. 2 and 3). On the other hand, this period allowed us to look at the spontaneous natural cyclic changes in platelet counts in congenital thrombotic thrombocytopenic purpura (see Supplementary Figs. S1–S3 available online at www.thrombosis-online.com). The low-dose i.v. infusion increased platelet counts in patient F1 (Fig. 2, Table 1), whereas platelet counts continued to fall in patient F2 (Fig. 3), although at a potentially slower rate as compared to the s.c. injections (Fig. 3). This might be due to the fact that the plasma concentrations of ARC1779 were lower in the non-responder (4.5 μg/ml vs. 8.7 μg/ml). Prolonged high-dose i.v. infusion of ARC1779 effectively inhibited VWF and increased platelet counts in two of three patients as long as ARC1779 was given (plasma concentrations between 19.6 and 68.8 μg/ml). Discontinuation of the infusion precipitated a fairly rapid drop in platelet counts (Fig. 3), which is due to the short half-life of ARC1779 (Fig. 1) (15), and in line with previous experience (18, 20). This shows that VWF inhibition, in contrast to ADAMTS-13 infusion with transfused plasma, does not eliminate the cause of congenital TTP, which is somewhat expected. Thus, i.v. infusion of ARC1779 increased platelet counts at least in some patients with congenital TTP. As the applied high i.v. dose already blocked free A1 domains of VWF completely (Fig. 1), we do not believe that a higher dose (>0.004 mg/kg/min) would be more effective. Also, shear-dependent platelet function was maximally inhibited.

There are few reports on platelet function in patients with TTP in general, and congenital TTP in particular. Moake et al. assessed VWF-mediated fluid shear stress-induced platelet aggregation before and after infusion of fresh frozen plasma (FFP) or S/D plasma in two children with congenital chronic relapsing TTP (32). Platelet aggregation before plasma infusion was excessive due to the presence of unusually large VWF multimers in patient plasma. Plasma transfusion decreased platelet aggregation and increased platelet counts to normal levels within one week. This indicates that ADAMTS-13 is present in FFP and S/D plasma (33), and that excessive in vivo platelet aggregation in chronic relapsing TTP and excessive in vitro VWF-mediated shear-induced aggregation may be similar phenomena.

Platelet function testing is not used in clinical routine care of TTP patients, also because thrombocytopenia can hamper interpretation of the results. However, for scientific purposes, we further evaluated the platelet function under high shear rates in our TTP patients.

Platelet function before ARC1779 was normal in two patients with moderate thrombocytopenia (platelet counts of 61 and 90x10^9/l) (see Supplementary Fig. S3 available online at www.thrombosis-online.com), which is in contrast to compromised platelet plug formation when platelet counts are <100x10^9/l in normal subjects (34). Hence, this reflects hyperfunctional VWF and is in line with Moake’s data (32). In our patients, i.v. infusion of ARC1779 blocked VWF-dependent platelet function and prolonged closure-times measured with the PFA-100 (see Supplementary Fig. S3 available online at www.thrombosis-online.com). Transfusion of plasma normalised platelet counts within two days, which is in accordance with the literature (1, 35). Closure-times already normalised when platelet counts increased but were still below normal range. Further, closure-times were often shortened to pathologically low values when platelet counts increased to or above normal after plasma transfusion (see Supplementary Figs. S2 and S3 available online at www.thrombosis-online.com): in fact CDP-CT values were even shorter than those values seen in patients with myocardial infarction (36) or those seen in patients whose myocardial infarction ended with a cardiac arrest (37). This is a possible explanation why one of our patients already had neurological symptoms since puberty, still in the presence of normal platelet counts, and also later between subsequent plasma transfusions, although she often presented with normal platelet counts. Thus, this massive increase in shear induced platelet function as a result of hyperactive VWF is a hallmark of congenital TTP, likely contributing to recurrent target organ damage. Thus, it will be exciting to examine the long-term effect of VWF blockade and to document the resolution of laboratory and clinical sequelae upon infusion of VWF inhibitors or recombinant ADAMTS13 (38) when these become available (see Supplementary Fig. S3 available online at www.thrombosis-online.com). The female patient (F2) received the anti von Willebrand factor aptamer ARC1779 either s.c. (vertical arrows, [A]), i.v. at a low dose [B], which were both ineffective) or iv. at a high and effective dose (C). Panel C also depicts the rapid drop in platelet counts after interruption of the infusion.
able online at www.thrombosis-online.com).

We further investigated whether ARC1779 interfered with agonist-induced platelet aggregation. In agreement with our previous in vitro data (16), in vivo infusion of ARC1779 did not inhibit TRAP-6 induced platelet aggregation. Quite the contrary, TRAP-induced aggregation increased 3–5 fold during high-dose i.v. infusion of ARC1779 when platelet counts increased. This indicates that the platelets being released from the bone marrow or potentially from intravascular aggregates during treatment exhibited a good platelet function. Hence, due to the specificity of action of ARC1779 for its target, VWF (16), ARC1779 may have a relatively low bleeding risk.

Limitations

This study does not support the use of ARC1779 in the current dosing or format for clinical use in congenital TTP as it was ineffective in correcting the clinical or laboratory features of TTP. As ARC1779 was only partially effective as a high-dose i.v. infusion, ARC1779 will not be useful for the treatment of such patients. Plasma was given after each treatment period to protect against early relapse of TTP, after discontinuation of ARC1779. Nevertheless, development of a 2nd generation of anti-VWF aptamers such as ARC15105 with higher s.c. bioavailability, higher affinity and longer half-life may be beneficial, especially in patients with congenital TTP, because they are treated in an outpatient setting.

What is known about this topic?

- Congenital thrombotic thrombocytopenic purpura (TTP) is an extremely rare microvascular occlusive disorder characterised by thrombocytopenia, microangiopathic haemolytic anaemia and systemic platelet thrombosis.
- It is due to a deficiency in the von Willebrand factor (VWF) cleaving enzyme (ADAMTS-13).
- Current standard treatment is the transfusion of plasma every 2–3 weeks.

What does this paper add?

- The aptamer ARC1779 is directed against VWF and has been developed for the use in TTP.
- This is the first drug trial ever in congenital TTP. It evaluated the safety, pharmacokinetics and pharmacodynamics of ARC1779 in the therapy of congenital TTP.
- Infusions of ARC1779 effectively inhibited VWF and increased platelet counts. The results of this trial provide support for the concept that VWF inhibition may be safe and beneficial in congenital TTP patients.

Conclusion

ARC1779 dose-dependently and specifically inhibits VWF-dependent platelet function in congenital TTP, and ARC1779 increased platelet counts in two out of three patients during infusion. However, the tested doses and particularly the s.c. injections did not correct all clinical or laboratory features of TTP.

Conflict of interest

This trial was sponsored by Archemix Corp. the employer of James C. Gilbert. Bernd Jilma and Paul Knöbl acted as consultants and members of advisory or steering committees.

References