The von Willebrand Inhibitor ARC1779 Reduces Cerebral Embolization After Carotid Endarterectomy: A Randomized Trial

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Background and Purpose—Inhibition of von Willebrand factor offers a novel approach to prevention of stroke and myocardial ischemia but has not yet been demonstrated to show efficacy on clinically relevant end points. ARC1779 is an aptamer that inhibits the prothrombotic function of von Willebrand factor by binding to the A1 domain of von Willebrand factor and thereby blocking its interaction with glycoprotein. Phase 1 studies suggest it inhibits platelet aggregation with less increase in bleeding than conventional antiplatelet agents. The effect of ARC 1779 on cerebral emboli immediately after carotid endarterectomy was investigated in a randomized clinical trial.

Methods—Patients undergoing carotid endarterectomy were randomized double-blind to ARC1779 or placebo administered intravenously. Transcranial Doppler recording, to detect cerebral embolic signals, was performed in the first 3 hours postoperatively. The primary end point was time to first embolic signals.

Results—Thirty-six patients were recruited, 18 in each arm. The Kaplan-Meier median time to first embolic signals was 83.6 minutes for ARC1779 compared with 5.5 minutes for placebo. Using Cox proportional hazards embolic signals occurred statistically significantly later on ARC1779 ($P=0.007$). Reduced embolic signals counts were correlated with inhibition of von Willebrand factor activity ($P=0.03$). Increased perioperative bleeding and anemia were seen with ARC1779.

Conclusions—von Willebrand factor inhibition reduces thromboembolism in humans. It may play a role in treatment of stroke and myocardial ischemia. The extent to which bleeding complications occur in nonoperated patients needs to be assessed in further studies.


Key Words: antiplatelet agents ■ antiplatelet drugs ■ antiplatelet RX ■ carotid endarterectomy ■ embolic stroke ■ embolism ■ transcranial Doppler

Cerebral embolism from large artery atherosclerosis is a major cause of stroke. Recent data have shown minor stroke and transient ischemic attack are followed by a high early risk of recurrent stroke, and risk is highest in large artery disease.¹ Aspirin reduces recurrent stroke risk but fails to prevent 80% of recurrences.² Combination therapy with dipyridamole, or monotherapy with clopidogrel, has greater efficacy but again fails to prevent many recurrences.³ It has been suggested more intensive antiplatelet regimes are required, particularly early after stroke or transient ischemic attack. One approach is to use combinations of existing therapies. The combination of aspirin with clopidogrel in early prevention has shown encouraging results in Phase 2 studies⁴–⁶ and is currently being tested in a Phase 3 study (http://clinicaltrials.gov/ct2/show/NCT00991029). The efficacy of triple therapy with aspirin, dipyridamole, and clopidogrel is also being explored.⁷ An alternative approach is to use novel antiplatelet therapies.

One promising approach is inhibition of von Willebrand factor (vWF). Initial recruitment of platelets to an injured arterial wall is mediated by reversible interaction between the platelet receptor glycoprotein (GPIb) and the large multimeric glycoprotein vWF bound to subendothelial collagen or the surface of activated endothelial cells followed by platelet activation and aggregation. Recent studies have shown that inhibition of GPIb or absence of vWF protects mice from brain infarction without causing intracerebral hemorrhage despite a prolongation of bleeding time.⁸ This suggests the GPIb–vWF axis may represent a suitable target for stroke prevention.
ARC1779 is an aptamer that inhibits the prothrombotic function of vWF by binding to the A1 domain of vWF and thereby blocking its interaction with GPIb. In healthy volunteers, it inhibits vWF activity and vWF-dependent platelet function but leaves the coagulation system and other pathways of platelet activation intact. It inhibits ex vivo platelet aggregation in myocardial infarction. One might expect ARC1779 to reduce thrombus formation on activated atherosclerotic plaque and reduce subsequent cerebral embolization. However, this has not yet been shown in humans.

A method of evaluating efficacy of antiplatelet medications in humans is transcranial Doppler ultrasound (TCD) detection of cerebral emboli. Embolic signals (ES) are much more common than clinical events, and therefore therapeutic efficacy can be examined in smaller patient numbers. ES independently predict stroke in symptomatic carotid artery disease. This technique has been used to evaluate novel antiplatelet regimens in single and multicenter studies. One situation that has been used is the setting of carotid endarterectomy (CEA). Excision of the plaque removes the endothe- lium creating a thrombogenic surface on which platelet aggregation occurs. ES are frequent after CEA and predict early postoperative stroke. The ES are believed to repre- sent platelet aggregates and this is supported by studies showing their frequency can be markedly reduced by anti- platelet agents. Because of the large number of ES, antiplatelet efficacy can be evaluated in relatively small patient numbers. This setting has been used to show the efficacy of aspirin and clopidogrel and GSNO, a novel nitric oxide inhibitor. The results found correlated well with those in subsequent studies in symptomatic carotid stenosis.

Therefore, we evaluated the efficacy of ARC1779 in reducing embolization in the immediate postoperative phase after CEA.

Methods

Overall Study Design

This was a randomized, double-blind, placebo-controlled study in patients undergoing CEA.

Administration of the study drug was begun 1 hour before induction of anesthesia and continued until 3.5 hours after skin closure. TCD recording from the ipsilateral middle cerebral artery was performed for 3 hours beginning 30 minutes postskin closure.

Inclusion and Exclusion Criteria

Inclusion criteria were planned CEA for symptomatic or asymptomatic carotid stenosis and 18 to 80 years of age. Exclusion criteria included lack of acoustic window allowing TCD recordings; unable/unwilling to consent; metallic prosthetic cardiac valve because these are associated with frequent ES due to presumed gaseous emboli; recent (<4 weeks) ischemic stroke involving more than one third of the middle cerebral artery territory; history of hemorrhagic stroke; thrombocytopenia; trauma or surgery within the preceding 30 days; history of a bleeding disorder, gastrointestinal ulcers, or other problem associated with increased bleeding risk; use of clopidogrel, warfarin, or any chronic antithrombotic therapy other than aspirin and/or dipyridamole; and fibrinolytic or GPIIb/IIIa inhibitor treatment within the preceding 24 hours.

All patients gave written informed consent. The study was approved by the local ethics committees and the Medicines and Healthcare products Regulatory Agency. EudraCT number was 2008-005246-23 (ClinicalTrials.gov identifier: NCT00742612).

Randomization Process

Patients were allocated randomly (1:1) to treatment with placebo or ARC1779 through an interactive voice randomization system using the method of permuted block randomization.

Treatment Schedule

ARC1779 injection or placebo was administered by intravenous infusion as an initial loading dose over 1 hour preoperatively with the rate of infusion increased gradually in 3 20-minute stepwise increments followed by continuous infusion of up to 2 hours intraoperatively and 3.5 hours postoperatively. The dosing regimen ARC1779 was 0.00015 mg/kg/min for 20 minutes, 0.003 mg/kg/min for 20 minutes, followed by 0.006 mg/kg/min for 20 minutes, followed by continuous infusion at 0.0006 mg/kg/min. It was expected this would result in a steady-state concentration of 3 μg/mL. The dose chosen for this study was predicted to deliver plasma concentrations of ARC1779 that would provide approximately 90% blockade of the binding site on vWF for platelets (ie, the A1 domain of vWF that binds to GPIb on platelets) and which had previously been demonstrated not to cause bleeding in healthy volunteers, in patients with acute coronary syndrome undergoing primary percutaneous coronary intervention, and in patients with acute thrombotic thrombocytopenic purpura undergoing plasma exchange.

Concomitant therapies, in addition to study drug treatment, which were permitted, included aspirin and unfractionated heparin during CEA. The use of medications that might increase the risk of bleeding (eg, antithrombotic agents other than aspirin, nonsteroidal anti-inflammatory drugs, etc) was prohibited.

TCD Recordings

TCD recordings were made from the middle cerebral artery ipsilateral to the operated carotid artery. All recordings were made using either EME/Nicolet Pioneer or Companion or DWL Multidop equipment with a 2-MHz transducer. Standard recording settings were used as recommended in International Consensus Criteria. The raw Doppler audio signal was recorded onto CD-ROM or other digital media for subsequent central analysis.

End Points

The primary end point was the time from onset of observation period until first ES.

Secondary end points included number of ES during the 3 hours after restoration of carotid circulation; hourly number of ES during each of 3 hours of postoperative recording; proportion of patients without ES overall and in each of the 3 hours of postoperative recording; intensity of ES in patients with ES; number of new lesions on postoperative MRI by diffusion-weighted imaging and proportion of patients with new postoperative diffusion-weighted imaging lesions; clinical transient ischemic attack and stroke during the first postoperative week; and concentration of S100β, a biomarker for central nervous system injury.

Follow-Up for New Transient Ischemic Attack or Stroke

All patients were examined postoperatively, at the time of hospital discharge, and again on Day 7. If new stroke was suspected clinically, it was confirmed on MRI.

TCD Analysis

All analysis of ES was performed in a central reading laboratory at St George’s University of London by a researcher blinded to patient and treatment identity. Recordings were reviewed in real time both visually and acoustically to identify ES as short-duration high-intensity signals accompanied by characteristic chirping sounds using standard criteria. All signals were reviewed by a second experienced researcher (H.S.M.). In addition, an intensity threshold of ≥7 dB was used; this improves interobserver reproducibility without excess loss of sensitivity.
Brain MRI Analysis
A neuroradiologist at the Core Laboratory of Perceptive Informatics (Lowell, MA) performed a blinded comparison of each patient’s preoperative and postoperative brain MRI to determine the presence or absence of new diffusion-weighted imaging lesions.

Pharmacodynamic and Pharmacokinetic Analysis
Blood samples were taken in the 24 hours before the operation, during the operation, hours 0 to 1.1 to 2.2 to 3, and 24 postoperatively, and then daily until hospital discharge. Enzyme-linked immunosorbent assay-based measurement of vWF activity (ie, free A1 domain sites available for binding) and of total vWF antigen was performed. Plasma samples were analyzed for quantification of ARC1779 concentrations using a validated high-performance liquid chromatography method.

Safety and Bleeding Assessment
Safety of ARC1779 was assessed through evaluation of adverse events, physical examination, clinical laboratory tests (serum chemistry, urinalysis), full blood count, and coagulation tests (prothrombin time, activated partial thromboplastin time).

Statistical Analysis
The protocol-specified analysis specified use of a proportional hazards regression model for recurrent events as described by Anderson and Gill. Due to distribution of the number of ES reported, there were convergence issues relating to using this model and therefore it was decided to analyze time to first occurrence of ES through a Cox proportional hazards regression model with factors for treatment group, center, and status (asymptomatic versus symptomatic). Subjects not reporting an ES had their time censored as the total time of their TCD recording.

Sample Size Estimation
A total sample size of 100 subjects, 50 per treatment group, was planned based on the following estimates. Considering the major secondary end point, total ES count in a 3-hour observation period, it was assumed that the expected mean count in the controls with nonzero counts was 15 based on published data. It was assumed treatment with ARC1779 would reduce ES count by 33% to a mean of 10. Twenty-four subjects per group was expected to give 80% power to detect a difference at 5% level (2-sided). We estimated the mean intensity of ES in patients with ES was lower in the ARC1779 treatment group, from 6 centers. Baseline characteristics of the 2 groups are shown in Table 1.

Results
Thirty-six subjects were randomized, 18 in each treatment group, from 6 centers. Baseline characteristics of the 2 groups are shown in Table 1.

Primary End Point
For 4 subjects in the placebo group, postoperative TCD recordings were not adequate for analysis; therefore, 32 subjects provided data.

On intention-to-treat analysis, time to first ES (the primary efficacy end point) was longer in the ARC1779-treated group (P = 0.007). The Kaplan-Meier median time to first ES was 83.6 minutes for ARC1779 compared with 5.5 for placebo. The terms for center (P = 0.59) and symptomatic status (asymptomatic versus symptomatic; P = 0.31) were not significant. The Figure shows the number of postoperative ES ranked in ascending order in the 2 treatment groups.

**Table 1. Baseline Characteristics of 2 Treatment Groups**

<table>
<thead>
<tr>
<th></th>
<th>ARC1779 (N=18)</th>
<th>Placebo (N=18)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y (mean; SD)</td>
<td>66.6 (10.1)</td>
<td>64.1 (9.1)</td>
<td>0.45</td>
</tr>
<tr>
<td>Male sex</td>
<td>13 (72.2%)</td>
<td>16 (88.9%)</td>
<td>0.21</td>
</tr>
<tr>
<td>Body mass index</td>
<td>26.2 (4.9)</td>
<td>27.7 (5.6)</td>
<td>0.39</td>
</tr>
<tr>
<td>Symptomatic carotid stenosis</td>
<td>10 (55.6%)</td>
<td>12 (66.7%)</td>
<td>0.49</td>
</tr>
<tr>
<td>Hypertension</td>
<td>14 (77.8%)</td>
<td>14 (77.8%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>13 (72.2%)</td>
<td>10 (55.6%)</td>
<td>0.30</td>
</tr>
<tr>
<td>Diabetes</td>
<td>4 (22.2%)</td>
<td>3 (16.7%)</td>
<td>0.62</td>
</tr>
<tr>
<td>Previous myocardial ischemia</td>
<td>4 (22.2%)</td>
<td>4 (22.2%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Aspirin at preoperative assessment</td>
<td>15 (83.3%)</td>
<td>14 (77.8%)</td>
<td>0.67</td>
</tr>
<tr>
<td>Dipyridamole at preoperative assessment</td>
<td>2 (11.1%)</td>
<td>3 (16.7%)</td>
<td>0.63</td>
</tr>
</tbody>
</table>

SD indicates standard deviation.

Secondary Efficacy End Points
**ES End Points**
Median (mean, SD) number of ES during the 3 postoperative hours was 1.0 (10.8, 20.7) in the ARC1779 group compared with 5.5 (19.2, 25.9) in the placebo group (P = 0.08).

The number of ES during each of the 3 hours of postoperative recording (0 to 1.1 to 2.2 to 3 hours) were: 15.8, 2.2, and 0.6 in ARC1779 group compared with 10.8, 3.4, and 5.1 in the placebo group.

Seven of 18 (38.9%) subjects in the ARC1779 group had no ES compared with zero in the placebo group (P = 0.0105). The treatment group differences were also significant during the first hour (P = 0.0449) in which the number of subjects without any ES was 9 (50.0%) in the ARC1779 group compared with 2 (14.3%) in the placebo group.

The mean intensity of ES in patients with ES was lower in the ARC1779 group: 9.9 versus 12.6 dB (P = 0.04).

**Stroke and MRI Lesions**
Two subjects sustained perioperative strokes. One on ARC1779 had a stroke secondary to carotid thrombosis with onset 26.9 hours after dosing and 1 in the placebo group had an ischemic stroke at 143.9 hours. In addition, 1 placebo subject had asymptomatic carotid occlusion.

None of 8 subjects in the ARC1779 group who had repeat diffusion-weighted imaging had a new lesion on the postoperative scan compared with 2 of 5 in the placebo group; of these 2, 1 had a single lesion (corresponding to a symptomatic stroke) and another had 6 asymptomatic new lesions.

**Blood Biomarkers**
There were no differences between the treatment groups at any time point in S100β.

There were significant differences (P < 0.0001) between the treatment groups for vWF activity intraoperatively and at 1, 2, and 3 hours postdose with much lower mean activity values for ARC-treated subjects (Table 2). There was a correlation between vWF activity and ES count (r = 0.42, P = 0.03) in the groups as a whole and also in the ARC group (r = 0.49, P = 0.048).

**Adverse Events**
There was no excess of any particular adverse event with ARC1779 except for bleeding, which was increased. One
The patient had a serious adverse event of intraoperative bleeding compared with none on placebo. There were 6 nonserious adverse events related to bleeding on ARC1779 and none on placebo, wound bleeding or hematoma in 2, anemia in 2, and anemia and postoperative hematoma in 1.

Mean (SD) time from start of loading dose to surgical wound closure was 169.8 (96.2) minutes for ARC1779 compared with 146.4 (52.3) minutes for placebo ($P=0.37$).

Local intervention to control neck bleeding was necessary in 2 patients on ARC1779 compared with none on placebo.

**Discussion**

The results of this study demonstrate that the novel vWF inhibitor ARC1779 reduces cerebral embolism after CEA. ARC1779 is a new antithrombotic representing both a novel therapeutic class (aptamer) and a novel therapeutic mechanism (vWF antagonism). Previous studies have demonstrated proof of mechanism, but this is the first study to demonstrate efficacy on thromboembolism in vivo.

**Table 2. Mean von Willebrand Factor Activity (%) Intraoperatively, at 1, 2, 3, and 24 Hours Postdose, at Day 7**

<table>
<thead>
<tr>
<th>Assessment</th>
<th>ARC1779, no.</th>
<th>Placebo, no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intraoperative</td>
<td>22.99 (18)*</td>
<td>103.09 (17)</td>
</tr>
<tr>
<td>1 h postdose</td>
<td>11.48 (17)*</td>
<td>105.19 (16)</td>
</tr>
<tr>
<td>2 h postdose</td>
<td>16.56 (17)*</td>
<td>107.85 (17)</td>
</tr>
<tr>
<td>3 h postdose</td>
<td>19.22 (17)*</td>
<td>115.39 (16)</td>
</tr>
<tr>
<td>24 h postdose</td>
<td>123.49 (12)</td>
<td>143.74 (12)</td>
</tr>
<tr>
<td>Discharge</td>
<td>183.41 (12)</td>
<td>132.62 (11)</td>
</tr>
<tr>
<td>Day 7</td>
<td>144.78 (17)</td>
<td>136.02 (15)</td>
</tr>
</tbody>
</table>

*Treatment comparisons statistically significant at 1% level using analysis of variance model (on ranked transformed data) with factors for treatment, center, and subject status (symptomatic/ asymptomatic).

In this first Phase 2 study, we chose to investigate the effect of ARC1779 on embolism in patients after CEA. This was because in this setting, there are frequent ES allowing efficacy to be demonstrated in relatively small patient numbers. ARC1779 resulted in a rapid reduction in ES frequency. The primary end point of time to first ES was highly significant. Secondary end points based on ES detection provided consistent results with either a significant difference or trend in favor of reduced ES in the ARC1779 group. In addition, ES mean intensity was reduced in the ARC1779 group. ES intensity is proportional to embolus size; previous studies have shown a reduction in ES intensity parallels a reduction in ES frequency and therapeutic efficacy.

Phase 1 studies suggested ARC1779 inhibited platelet aggregation without a significant increase in bleeding. This study, in patients undergoing operation, showed an increased bleeding risk with ARC1779. Many of these were related to local wound hematoma and therefore the proportion of patients with bleeding complications is likely to be lower in nonoperated patients. A previous study using similar methodology in patients undergoing CEA found clopidogrel and aspirin were associated with increased time to wound closure, but the same combination was not associated with increased bleeding in nonoperative patients with symptomatic carotid stenosis. However, anemia was also reported in the ARC1779 arm in this study. Previous studies in normal volunteers showed a low bleeding risk, but this was in younger individuals. Therefore, further studies and more safety data are required in nonoperative older patients to determine the bleeding risk associated with ARC1779.

Aptamers are a novel therapeutic class of oligonucleotides with drug-like properties that share some attributes of monoclonal antibodies as well as some of those of low-molecular-weight chemically synthesized drugs. ARC1779 is an aptamer antagonist that blocks the A1 domain of vWF and inhibits vWF-dependent platelet functions.
vWF inhibition offers a number of potential advantages over other antiplatelet agents. First, the vWF platelet interaction pathway is independent of other pathways leading to platelet aggregation through the P2Y12 receptor; therefore, the effect of vWF antagonism should be additive to the effects of P2Y12 antagonists such as clopidogrel. Second, vWF is only active in the presence of the intravascular shear forces found in the arterial-side circulation, and therefore vWF inhibition antagonist should act with spatial specificity potentially reducing hemorrhagic complications. Finally, a vWF antagonist should modulate the cascade of reactions that culminates in thrombus growth at several of its component steps, unlike existing antiplatelet agents that act at a single stage.

The planned sample size for this study was 100 patients. However, the study was terminated after 36 patients due to cessation of funding before any unblinded analysis. Despite this reduction in sample size, we were still able to show a significant effect of ARC1779 in reducing embolization emphasizing the sensitivity of TCD ES detection in evaluating therapeutic agents.

In summary, our results show for the first time that vWF inhibition improves a clinically relevant end point, cerebral embolization in humans. Further studies are now required to determine both whether this treatment approach reduces clinical cardiovascular events and to assess the risk of bleeding complications in a nonoperative situation.

Appendix

Study Personnel

Study sites (with number of patients entered): University Hospital of South Manchester, Manchester, UK (Charles McCollum, Angela Chrisopoulou, Rockesh Gurtu, Richard Pole [13]); University Hospitals Coventry and Warwickshire NHS Trust (Christopher Imray, Colette Marshall, Carl Tirvas [9]); St George’s Healthcare NHS Trust, London, UK (Matt Thompson, Ian Loftus, Gillian Horne, Leanne Seguira [5]); Vascular Specialty Associates, Baton Rouge, Los Angeles, CA (Albert D. Sam [4]); The Methodist Hospital, Houston, Texas, Weill Cornell Medical College, TX (David Chiu, C David McCane, Zsolt Garami, Alan Lumsden [3]); Heart and Vascular Hospital, Hackensack University Medical Center, Hackensack, NJ (Massimo M. Napolitano, Diane Agar [2]).


Data analysis: TCD: Adam Osman, Alice King, Hugh Markus, St George’s University of London. MRI: Core Laboratory of Perceptive Informatics (Lowell, MA).

Data Safety and Monitoring Committee: Kennedy Lees, University of Glasgow, UK (chair), Ross Naylor, University of Leicester, UK; Alan Skeene, Nottingham, UK.

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Disclosures

H.S.M. has received consultancy fees from Archimex Corp. J.G. is an employee of Archimex Corp.

References
