Development of a recalcitrant, large clot burden, bifurcation occlusion model for mechanical thrombectomy

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OBJECTIVE Stroke is a major cause of disability and death in adults. Several large randomized clinical trials have shown the significant benefit of mechanical thrombectomy with modern stent retrievers in the treatment of large-vessel occlusions. However, large clots located at bifurcations remain challenging to treat. An in vivo model of these recalcitrant clots needs to be developed to test future generations of devices.

METHODS Autologous blood was drawn from anesthetized swine via a femoral sheath. Blood was then mixed with thrombin, calcium chloride, and saline, and injected into silicone tubing to form cylindrical clots in the standard fashion. Matured clots were then delivered in an unfragmented fashion directly into the distal extracranial vasculature, at branch points where vessel sizes mimic the human middle cerebral artery, by using Penumbra aspiration tubing and the Penumbra ACE68 reperfusion catheter.

RESULTS A total of 5 adult swine were used to develop the model. The techniques evolved during experiments in the first 3 animals, and the last 2 were used to establish the final model. In these 2 swine, a total of 8 autologous clots, 15–20 mm, were injected directly into 8 distal extracranial vessels at branch points to mimic a bifurcation occlusion in a human. All clots were delivered directly at a distal bifurcation or trifurcation in an unfragmented fashion to cause an occlusion. Ten revascularization attempts were made, and none of the branch-point occlusions were fully revascularized on the first attempt.

CONCLUSIONS Using novel large-bore distal access catheters, large unfragmented clots can be delivered into distal extracranial vessels in a swine occlusion model. The model mimics the clinical situation of a recalcitrant bifurcation occlusion and will be valuable in the study of next-generation stroke devices and in training settings.

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KEY WORDS stroke; model; large-vessel occlusion; swine; thrombectomy; stent retriever

CEREBROVASCULAR disease affects more than 6 million US adults, and the number is expected to increase further with an aging population.^{8,13} Stroke is the fourth leading cause of death in the US, and the primary cause of significant disability.¹³ Ischemic stroke accounts for 87% of all strokes, with large-vessel occlusion (LVO) making up 40% of these. In 2015, several large ran-

domized controlled trials demonstrated a marked benefit of using newer-generation stent retrievers to treat patients with LVOs.^{1,2,5,9,14}

Currently available and commonly used stent retrievers include Solitaire (Medtronic) and Trevo (Stryker Neurovascular); both devices are currently in their second generation, with further iterations in development.

ABBREVIATIONS CCA = common carotid artery; DAC = distal access catheter; ECA = external carotid artery; ICA = internal carotid artery; ID = inner diameter; IMA = internal maxillary artery; LVO = large-vessel occlusion; MCA = middle cerebral artery; TICI = Thrombolysis in Cerebral Infarction.

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The current *in vivo* model for LVO, published in 2006, was based on a 7-Fr guiding catheter delivery platform with an inner diameter (ID) of 0.073 in. However, guiding catheters are difficult to navigate into the distal vasculature, and thus clots either fragment or are uncontrolled in their delivery, and lodge at their destination in an unpredictable manner.

The model described and tested in this study is unique. We used a large-bore distal access catheter (DAC), the ACE68 reperfusion catheter (Penumbra), to deliver an intact clot at a distal bifurcation in the swine's external carotid artery (ECA).

Methods

Experimental Procedures

All cerebral angiographic studies and cerebrovascular interventions were performed at the large animal angiography laboratory at Baylor College of Medicine. All procedures were performed in accordance with the protocol approved by the College's Institutional Animal Care and Use Committee. Two swine were used for final evaluation of the model, using refinements developed in the first 3 animals.

Clot Preparation

Silicone tubing with an ID of 0.188 in was rinsed with saline prior to use. Autologous whole blood (15 ml) was collected from the anesthetized pig via a femoral sheath. Thrombin solution was created by dissolving 7 g of CaCl_2 in 250 ml of phosphate-buffered saline, 3.3 ml of which was then mixed with 100 U of thrombin. This thrombin solution was then mixed at a 1:5 ratio with the autologous blood. The blood and thrombin mixture was quickly injected into silicone tubing, prior to initiation of clotting. The clotted material was matured in the tube for 1 hour prior to use. The clot was cut to the desired length (between 15 and 20 mm) for creation of the vascular occlusion (Fig. 1A). This technique was modified from that used by Gounis et al.⁴

Animal Procedure

Atropine (0.12 mg/kg) was administered as an intramuscular preanesthetic agent. Anesthesia was induced by the veterinary care team, using a mix of Telazol (4.4 mg/kg) and xylazine (2.5 mg/kg). Mechanical ventilation was given with oxygen mixed with isoflurane (1%–3%). Vital signs were monitored throughout the procedure. Female Yorkshire swine, approximately 7 months old and weighing 60–70 lbs each, were used.

A 9-Fr sheath was placed in the right femoral artery by using a modified Seldinger technique following a cutdown. The short sheath was then secured in place. A 7-Fr Cook Shuttle Guiding catheter (Cook Medical) was advanced over an 0.035-in Glidewire (Terumo IS) under fluoroscopy. Heparin (50 IU/kg) was delivered intravenously. Intraarterial verapamil (10 mg) was infused continuously through the guiding catheter.

Under roadmap guidance, the guiding catheter was placed in the common carotid artery (CCA) and a triaxial system was established with a Synchro Standard microwire

FIG. 1. Clot preparation and delivery. **A:** Clot after 1-hour maturation, cut to 2-cm length. **B:** Clot, kept completely intact, loaded into the back end of the Penumbra suction tubing. **C:** Clot traveling retrograde through the Penumbra suction tubing, which is connected to the reperfusion catheter for delivery.

(Stryker), Trevo 18 microcatheter (Stryker Neurovascular), and ACE68 reperfusion catheter.

After the ACE68 catheter was positioned in the desired location, the clot was backloaded into the device through the Penumbra suction tubing (ID 0.110 in), and then flushed antegrade through the system (Fig. 1B and C). Care was taken throughout this procedure to minimize any damage to the clot, and any clots that underwent fragmentation prior to loading into the ACE catheter were discarded so as to use the best possible intact clot for injection. Diagnostic angiography was performed to confirm the occlusion. The clot was then traversed with the microwire and microcatheter in the usual fashion. The Trevo device (4 × 20 mm, Stryker Neurovascular) was used to retrieve the clot under aspiration through the ACE68 catheter, and follow-up angiography was performed to assess reperfusion. Additional attempts were made to effect further recanalization. In trying to develop a “recalcitrant” model, we defined recalcitrance as failure of first-pass revascularization to provide a Thrombolysis in Cerebral Infarction (TICI) Grade 2b or 3 result.

Results

Experimental Animals

A total of 5 swine were used in the development of this model. With the first 3, changes were made to the protocol as follows: barium was eliminated from the initial clot formation to reduce fragmentation; intravenous heparin was added to reduce unrelated thromboembolic events; and an intraarterial vasodilator was added to reduce the incidence of device-related vasospasm. The basic setup of clot backloading through Penumbra tubing (Fig. 1C) into the ACE68 DAC and retrieval with the Trevo device under aspiration was constant across all animals.

The results in the last 2 swine are presented; the meth-

FIG. 2. Example case of clot injection into the distal IMA. **A:** The distal IMA was selected for the ACE68 catheter (white arrow). The 7-Fr guide catheter, used in the classic model, was placed in the distal CCA (black arrow). **B:** Clot has been injected, showing an angiographic “stump.” The shadow of the clot, leading up to the selected trifurcation, can be seen with a faint outline of contrast (notched arrow). **C:** Digital subtraction angiography study obtained via the DAC, with the Trevo deployed within the clot (arrowhead), showing lack of flow through the device. This was concluded to be due to the further clot that was present distally, reaching into all branches off the trifurcation. **D:** Digital subtraction angiography study obtained via the microcatheter, now located more distally, showing flow into this branch vessel beyond the distal branch occlusion. **E:** After the second revascularization attempt, faint flow is seen in 2 branches off the trifurcation, limited by vasospasm that was treated with verapamil. **F:** Recalcitrant clot that was retrieved.

odology described was unchanged between these animals, and comprises our new model. A total of 8 vessels were injected with 8 clots between the 2 animals. The occlusions were all at distal branch points (either bifurcation or trifurcation) of the internal maxillary artery (IMA) (Fig. 2A). Clot fragmentation was not observed in any cases. All occlusions were categorized as “recalcitrant,” which was defined by a recanalization less than TICI Grade 2b after the first pass. Ten revascularization attempts were made by 2 experienced neurointerventionalists, and none of the bifurcation or trifurcation occlusions were successfully revascularized on the first attempt.

Postprocedure Complications

Angiographic vasospasm, ranging from mild to nearly occlusive, was observed in 100% of cases after Trevo retrievals, but no incidences of severe vasospasm were seen secondary to manipulation of the DAC. All vasospasms responded to intraarterial verapamil treatment. No animals exhibited evidence of dissection or perforation related either to the surgical procedure or to the use of equipment. Perioperative mortality was zero; all pigs survived the duration of the procedure prior to euthanasia.

Discussion

In this study, we present the development and initial

evaluation of a novel model for mechanical thrombectomy that simulates the clinical situation of a recalcitrant clot at a bifurcation (such as the internal carotid artery [ICA] terminus, middle cerebral artery [MCA] bifurcation, or basilar apex). This model makes use of newly released large-bore DACs that allow accurate selection of distal vessels to deliver large, unfragmented clots. We sought to develop a bifurcation model because that is often the most challenging clinical situation, requiring separate revascularization of each individual branch.

The swine extracranial model was selected as previously popularized by Gralla et al.,⁶ due to the animals' similarity in vessel size and coagulation parameters relative to humans.¹⁸ This would allow testing of standard human reperfusion platforms in the new model. Others have popularized rabbit models, which more closely mirror human responses to thrombolytics, but involve smaller rabbit blood vessels.^{4,7} The swine model, as used by many others, allows for easy translation of skills from the model to patients, based on similar size and vasculature. After insertion of the femoral sheath via cutdown, the procedure was otherwise a high-fidelity replication of the procedural environment in stroke intervention.

Benefits of the DAC

The strengths of new DACs have been previously described for their clinical utility.^{10,16} The enhanced stability

FIG. 3. An example of the original barium clots after injection. This clot was injected from a similar location to the one in Fig. 2. However, the barium mixed poorly and was thus heterogeneously interspersed through the clot, leading to fragmentation (multiple fragments highlighted by arrows).

and atraumatic trackability of the ACE68 catheter used in this study allowed safe selection of distal arterial bifurcations that could simulate the human MCA, ICA, or basilar bifurcations. Previous studies have used 6-Fr or 7-Fr guide catheters, which have IDs large enough to deliver unfragmented clots, but lack the navigability, trackability, and length to reach an appropriate distal location. The ID of the ACE68 is 0.068 in, which is only marginally smaller than the 7-Fr guide catheter used in previous studies, but with all the additional beneficial characteristics mentioned. The DAC allowed clot injection at locations where vessel sizes had tapered to the size of the human MCA—

approximately 2–3 mm. The ICA and basilar termini are mentioned as potential extensions of the technique used here, although our model is most suited to simulate the human MCA bifurcation or trifurcation based on verisimilitude of vessel size. The guide catheter, which provided good support, was placed safely in the CCA or proximal ECA, so as to avoid vascular trauma or vasospasm.

Clot Selection

One of the lessons learned in the initial 3 animals was simple but important—any minor fragmentation of clot while loading will lead to overt fragmentation distally. Thus, in later experiments, we applied a more stringent quality control check when clots were backloaded into the Penumbra suction tubing before it was connected to the DAC. Occasionally, up to 3 clots may be loaded but show signs of fragmentation before reaching the DAC, but these are flushed out on the table and not delivered. More than enough clots are made in the silicone tubing to apply this stringent quality check. Despite this, clots may show some fragmentation distally based on the force with which they are flushed (Fig. 2C). This can be moderated by using shorter clots (15 mm vs 20 mm) and controlled injection.

Barium and Radiopacity

Previous models^{3,6,11,15,20} have included the addition of barium during clot preparation to enhance the radiopacity of the clot. Although this helps to identify the clot location easily on fluoroscopy and to determine its engagement into the destination vessel, it also weakens clot elasticity³ and, in our experience, led to fragmentation (Fig. 3). Some groups have advocated for other solutions such as the use of fibrinogen,¹¹ which we did not pursue. We found that the benefits of visualization were far outweighed in this regard, because we could visualize the clot quite well with the angiographic “stump” (Fig. 2B).

Procedure-Related Vasospasm

One of the major challenges encountered in working with the swine model is vasospasm.⁴ We found that vasospasm occurred in 100% of the swine subjects, to at least some extent on all stent retriever passes. Vasodilators (both verapamil and nitroglycerin) ultimately helped to resolve this. Our protocol has evolved to include a continuous verapamil infusion through the guide catheter and additional intraarterial infusion as necessary. Vasospasm also occurred in the first 3 animals secondary to the use of the 0.035-in Glidewire in the distal ECA. This was easily mitigated by the use of a microwire and microcatheter over which to advance the DAC.

Recalcitrance of Occlusion

All 8 clots injected with subsequently attempted retrieval failed the first pass for full recanalization, meeting our definition for recalcitrance. This was due to the large clot burden delivered at a bifurcation. In the example case shown in Fig. 2, the Trevo crossed the clot and entered into one of the branch vessels coming off the trifurcation (Fig. 2D), and there was no flow after the first attempt of revascularization. Only after a second pass was faint revascularization in 2 branches achieved (Fig. 2E), after removal of

FIG. 4. Angiography studies showing an attempt at clot aspiration. **Left:** The location of clot injection at an arterial bifurcation is shown, with complete occlusion of one branch and trace flow into the other (arrow). **Right:** The panel shows partial recanalization with moderate vasospasm after primary aspiration thrombectomy.

the recalcitrant clot (Fig. 2F). The recalcitrance of clots delivered in this manner is due to their lodging across an arterial branch point, rather than a characteristic intrinsic to the clots, which were produced by established techniques.

Future Experiments

We describe the initial experience with our model of bifurcation occlusion with 5 animals. Further experiments will help to validate and confirm the recalcitrance of our bifurcation model. This includes the evaluation of different established modalities for thrombectomy, such as the ADAPT (A Direct Aspiration First Pass Technique)¹⁷ and ARTS (Aspiration-Retriever Technique for Stroke)¹² methods, or primary suction thrombectomy (Fig. 4). Other areas of interest would include analysis of the retrieved and injected clot, including variations of the clot production, such as the use of fibrin-rich clot.¹⁹ Analysis of distal clot propagation and time to recanalization of bifurcation occlusions may also be of interest in future studies.

Conclusions

Large-bore DACs allow accurate delivery of large, intact clots at distal arterial bifurcations in a swine model of LVO. This recalcitrant model can be used in training and to test next-generation thrombectomy devices.

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References

- Berkhemer OA, Fransen PS, Beumer D, van den Berg LA, Lingsma HF, Yoo AJ, et al: A randomized trial of intraarterial treatment for acute ischemic stroke. *N Engl J Med* **372**:11–20, 2015
- Campbell BC, Mitchell PJ, Kleinig TJ, Dewey HM, Churilov L, Yassi N, et al: Endovascular therapy for ischemic stroke with perfusion-imaging selection. *N Engl J Med* **372**:1009–1018, 2015
- Chueh JY, Wakhloo AK, Hendricks GH, Silva CF, Weaver JP, Gounis MJ: Mechanical characterization of thromboemboli in acute ischemic stroke and laboratory embolus analogs. *AJNR Am J Neuroradiol* **32**:1237–1244, 2011
- Gounis MJ, Nogueira RG, Mehra M, Chueh J, Wakhloo AK: A thromboembolic model for the efficacy and safety evaluation of combined mechanical and pharmacologic revascularization strategies. *J Neurointerv Surg* **5** (Suppl 1):i85–i89, 2013
- Goyal M, Demchuk AM, Menon BK, Eesa M, Rempel JL, Thornton J, et al: Randomized assessment of rapid endovascular treatment of ischemic stroke. *N Engl J Med* **372**:1019–1030, 2015
- Gralla J, Schroth G, Remonda L, Fleischmann A, Fandino J, Slotboom J, et al: A dedicated animal model for mechanical thrombectomy in acute stroke. *AJNR Am J Neuroradiol* **27**:1357–1361, 2006
- Haider T, Plasenzotti R, Bergmeister H, Mach G, Kleinpeter G, Aguilar-Perez M, et al: New mechanical thrombectomy model in the rabbit: A feasibility study. *J Neurosci Methods* **271**:139–142, 2016
- Heidenreich PA, Trogon JG, Khavjou OA, Butler J, Dracup K, Ezekowitz MD, et al: Forecasting the future of cardiovascular disease in the United States: a policy statement from the American Heart Association. *Circulation* **123**:933–944, 2011
- Jovin TG, Chamorro A, Cobo E, de Miquel MA, Molina CA, Rovira A, et al: Thrombectomy within 8 hours after symptom onset in ischemic stroke. *N Engl J Med* **372**:2296–2306, 2015
- Lin LM, Colby GP, Huang J, Tamargo RJ, Coon AL: Ultra-distal large-bore intracranial access using the hyperflexible Navien distal intracranial catheter for the treatment of cerebrovascular pathologies: a technical note. *J Neurointerv Surg* **6**:301–307, 2014
- Luo ZH, Chung A, Choi G, Lin YH, Uchida BT, Pavcnik D, et al: Creation of fibrinogen-enhanced experimental blood clots to evaluate mechanical thrombectomy devices for treatment of acute stroke: an in vitro study. *J Vasc Interv Radiol* **23**:1077–1083, 2012
- Massari F, Henninger N, Lozano JD, Patel A, Kuhn AL, Howk M, et al: ARTS (aspiration-retriever technique for stroke): initial clinical experience. *Interv Neuroradiol* **22**:325–332, 2016
- Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, et al: Heart disease and stroke statistics—2016 update: a report from the American Heart Association. *Circulation* **133**:e38–e360, 2016
- Saver JL, Goyal M, Bonafe A, Diener HC, Levy EI, Pereira VM, et al: Stent-retriever thrombectomy after intravenous t-PA vs. t-PA alone in stroke. *N Engl J Med* **372**:2285–2295, 2015
- Shao Q, Zhu L, Li T, Li L, Li D, Zhang J, et al: New method of thrombus preparation using a fluid model for evaluation of thrombectomy devices in a swine model. *Thromb Res* **134**:1087–1092, 2014
- Turk A, Manzoor MU, Nyberg EM, Turner RD, Chaudry I: Initial experience with distal guide catheter placement in the treatment of cerebrovascular disease: clinical safety and efficacy. *J Neurointerv Surg* **5**:247–252, 2013
- Turk AS, Frei D, Fiorella D, Mocco J, Baxter B, Siddiqui A, et al: ADAPT FAST study: a direct aspiration first pass technique for acute stroke thrombectomy. *J Neurointerv Surg* **6**:260–264, 2014
- Windberger U, Bartholovitsch A, Plasenzotti R, Korak KJ,

Heinze G: Whole blood viscosity, plasma viscosity and erythrocyte aggregation in nine mammalian species: reference values and comparison of data. **Exp Physiol** **88**:431–440, 2003

19. Yuki I, Kan I, Vinters HV, Kim RH, Golshan A, Vinuela FA, et al: The impact of thromboemboli histology on the performance of a mechanical thrombectomy device. **AJNR Am J Neuroradiol** **33**:643–648, 2012
20. Zhu L, Shao Q, Li T, Saver JL, Li L, Li D, et al: Evaluation of the JReCan device for thrombus retrieval: efficacy and safety in a swine model of acute arterial occlusion. **J Neurointerv Surg** **8**:526–530, 2016

Disclosures

Dr. Chintalapani is an employee of Siemens Medical Solutions

USA, Inc. Dr. Kan is a consultant for Stryker Neurovascular and for Medtronic.

Author Contributions

Conception and design: Kan, Srinivasan, Chen. Acquisition of data: all authors. Analysis and interpretation of data: Kan, Srinivasan, Chen, Camstra. Drafting the article: Kan, Srinivasan, Chen, Camstra. Critically revising the article: Kan, Srinivasan, Chen. Reviewed submitted version of manuscript: Kan, Srinivasan, Camstra, Chintalapani. Statistical analysis: Kan, Srinivasan. Study supervision: Kan, Chen.

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