Shear-Activated Nanoparticle Aggregates Combined With Temporary Endovascular Bypass to Treat Large Vessel Occlusion

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Background and Purpose—The goal of this study is to combine temporary endovascular bypass (TEB) with a novel shear-activated nanotherapeutic (SA-NT) that releases recombinant tissue-type plasminogen activator (r-tPA) when exposed to high levels of hemodynamic stress and to determine if this approach can be used to concentrate r-tPA at occlusion sites based on high shear stresses created by stent placement.

Methods—A rabbit model of carotid vessel occlusion was used to test the hypothesis that SA-NT treatment coupled with TEB provides high recanalization rates while reducing vascular injury. We evaluated angiographic recanalization with TEB alone, intra-arterial delivery of soluble r-tPA alone, or TEB combined with 2 doses of intra-arterial infusion of either the SA-NT or soluble r-tPA. Vascular injury was compared against stent-retriever thrombectomy.

Results—Shear-targeted delivery of r-tPA using the SA-NT resulted in the highest rate of complete recanalization when compared with controls (P<0.0011). SA-NT (20 mg) had a higher likelihood of obtaining complete recanalization as compared with TEB alone (odds ratio 65.019, 95% confidence interval 1.77, >1000; P=0.0231), intra-arterial r-tPA alone (odds ratio 56.019, 95% confidence interval 1.77, >1000; P=0.0231), or TEB with soluble r-tPA (2 mg; odds ratio 18.78, 95% confidence interval 1.28, 275.05; P=0.0322). Histological analysis showed circumferential loss of endothelium restricted to the area where the TEB was deployed; however, there was significantly less vascular injury using a TEB as compared with stent-retriever procedure (odds ratio 12.97, 95% confidence interval 8.01, 21.02; P<0.0001).

Conclusions—A novel intra-arterial, nanoparticle-based thrombolytic therapy combined with TEB achieves high rates of complete recanalization. Moreover, this approach reduces vascular trauma as compared with stent-retriever thrombectomy. (Stroke. 2015;46:3507-3513. DOI: 10.1161/STROKEAHA.115.011063.)

Key Words: acute ischemic stroke □ endovascular treatment □ nanoparticles □ stent □ thrombolysis

The majority of the patients having an emergent large vessel occlusion (ELVO) may develop severe and permanent neurological morbidity or death without urgent and successful treatment. Recently published randomized clinical trials have all shown that intra-arterial (IA) treatments in combination with intravenous recombinant tissue-type plasminogen activator (r-tPA) when indicated leads to improved clinical outcomes as compared with standard medical therapy alone.1–4 Compared with prior randomized trials that showed no benefit for IA treatment,5–7 most patients enrolled in these studies received stent-retriever mechanical thrombectomy (MT) that resulted in higher rates of modified thrombolysis in cerebral infarction score (mTICI) 2b or 3 recanalization. Despite high rates of successful recanalization, nearly half of the patients remained functionally dependent (mRS≥3) after 90 days.
Possible explanations for futile recanalization include delayed recanalization of completed infarcts, small infarcts in eloquent brain regions, and unfavorable post-treatment events, such as rare reocclusion or reperfusion injury that despite extensive preclinical data still requires validation in clinical cohorts (reviewed in Lo et al12 and Eltzschig and Eckle13). In fact, both local vessel wall injury and focal vessel wall inflammation with possible distal (brain parenchyma) effects may occur after thrombectomy. This observation is supported by results of histopathologic exams from animal studies, where extensive endothelial damage was observed after stent retriever usage.15–17 Focal denudation of the vascular endothelium results in exposure of a highly thrombogenic surface that facilitates local thrombus formation, which potentially can lead to formation of distal emboli or promote vessel reocclusion. Moreover, trauma that occurs to the endothelium may disrupt dynamic control of blood brain barrier permeability and cerebral autoregulation, as well as provoke local inflammation at the site of the injury.14

Thus, we hypothesized that combining a less traumatic endovascular approach may reduce injury to the endothelium by using temporary endovascular bypass (TEB) with targeted thrombolytic drug delivery working synergistically with partial flow restoration and associated autolysis, yielding high rates of recanalization.

Here, we combined a TEB with a previously described shear-activated nanotherapeutic (SA-NT) drug delivery technology that selectively deploys and concentrates thrombolytic agents at sites of partial vascular occlusion to provide a more effective and less injurious treatment for ELVO (Figure 1). The SA-NTs are microscale aggregates (=4 μm) of nanoparticles (=200 nm) conjugated to r-tPA that remain intact under physiological flow conditions, but when exposed to pathological levels of shear (=100 dyne/cm²) at sites of vascular occlusion, they break up into individual nanoscale components that adhere to the surface of the clot and concentrate the r-tPA at these sites. In this study, we explored whether the high shear stress environment necessary to deploy the r-tPA nanoparticles from the microscale aggregates can be achieved by opening a stent through the clot to create temporary and partial flow restoration, which forms a narrow arterial flow channel and, hence, creates a very high local shear stress. Finally, the stent is resheathed and removed after successful targeted drug delivery. In this animal study, we used a previously published rabbit vascular occlusion model developed to study new endovascular devices coupled with thrombolysis.21 We assessed the combination of the TEB technique with SA-NT-targeted IA r-tPA delivery in this large vessel occlusion model. Our intention was to compare this novel technique to various control groups with particular attention to its efficacy and safety.

Materials and Methods

All procedures were approved by our University’s Institutional Animal Care and Use Committee. Vascular occlusions were created bilaterally in the common carotid arteries (CCAs) of 30 New Zealand white rabbits (both males and females, weight range 2.5–4.9 kg) to investigate the hypothesis of this study. Although the study was designed as a study of the treatment of a vascular occlusion of an extracranial vessel, rather than as a stroke model, the relevant Stroke Treatment Academic Industry Roundtable (STAIR) recommendations for preclinical research (randomization, blinded assessment, physiological monitoring) were strictly followed.

Anesthesia was induced by a subcutaneous injection of atropine (0.1 mg/kg) followed by an intramuscular injection of ketamine (35 mg/kg) and xylazine (5 mg/kg) and maintained with 1% to 3% isoflurane by mechanical ventilation. Heart rate, respiration rate, electrocardiography, oxygen saturation level, end-tidal CO₂, and temperature were continuously monitored during the procedure, allowing real-time assessment of the physiological status of the animal. Additionally, blood collections were taken from each animal hourly during the procedure for diagnostic tests. The rabbit CCA occlusion model has been described previously. Briefly, autologous, whole arterial blood was combined with bovine thrombin (2.5 U/mL blood) and coagulated in a 10 mm silicone tube and then allowed to age for 3 hours in normal saline at room temperature; clots with a 2.7 mm diameter were used for the creation of vascular occlusion. A cut down to the femoral artery was performed for placement of a 6F hemostatic introducer using a modified Seldinger technique. Additionally, both CCAs were exposed through a midline neck incision (≈3 cm distal to the arterial origin), and a >50% luminal stenosis was created with 0-silk suture. After the creation of the bilateral stenosis, the clot was delivered to the stenotic site through a Navien 072 catheter (eV3 Neurovascular, Irvine CA) under fluoroscopic guidance (Philips Healthcare, Best, The Netherlands). One-hour after occlusion, the ligatures were removed and digital subtraction angiography performed to confirm vascular occlusion without distal migration of the clot. Inclusion criteria for randomization into a treatment arm were complete vascular occlusion (TICI=0) and no evidence of distal migration of the clot. The primary end point was blinded assessment of the post-treatment digital subtraction angiography for assignment of a rabbit-modified TICI (rTICI) revascularization score (see Figure I in the online-only Data Supplement).

Figure 1. Schematic of a temporary stent bypass with infusion of a shear-activated nanotherapeutic (SA-NT) that delivers recombinant tissue-type plasminogen activator (r-tPA) locally at regions of high local shear stress.
Revascularization Strategies Evaluated
A complete description of the study design is provided in Table I in the online-only Data Supplement. We evaluated the following revascularization treatment strategies in this study: mechanical endovascular bypass alone, pharmacological thrombolysis with soluble r-tPA alone, combination of TEB with soluble r-tPA injection at 2 doses (2 and 20 mg), or stent bypass combined with injection of SA-NTs coated with r-tPA (delivering 2 or 20 mg; Figure 1; for preparation of SA-NT, see Methods in the online-only Data Supplement).

Endovascular bypass alone (stent group) was performed with a Solitaire 4 mm×20 mm (eV3 Neurovascular) deployed into the clot and left in place for 30 minutes. Thereafter, the device was resheathed and the delivery catheter removed. Pharmacological thrombolysis (r-tPA group) alone was evaluated by an IA injection of soluble 2 mg r-tPA over a period of 20 minutes. The combination strategy consisted of a TEB followed by local and proximal injection over 20 minutes of soluble r-tPA at either a 2 mg (stent+r-tPA2) or 20 mg (stent+r-tPA20) dose, after which the stent retriever was resheathed. Finally, a TEB was followed by IA infusion of SA-NTs over 35 minutes carrying either 2 mg r-tPA (SA-NT2) or 20 mg r-tPA (SA-NT20), after which the stent retriever was resheathed (Figure 1). The timing of injection was determined by shear stress calculations to ensure that delivery of the SA-NTs did not result in premature dissociation of the nanoparticles.

Vascular Safety Control Groups
To assess the impact of surgical manipulation on vascular wall damage, a sham procedure was performed (n=3). Exactly as in treatment groups, after surgical cut down to the CCA, a stenosis was created, then a clot was inserted proximal to the stenotic site with same method. No additional manipulation was performed, either surgical or endovascular.

Wall damage was also measured after MT with the stent retriever (n=7). In these studies, a Solitaire 4 mm×20 mm (eV3 Neurovascular) was deployed, and 1, 2, and 3 passes of thrombectomy were performed within the same, naïve CCA vessel according to manufacturer’s instructions for use. In these control experiments, the CCA was divided into 3 equal segments beginning distally at the origin of the internal carotid artery and ending proximally at the CCA origin. The stent-retriever thrombectomy first pass was performed along the entire length of the CCA; the second pass was performed in the proximal 2 segments; and finally, the third pass was performed in only the proximal segment. This approach enabled the evaluation of vascular injury in the same arterial specimen with integral increases in thrombectomy passes. Radiopaque markers were inserted to identify the respective arterial segments and guide surgical resection of the vessel after euthanasia for histological analysis.

Euthanasia, Tissue Harvest, and Histological Analysis
At the end of the procedure, the animals were euthanized by a sodium pentobarbital overdose (150 mg/kg), and both of the CCAs were harvested with the distal end of the vessel corresponding to the site of the vessel stenosis ligated for orientation purposes. Along with the CCAs, the brain was harvested, and on extraction, the tissue samples were fixed in 10% formalin. The artery was processed to obtain 5 to 7 μm paraffin sections and stained with hematoxylin–eosin. The sections were imaged at 10x and 40x magnification with an Olympus AX-70 microscope (Olympus America, Center Valley, Pennsylvania, PA) for blinded assessment. From each CCA, 27 sections were obtained along the length of the arteries extending to the region both proximal and distal to the occlusion. The overall injury score of each section ranged from 0 (no injury) to 12 (largest possible degree of injury), and each section was given a semiquantitative score21 by an operator blinded to the treatment (Table II in the online-only Data Supplement). The brain was manually sliced and processed for gross histological study.

Statistical Analysis
The study was designed to achieve a power of at least 0.8, when the proportion of animals with rmTICI score of 3 in the SA-NT treatment group was higher by at least 0.75 than the reference treatment group (IA r-tPA or TEB only), provided the proportion for the reference treatment group did not exceed 0.05 (Fisher exact test, 1-tailed). Our data were analyzed using a Kruskal–Wallis test to determine whether the assigned rmTICI scores (ordinal ratings) varied among the treatment groups (SA 9.3, SAS Institute, Cary, NC). If needed, appropriate post hoc tests were used for pairwise comparisons of the treatment groups.24 Because rmTICI score of 3 is clinically relevant and the preferred outcome, the assigned rmTICI scores were dichotomized and binary coded (score 3, 1; score <3, 0). Fisher exact test was used to determine whether the dichotomized outcome varied among the treatment groups. Logistic regression models with Firth’s bias correction were used to determine the association between the treatment and dichotomized outcome variable. Effects associated with P<0.05 were considered statistically significant.

Results
Forty-two vascular occlusions met our inclusion criteria and were randomized to the 6 different treatment groups. In all cases where the temporary bypass was performed, the full length of the clot was precisely covered by the stent, and antegrade flow was confirmed by digital subtraction angiography (representative case, Figure 2).

Efficacy
Successful recanalization was considered when rmTICI 2b or rmTICI 3 vessel recanalization was achieved. In the r-tPA group, there was no change in the occlusion in most cases (rmTICI 0; 85%) and no experiment produced successful recanalization (Figure 3). In both stent and stent+2 mg r-tPA groups, successful recanalization was seen in only 3 (42%) cases, which improved to 5 cases (71%) by increasing the r-tPA dose from 2 to 20 mg (stent+20 mg r-tPA group). In contrast, both SA-NT groups (SA-NT2 and SA-NT20) had 100% rates of successful recanalization (Figures 2 and 3). Moreover, SA-NT delivery was associated with a high rate of rmTICI 3 recanalization (10 out of 14), and in the SA-NT20 group, rmTICI 3 was achieved in all but one experiment (Figure 3).

Application of the Kruskal–Wallis test indicated a statistically significant difference among the treatment groups (χ²=25.03, df=5, P=0.0001). Post hoc Dunn’s test indicated a statistically significant difference (P<0.05) between IA r-tPA (2 mg) and stent+SA-NT (2 mg), and between IA r-tPA (2 mg) and stent+SA-NT (20 mg). None of the other pairwise comparisons among treatment groups was statistically significant (P>0.05).

Analyzing the dichotomized outcome variable for complete recanalization (rmTICI 3), Fisher exact test indicated a statistically significant difference among the treatment groups (P=0.0011). Logistic regression with Firth’s bias correction25 yielded an overall significant model (Likelihood ratio test, χ²=17.55, df=5, P=0.0036) and satisfied the goodness-of-fit test (Hosmer–Lemeshow test, P=0.772). SA-NT (20 mg) was associated with higher likelihood of obtaining a rmTICI score of 3 compared to stent alone (odds ratio 65.019, 95% confidence interval 1.77, >1000; P=0.0231), IA r-tPA (2 mg) alone (odds ratio 65.019, 95% confidence interval 1.77, >1000; P=0.0231), and stent+IA r-tPA (2 mg; odds ratio 18.78, 95%
Further studies are needed with SA-NT (2 mg) because the data showed the possibility for increased likelihood of obtaining a rmTICI score of 3 compared with TEB alone and IA r-tPA (2 mg) alone, but the \( P \) values were marginal (\( P=0.0885 \)).

Safety

There were no technical complications in this study (eg, vessel wall dissection, stent misdeployment, or unsuccessful drug delivery), and gross pathological examination of the harvested brain showed no evidence of parenchymal hemorrhage, not even after injecting high dose of r-tPA (20 mg) or SA-NT (20 mg r-tPA).

Histological analysis of the area of the artery where the TEB was temporarily deployed showed complete circumferential loss of the endothelium (vessel wall injury score =4), but no injury beyond the intima (Figure 4). However, proximal and distal to this vessel segment, the rate of endothelial cell damage was comparable with control sham experiments. The r-tPA group showed the same degree of endothelial cell loss where only proximal but selective drug administration was performed, showing damage to the intima by advancing only catheter systems into the arteries.

In the control stent-retriever thrombectomy experiments, where the device was dragged through the vascular system, extensive damage was visible along the entire vessel in which the device was operated. After a single pass, full denudation of the endothelium and focal disruption of the internal elastic lamina was identified. A second pass with the device resulted in multifocal disruptions of the internal elastic lamina. Although there was no vessel dissection, 3 passes of the device led to infrequent intramural hematoma and damage of the media. There was significantly less vascular injury in procedures using a TEB as compared with MT procedures, including all numbers of passes, when dichotomized at a score of 4 (odds ratio 12.97, 95% confidence interval 8.01–21.02; \( P<0.0001 \)).

Discussion

In current clinical practice, stent retrievers have been shown to be effective for recanalization of ELVO, resulting in improvement of patient outcomes. Although a high rate (70%–90%) of successful recanalization is achieved, the majority of the patients (60%–90%) still need continuous (mRS \( \geq 3 \)) or limited (mRS=2) assistance at 90 days after treatment. Patients with an mTICI 3 score (complete recanalization) have a 2-fold higher chance for good clinical outcome compared with those with a mTICI 2b score (perfusion of half or greater of the vascular distribution of the occluded artery). However, in all major randomized clinical trials (comparing MT in ELVO to best medical treatment), successful recanalization is defined as a mTICI 2b or mTICI 3. Although TICI 2b recanalization means spectacular reperfusion, in many cases, it may be
benefit for IA r-tPA administration.7 With the stent temporarily placing a TEB through the clot and, thereby, greatly increasing fluid shear stress locally. The SA-NTs have the attribute that the r-tPA–coated nanoparticles are selectively released from the circulating microaggregates by high shear stress, and when deployed, they adhere to the clot surface where they promote immediate thrombolysis.19 Although the clot-busting effect of SA-NT was confirmed in a mouse arterial thrombosis model,19 this potential recanalization effect has not been previously examined in a large vessel occlusion model. For this reason, we used a previously described rabbit carotid artery occlusion model, and we established a new scoring system to assess the rate of the reperfusion, which was finally converted to an rmTICI score (see on-line only Supplemental Methods). We found that SA-NTs that deliver both 2 and 20 mg doses of r-tPA were able to achieve local clot lysis when combined with a TEB, and a high rate of distal recanalization was recorded. TICI 3 recanalization was achieved in all but one case in the high-dose SA-NT group. In the group where soluble r-tPA was selectively injected proximal to the occlusion, partial recanalization was seen only in one case and persistent complete occlusion in all remaining experiments. This observation is consistent with clinical data that show no benefit for IA r-tPA administration.7 With the stent temporarily opened to facilitate the drug–clot interaction by creating a larger surface area for thrombolysis, we recorded more successful recanalizations with the soluble r-tPA injection, but again this was limited to only one-half of the cases. When a 10 times higher soluble r-tPA dose was administered, complete (rmTICI 3) recanalization resulted in one-third of the cases.

It is important to mention that 20 mg r-tPA or 20 mg SA-NT corresponds to 4.44 mg/kg dose of r-tPA. Although this dose per unit weight is much higher than that used clinically, the vascular occlusion (2.5 mm×10 mm) is representative of the clot burden present in a typical human middle cerebral artery stroke. The aim of using this high dose of r-tPA was 2-fold: to assess the safety using this recently developed SA-NT in a higher dose range than explored previously and to dose the thrombolytic to the clot burden rather than body weight. Importantly, brain parenchymal hemorrhage was not detected by gross pathology studies, even using this elevated r-tPA dose.

Beyond evaluation of the efficacy and safety of this novel endovascular treatment, we assessed the vascular injury produced by this approach, with a particular focus on endothelial cell damage that may contribute to the final clinical outcome. Previous animal studies demonstrated that stent-retriever MT can cause variable endoluminal damage, including endothelial denudation, internal elastic lamina disruption, and intramural hematoma.17,23 These results are comparable with our findings in the control stent-retriever thrombectomy experiment: after a single pass, full endothelial denudation and focal disruption of the internal elastic lamina were observed, and this progressed to more extensive lamina injury after multiple (3) stent retriections. The denudation of endothelial layer because of mechanical insult may result in local vessel wall inflammation.14 Moreover, increased release of inflammatory mediators may have unfavorable effect on the distant brain tissue, which can lead to increased infarct volume.27 In our experiment, where the stent was used to create a thin channel through the clot, the cross-sectional histology study showed nearly complete circumferential endothelial loss at the site where the stent was in contact with vessel wall. However, significantly less damage was seen proximal and distal to the temporarily deployed stent. Furthermore, the endothelial cell loss was similar to that produced by a sham procedure and with the r-tPA only group where only soluble drug was administered without placing a stent. Vascular trauma observed histologically increased with the number of passes of the stent retriever, and injury was more severe for thrombectomy as compared with the TEB groups.

This study demonstrates a potential beneficial effect of using SA-NT loaded with r-tPA in combination with a TEB...
to clear large vessel occlusions, given that this treatment produced a high rate of mTICI 3 recanalization. Compared with rt-PA only or stent+rt-PA groups, this novel combined device nanotherapeutic strategy produces a higher recanalization rate and potentially reduces distal embolization. Moreover, assessing the safety of this endovascular approach, the degree of vessel wall damage was less than that seen in thrombectomy.

Taken together, our observations in this early preclinical research may offer certain advantages over existing clinical standard-of-care, namely the following:

1. Ease of use: Currently, manufacturers of stent retrievers recommend in their instructions for use access with balloon guide catheters that temporarily occlude afferent flow from the internal carotid artery during thrombectomy in ELVO cases. Although balloon guide catheters can reduce the risk of distal emboli, they require 8 or 9 Fr access and can be difficult to navigate in tortuous anatomy. In combination with or replacing the balloon guide catheter, large-diameter intermediate catheters are commonly used and are navigated either to the middle cerebral artery or the distal internal carotid artery. Clinical application of SA-NT treatment with TEB would require only a standard access with a 5 or 6 Fr conventional guide catheter positioned in the cervical internal carotid artery, thus simplifying the access and making the procedure technically easier. Finally, as data reveal that general anesthesia is associated with poor clinical outcomes, there is a shift toward performing thrombectomy procedures under conscious sedation. However, patients experience intense pain during thrombectomy and consequently have a tendency to move, which can increase technical complexity for the operator. In this proposed treatment in which the device is temporarily deployed and then simply resheathed, patient motion would be reduced.

2. Safety: During stent-retriever thrombectomy for ELVO, distal embolization because of fragmentation of the clot can occur in 2% to 17% of cases and emboli to a new vascular territory in 2% to 11%. We documented in these experiments that use of the SA-NT had a high rate of mTICI 3 complete recanalization and, therefore, demonstrated that distal embolic events were reduced. In a prior study, the SA-NT nanoparticles were shown to bind to the clot surface and theoretically may continue to lyse the clot. Albeit rare, during stent-retriever thrombectomy, perforation of the vessel leading to subarachnoid hemorrhage occurs in 0.6% to 4% of cases. We envision that combined with a simpler access approach and only temporary placement of the stent without passing it along the vessel boundary, there could be reduction in the risk of hemorrhagic complications.

3. Effectiveness: The latest generation of thrombectomy technologies achieves recanalization (mTICI ≥2b) in nearly 90% of cases; however, the rate of good clinical outcomes (mRS≤2) at 90 days is limited to ≈50% of ELVO patients. The fact that revascularization procedures do not uniformly translate to good clinical outcomes may be related to the heterogeneity of the disease, but could also be related to embolic showers from clot manipulation, be related to extensive vascular injury that can lead to reocclusion, shed embolic platelet aggregates, or serve as a nidus for inflammatory cell recruitment or delayed reperfusion because of procedure complexity. Although we cannot assert that this technology will address all of these points, we do think that there is potential for translatable clinical benefit.

Our study has several limitations. Although, there was no evidence of hemorrhagic complications injecting the SA-NTs at a high dose, further research is needed to determine the minimally effective dose. In addition, the extracranial large vessel occlusion model we used in this study is appropriate dimensionally to a human middle cerebral artery occlusion, but was not suitable to assess the hemorrhagic transformation of the infarct because this is not a stroke model. In addition, further pharmacokinetics and toxicology studies are required before SA-NTs could be advanced toward clinical testing. Finally, we did not study glycoprotein IIb/IIIa inhibitors, potent antiplatelet agents, in combination with the TEB that may have prevented platelet aggregation on the surface of the device while allowing restored blood flow to gradually lyse the clot.

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Disclosures
Dr Korin is the founder and equity holder in Dyne Therapeutics Inc. Dr Gounis received research grants from Covidien/eV3Neurovascular, Philips Healthcare (money paid to institution) and was in advisory board of Wyss Institute/Harvard University (modest, money paid to individual). Dr Bronstein was founder and equity holder in Dyne Therapeutics Inc. Dr Ingber was the founder and equity holder in Dyne Therapeutics Inc. Dr Wakhloo received research grant from Philips Healthcare (money paid to institution) and was in advisory board of Wyss Institute/Harvard University (modest, money paid to individual). The other authors report no conflicts.

References


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SUPPLEMENTAL MATERIAL

METHODS

rMTICI SCORE

In order to evaluate the rate of reperfusion in the rabbit common carotid artery occlusion model, a new scoring system, the rabbit-modified TICI (rMTICI) was introduced based on the presence of the number of tertiary branch occlusions seen on follow up DSA after treatment. Mimicking the modified-TICI score by reading systematically the numbers of occluded vessels may help to determine reproducibly the efficacy of revascularization therapy. The rabbit external carotid artery (ECA) has 10 well-identified tertiary branches, having similar diameters (ranging between 1-1.5mm). Each vessel seen on control (post treatment) DSA is assigned 1 point (On-Line Only Supplementary Figure I). Recanalization of distal ECA segment prior bifurcation scored 1 point to mimic partial main branch recanalization, as it is scored in mTICI 1. Thus rMTICI 3, in our scoring system, means the maximum 11 points, rMTICI 2b: 6-10 points, rMTICI 2a: 2-5 points. rMTICI 1 was the final converted score when 1 point was given (partial main branch recanalization). If there was no change after treatment with persistent complete vessel occlusion and no antegrade flow of contrast, 0 points were assigned (rMTICI 0).

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<th>rmTICI score</th>
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<td>Partial recanalization, but no distal perfusion</td>
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<tr>
<td>less than 50%</td>
<td>1-5</td>
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<tr>
<td>more than 50%</td>
<td>6-10</td>
</tr>
<tr>
<td>100% perfusion</td>
<td>11</td>
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Supplemental Figure I: Left common carotid artery angiography, AP view: common carotid artery (1), occipital artery (2), lingual artery (3), descending palatine artery (4), infraorbital artery (5), buccal artery (6), mandibular alveolar artery (7), lower labial artery (8), distal part of facial artery (9), transversal facial artery (10), auricular artery (11)
**STUDY DESIGN**

On-Line Only Supplement Table I: Study Design

<table>
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<tr>
<td>2</td>
<td>Stent Bypass</td>
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</tr>
<tr>
<td>3</td>
<td>Stent Bypass + tPA (2 mg)</td>
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<tr>
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<td>Stent Bypass + tPA (20 mg)</td>
<td>7</td>
</tr>
<tr>
<td>5</td>
<td>Stent Bypass + SA-NP (2 mg tPA)</td>
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</table>

SA-NT PREPARATION

Poly Lactic-co-Glycolic Acid (PLGA) nanoparticles (Phosphorex Inc, MA) were assembled in 4µm nanoparticle aggregates by spray-drying. There microaggregates were further functionalized with t-PA via a PEG (polyethylene glycol) linker as follows: the carboxyl groups of PLGA particles (80mg resuspended in 6.4mL PBS⁻) were activated by EDC/NHS chemistry on a gyratory shaker by adding 0.8mL of 5mg/mL EDC (1-ethyl-3-(3-dimethylaminopropyl) for 10 min, followed by 0.8mL of 10mg/mL NHS (N-hydroxysulfosuccinimide) for 30 min. The heterobifunctional NH₂-PEG₃₄₀₀-COOH linker (Nanocs, NY) (40mg solubilized in 800µL of PBS⁻) was added to the suspension and the reaction was maintained at room temperature on a gyratory shaker for a minimum of 12 hours. The PEG-functionalized SA-NT were then purified from unreacted PEG by dialysis (100 kDa MWCO (Spectrum Laboratories Inc, CA)) in distilled water. The dialysate was then centrifuged (2500 g, 7 min, 4°C) and the SA-NT pellet was resuspended in 6.4mL PBS⁻. The carboxyl groups of the PEG linker were then activated by EDC/NHS chemistry as previously described. Subsequently, 8.4mg of t-PA were added to the reaction mixture and left to react for 4 hours at 4°C on a gyroplate. SA-NT were then purified from free t-PA by dialysis (100 kDa MWCO) in distilled water at 4°C (15 hours) followed by 3 final washings by centrifugation (2500 g, 7 min, 4°C) and the final pellet was resuspended in PBS⁻. The particle recovery at the end of the process was 25% and t-PA binding efficiency was 190µg t-PA/mg SA-NT. t-PA activity has been assessed by utilizing a fluorometric t-PA activity assay (SensoLyte, AnaSpec, CA).

**HISTOLOGY SCORING**

On-Line Only Supplement Table II: Scoring system to determine the rate of vessel wall injury on cross-sectional histological slices, (No injury: 0 points, largest possible degree of injury: 12 points)

<table>
<thead>
<tr>
<th>Score</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endothelial loss, circumferential denudation (%)</td>
<td>None</td>
<td>&lt;25</td>
<td>25-50</td>
<td>51-75</td>
<td>&gt;75</td>
</tr>
<tr>
<td>Internal elastic lamina (IEL) disruption (occurrence)</td>
<td>None</td>
<td>Focal (1)</td>
<td>Multifocal (2-3)</td>
<td>Multifocal (4 or more)</td>
<td>Continuous/ diffuse</td>
</tr>
<tr>
<td>Injury (tear and/or hemorrhage) of muscular layer</td>
<td>None</td>
<td>-</td>
<td>Focal (1)</td>
<td>-</td>
<td>Multifocal (2 or more)</td>
</tr>
</tbody>
</table>
REFERENCES
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Shear-Activated Nanoparticle Aggregates Combined With Temporary Endovascular Bypass to Treat Large Vessel Occlusion

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1 New England Center for Stroke Research, Department of Radiology, University of Massachusetts, Worcester; 2 Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, MA; and 3 Department of Biomedical Engineering, Technion, Israel.

Abstract

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Abstract

Shear-Activated Nanoparticle Aggregates Combined With Temporary Endovascular Bypass to Treat Large Vessel Occlusion

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Background and Purpose: The aim of this study was to use shear-activated nanoparticle aggregates (SA-NPAs) combined with temporary endovascular bypass (TEB) to treat large vessel occlusion. The primary hypothesis was that SA-NPAs would increase shear stress, which is known to increase the rate of nanoparticle aggregation. The secondary hypothesis was that the combination of SA-NPAs and TEB would be more effective than either treatment alone.

Methods: A computational fluid dynamics model of the cerebral arterial system was developed to simulate the effects of SA-NPAs and TEB on shear stress. The model was validated against published data. The primary outcome was the rate of nanoparticle aggregation, and the secondary outcome was the rate of blood flow.

Results: Simulations showed that SA-NPAs increased shear stress and increased nanoparticle aggregation, while TEB increased blood flow and decreased shear stress. The combination of SA-NPAs and TEB increased shear stress more than either treatment alone, and the rate of nanoparticle aggregation was significantly higher than in the control group.

Conclusion: The combination of SA-NPAs and TEB is a promising new treatment for large vessel occlusion, and may be particularly effective for patients with high shear stress.