Aptamer Inhibitor of Von Willebrand Factor Induces Thrombolysis and Improves Vascular Patency Upon Immediate and Delayed Treatment of Stroke in an *in vitro* Microfluidic Model

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Introduction

- Von Willebrand factor (VWF) is critical in arterial thrombosis.
- Previous studies have shown that aptamer BB-031, a VWF inhibitor, induces thrombolysis *in vivo* in ischemic stroke models, making it a promising stroke therapeutic [1].
- Microfluidic models have demonstrated a dose dependent VWF inhibition by BB-031 correlates with thrombolysis [2].
- This study aims to further investigate BB-031 thrombolysis and BB-031 efficacy in immediate and delayed treatment of stroke via a novel microfluidic platform, in addition to comparison with current standard of care stroke therapeutics.
- We hypothesized that BB-031 will induce thrombolysis in an *ex vivo* ischemic stroke model and that advanced imaging techniques will provide mechanistic information.

Methods

- A custom-made microfluidic model was utilized to form an *ex vivo* ischemic stroke clot and facilitate drug delivery immediately upstream of the occluded thrombus without disruption (Figure 1).
- Heparinized whole blood was obtained from healthy volunteers (N=10).
- Gravity-based constant pressure system mimicking arterial perfusion induced occlusive thrombosis at a collagen-coated region of stenosis.
- After 0, 3, or 6 hours “resting” to mimic delayed treatment and allow for clot retraction, *in situ* thrombi were reperfused with blood containing 1692nM (~1mg/kg) BB-031, 0.7nM Alteplase (ALT), 0.7nM Tenecteplase (TEN), or vehicle 500 microns upstream of the thrombus.
- Platelets were labelled prior to thrombus formation and reperfusion using fluorescent anti-CD41 for visualization.
- Thrombolysis and patency were monitored for 2 hours via outlet mass recording and z-stacked immunofluorescent imaging.

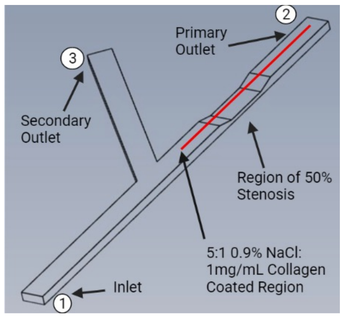

Figure 1. Microfluidic Channel Design. Sample is perfused into the inlet (1) through the primary outlet (2), while the secondary outlet (3) is closed. The red line indicates the stenosed region functionalized with a collagen coating where occlusive thrombus forms at the stenosis throat. The primary outlet (2) is then closed, and new sample can be perfused via the secondary outlet (3) without disturbing the thrombus. The secondary outlet is then closed again, the primary outlet reopened to allow natural perfusion, if achieved, of the occlusion.

Results

- Upon immediate reperfusion (0 hours resting), BB-031 increased mean mass output by 0.245 ± 0.631g compared to 0.047 ± 0.068g with vehicle, 0.080 ± 0.062g with ALT, and 0.088 ± 0.124g with TEN.
- After a 3-hour retraction period, BB-031 reperfusion mean mass output was 0.951 ± 1.279g compared to 0.156 ± 0.266g with vehicle, 0.487 ± 0.597g with ALT, and 0.249 ± 0.329g with TEN.
- After a 6-hour retraction period, reperfusion mass output increased by 1.354 ± 1.433g with BB-031, 0.297 ± 0.497g with vehicle, 0.482 ± 0.725g with ALT, and 0.326 ± 0.373g with TEN.
- A subset of donor matched experiments show enhanced clot volume reduction with BB-031 when utilizing z-stacked renderings of the thrombus pre and post reperfusion.
- In a donor-matched data set (n=4), after a 6-hour retraction period, BB-031 decreased volumetric thrombus size by an average of 51%, whereas vehicle thrombus size decreased 16%, and ALT and TEN both averaged increases in thrombus size.

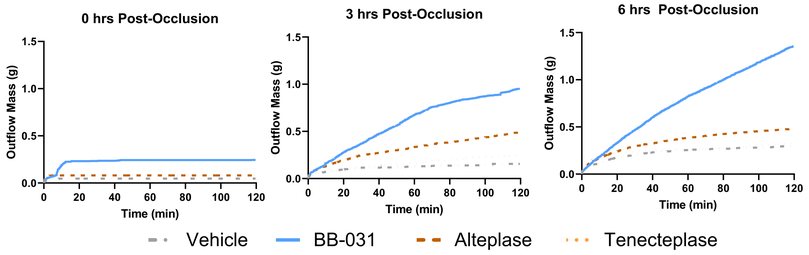

Figure 2. Outflow Mass During Reperfusion. After occlusive thrombus formation, new sample containing vehicle, BB-031, Alteplase, or Tenecteplase was perfused immediately upstream and a gravity-based constant pressure system allowed for natural perfusion over 2 hours. Increase in outflow mass indicates recanalization of the occlusive thrombus.

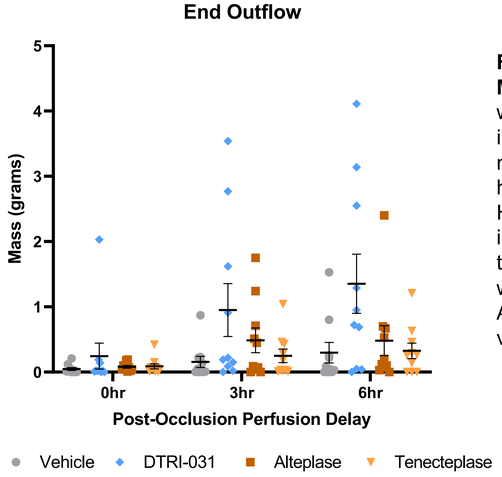

Figure 3. End Outflow Mass. Patency is illustrated with the final mass collected in the outlet of the microfluidic model after a 2-hour reperfusion period. Higher outflow mass indicates occlusive thrombi that achieved recanalization with BB-031 compared to Alteplase, Tenecteplase, or vehicle.

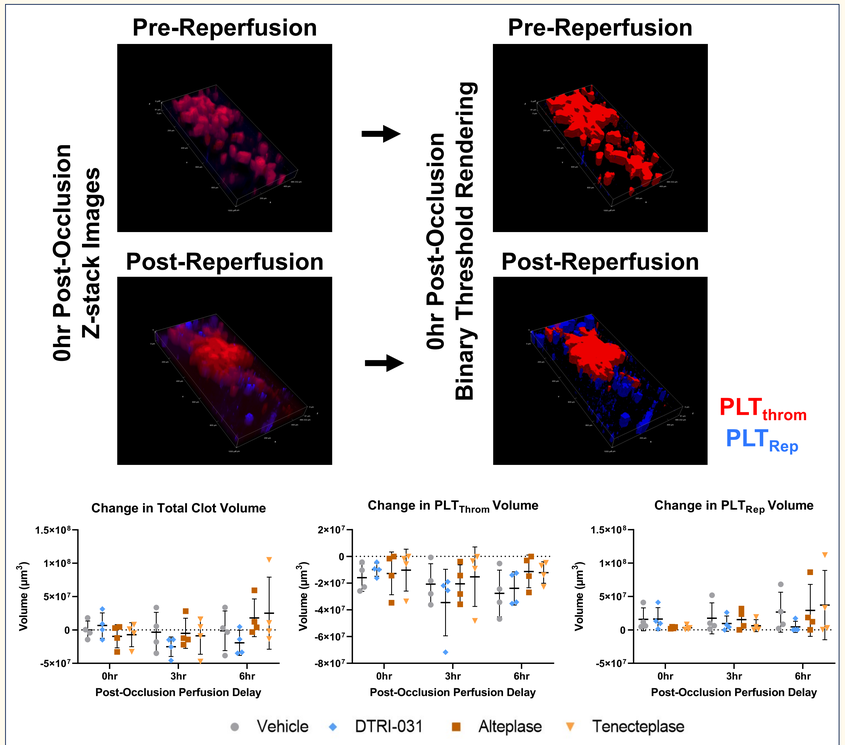

Figure 4. Changes in 3D Structure. Pre- and post-reperfusion z-stack images, representative 0-hour resting vehicle treatment shown above, allowed for analysis of binary threshold renderings where total clot volume, and the contribution of the original thrombus (PLT_{Throm}) and reforming thrombus from the reperfusion sample (PLT_{Rep}) were quantified.

Conclusions

BB-031 improves patency in a microfluidic model of biofidelic thrombolysis compared to current standards of care, which is enhanced at 3 and 6 hours post-occlusion. This may be due to VWF-dependent lysis resulting in reduced thrombus volume.

References

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