Making Clots and Breaking Clots: Modelling Arterial Occlusion to Test Stroke Sonothrombolysis

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The thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to the final version of my thesis being made available worldwide when deposited in the University's Digital Repository*, subject to the provisions of the Copyright Act 1968. * Unless an embargo has been approved for the determined period.

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I hereby certify that the work embodied in this thesis has been done in collaboration with other researchers, and carried out in other institutions. I have included as part of the thesis a statement clearly outlining the extent of collaboration, with whom and under what auspices.

See Appendix A.

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I hereby certify that the work embodied in this thesis contains published papers of which I am a joint author. I have included as part of the thesis a written statement, endorsed by my supervisor, attesting to my contribution to the joint publications.

See Appendix A.

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Amelia Tomkins *Date*

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ABBREVIATIONS

Please note, this thesis uses British English unless directly citing a paper or clinical trial name.

ACA	Anterior cerebral artery
ACEC	Animal Care and Ethics Committee
AIC	Acute ischaemic changes
BPU	Blood perfusion units (for laser Doppler flowmetry)
CCA	Common carotid artery
CT	Computed Tomography
ECA	External carotid artery
GP IIb/IIIa	Platelet glycoprotein IIb/IIIa receptor
HU	Hounsfield units
Hz	Hertz
ICA	Internal carotid artery
JLU	Justus-Liebig University, Giessen, Germany
kHz	Kilohertz
LDF	Laser doppler flowmetry
MCA	Middle cerebral artery
mg/kg	Milligrams per kilogram of body weight
MHz	Megahertz
MRI	Magnetic resonance imaging
mRS	Modified Rankin Scale
mW/cm^2	Milliwatts per square centimetre
NIHSS	National Institute of Health Stroke Score
o.d.	Outer diameter
PAI-1	Plasminogen inhibitor-1
PRC	Platelet rich clot
RBC	Red blood cell
rCBF	Regional cerebral blood flow
ROI	Region of Interest
SAH	Subarachnoid haemorrhage
SD	Standard deviation
SHR	Spontaneously hypertensive rat
sICH	Symptomatic intracerebral haemorrhage
TCCD *or* TCCS	Transcranial color-coded Doppler/sonography

TCD	Transcranial Doppler ultrasound
tPA *or* rt-PA	(Recombinant) tissue plasminogen activator
TTC	2,3,5-triphenyl-tetrazolium chloride
U/S	Ultrasound
UoN	University of Newcastle, Australia
VV	Vascular volume
VVF	Vascular volume fraction

CLINICAL TRIALS REFERENCED IN THIS THESIS

Clinical trial names are defined in footnotes at first mention throughout the text.

CLOTBUST	Combined Lysis Of Thrombus in Brain ischaemia using transcranial Ultrasound and Systemic tPA (Clinical sonothrombolysis Trial)
CLOTBUST-ER	Combined Lysis of Thrombus With Ultrasound and Systemic Tissue Plasminogen Activator (tPA) for Emergent Revascularization in Acute Ischemic Stroke
ECASS III	European Cooperative Acute Stroke Study III
ESCAPE	Endovascular Treatment for Small Core and Anterior Circulation Proximal Occlusion with Emphasis on Minimizing CT to Recanalization Times
EXTEND-IA	Extending the Time for Thrombolysis in Emergency Neurological Deficits - Intra-Arterial
MR CLEAN	Multicenter Randomized CLinical trial of Endovascular treatment for Acute ischemic stroke in the Netherlands
NINDS	National Institute of Neurological Disorders and Stroke rtPA Stroke study
REVASCAT	RandomizEd Trial of reVascularizAtion With Solitaire FR® Device Versus Best mediCal Therapy in the Treatment of Acute Stroke Due to anTerior Circulation Large Vessel Occlusion Presenting Within 8 Hours of Symptom Onset
SWIFT-PRIME	Solitaire With the Intention For Thrombectomy as PRIMary Endovascular Treatment
TRUMBI	TRanscranial low-frequency Ultrasound-Mediated thrombolysis in Brain Ischemia (Clinical sonothrombolysis Trial)
TUCSON	Transcranial Ultrasound in Clinical Sonothrombolysis (Clinical sonothrombolysis Trial)

Abstract

Background: Acute ischaemic stroke is caused by occlusion of a major cerebral artery and is a major cause of death and disability worldwide. Early reopening of the occluded artery (recanalization) to restore blood flow to the ischaemic tissue is the best known approach to improving patient outcome after stroke. However, the current approved thrombolytic drug, tissue plasminogen activator (tPA), causes recanalization in <50% of cases treated, indicating a need for better recanalization approaches. Recent studies have revealed benefit of endovascular intervention to remove the occluding clot, but with limited endovascular centres, improving tPA's effect may be a better and more cost effective approach for most centres. One such approach to enhanced tPA recanalization is with the application of continuous ultrasound insonation during tPA infusion (sonothrombolysis). Small scale trials of sonothrombolysis for stroke have shown improved recanalization and patient outcome over tPA alone. Added infusion of microbubbles has also been suggested to enhance sonothrombolysis. However, much is still unknown regarding sonothrombolysis efficacy in different situations of stroke and in preclinical models of clinically relevant scenarios. Clinical trials of sonothrombolysis have thus far focused on intracranial large vessel occlusions. However, stroke is a heterogeneous condition whereby patient outcome is affected by clot compositions, the occlusion site, and vascular stenosis. Efforts to model the clinical situation have also resulted in a large variety of experimental clots used for preclinical thrombolytic testing. Preclinical studies of sonothrombolysis to-date have tended to use models with clot compositions that are more susceptible to thrombolysis than a more clinically common platelet rich clot (PRC) composition. The effect of sonothrombolysis on PRC is yet to be tested *in vivo*. The middle cerebral artery (MCA) is the most commonly affected vessel in stroke, yet occlusions of the internal carotid artery (ICA), often associated with vascular stenosis, are associated with poorer patient outcome and lower rates of tPA recanalization. The recanalization potential of sonothrombolysis for extracranial carotid occlusions has not previously been tested, nor the effect of varying degrees of stenosis underlying an occlusion. While recanalization of the large occluded arteries is known to be a predictor of good patient outcome, reperfusion of the ischemic tissue has recently been shown to be a better predictor of good outcome than recanalization. Perfusion deficits caused by occlusions of the microvasculature can persist even after recanalization due fibrin and platelet deposits causing a "no-reflow phenomenon". Improving therapies to target not only large vessel recanalization, but also microvascular reperfusion should improve the overall rates of patient outcome after stroke. To date, there has only been one preclinical study of sonothrombolysis of the microvasculature that suggested a benefit of this therapy for stroke reperfusion. Sonothrombolysis ± microbubbles may therefore, be a potential therapy to target both large vessel recanalization and microvascular reperfusion. To

obtain meaningful data of the potential of sonothrombolysis for stroke, it needs to be compared against the current standard, tPA. Rodent doses of tPA are conventionally 10-fold higher than human doses, due to some evidence that the rat fibrinolytic system is less sensitive than humans. However in some models, this dose causes high rates of recanalization that are not representative of the clinical response to thrombolysis. An appropriate "human equivalent" tPA dose that models the clinical response and allows room for improvement with sonothrombolysis is not known.

Aims: The overall aim of this thesis was to test sonothrombolysis (± microbubbles) for recanalization in different models of stroke in rats. The individual aims of the studies were: **1)** To develop a model of MCA occlusion with PRC and to test for recanalization with sonothrombolysis and microbubbles (Chapter 2), **2)** To test the effect of sonothrombolysis with microbubbles on restoring microvascular patency after large vessel recanalization and to directly compare two microbubble formulations at high and low doses (Chapter 3), **3)** To develop a model of extracranial carotid artery occlusion with variable stenosis and to test sonothrombolysis for recanalization in this model (Chapter 4), and **4)** To compare different doses of tPA ranging from the clinical dose (0.9 mg/kg) to the conventional rat dose (10 mg/kg) in a model of carotid artery occlusion to identify a "human equivalent" tPA dose that mimics clinical recanalization rates (Chapter 5).

Methods: 1) Preformed PRC were injected via the extracranial carotid arteries to occlude the origin of the MCA. Treatment groups were sonothrombolysis with microbubbles (BR38), tPA alone, or control. Recanalization was monitored by laser Doppler flowmetry and macroscopic visualization of the cerebral vasculature post-mortem. **2)** Microvascular occlusion was achieved in a model of thread occlusion of the MCA with recanalization. Treatment groups were sonothrombolysis with SonoVue or BR38 microbubbles at full or half doses, tPA alone, or saline control. Patency of the microvasculature was assessed by micro-computed tomography. **3)** The carotid artery was crushed to injure the endothelium and stenosed with a ligature to induce local thrombosis. Following occlusion, the stenotic ligature was released for a model of mild stenosis, or left in place for a model of severe stenosis. Doppler flow was used to confirm occlusion and to monitor for recanalization. Treatment groups were sonothrombolysis or tPA-alone in both stenosis models. **4)** The mild stenosis model was used for testing the recanalization rates of different tPA doses, monitored by Doppler flow. tPA doses were: clinical dose (0.9 mg/kg), 2x the clinical dose (1.8 mg/kg), 5x the clinical dose (4.5 mg/kg), or the conventionally used rat dose (10 mg/kg).

Results: 1) No recanalization was observed in any treatment group (sonothrombolysis with microbubbles, tPA or control) in a model of MCA occlusion with PRC. **2)** Microvascular patency was restored by sonothrombolysis with microbubbles after large vessel recanalization, regardless

of microbubble formulation or dosage. tPA alone did not restore microvascular patency. **3)** High rates of recanalization were observed in a model of carotid artery occlusion with a mild stenosis, regardless of treatment: sonothrombolysis or tPA alone. No recanalization was observed in the severe stenosis model, regardless of treatment. **4)** The clinical tPA dose did not cause recanalization, and the conventional rat dose caused recanalization rates too high to mimic clinical recanalization in a carotid artery occlusion model. In this model, 1.8 mg/kg tPA (2x the clinical dose) more closely mimicked recanalization rates of clinical carotid artery occlusion.

Conclusions: My results raise doubts regarding the overall efficacy of sonothrombolysis (± microbubbles) as a recanalization therapy for stroke. In different models of stroke, I determined that characteristics of clot composition and vessel stenosis will affect the success of thrombolysis (and sonothrombolysis). PRC were found to be completely resistant to microbubble-enhanced sonothrombolysis. My results suggest that sonothrombolysis will not be beneficial for this subpopulation of stroke patients. The degree of carotid artery stenosis associated with an occlusion was found to be predictive of thrombolytic success. My study was inconclusive regarding sonothrombolysis for recanalization in a model of mild stenosis and occlusion due to high recanalization rates with tPA alone. Sustained recanalization with any thrombolytic or thrombolytic-enhancer is unlikely to occur in the presence of a severe stenosis. Sonothrombolysis with microbubbles restored microvascular perfusion after mechanical recanalization of the large vessel occlusion. This effect was regardless of microbubble formulation or dose, suggesting a class effect of microbubbles to enhance sonothrombolysis, rather than individual microbubble properties or concentration. With recent evidence that reperfusion is a better predictor of good outcome than recanalization, microbubble-enhanced sonothrombolysis of microvascular occlusion is a potential approach to enhance the reperfusion clinically. Finally, the conventional tPA doses (human and rat) used preclinically, were found to be poor mimics of clinical recanalization rates in a model of carotid artery occlusion. For any thrombolytic-enhancer therapies, recanalization is an important outcome measure as it is the main mechanism by which tPA causes good patient outcome (by clot lysis and flow restoration). Using doses that mimic clinical recanalization rates may allow better translation of therapies. This finding should be considered for any future testing of thrombolytic-enhancers. Overall, this thesis presents data to indicate that sonothrombolysis (± microbubbles) is unlikely to be a suitable enhancer of tPA for large artery recanalization in stroke patients. However, there could be a potential for this strategy as an enhancer of reperfusion to improve patient outcome.