Identification of new approaches for the treatment of stroke

[Progress Report – Brain Foundation Gift]

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SUMMARY: Despite the devastating impact Acute Ischaemic Stroke places on global health, wellbeing and health economics, only a small proportion of patients benefit from current thrombolytic regimens. Whilst advances in mechanical clot retrieval have improved stroke outcomes, this procedure is limited to ~10% of stroke patients and is only available in specialised stroke units. The importance of developing innovative new treatments for stroke has been highlighted by the National Institute of Neurological Disorders and Stroke (NINDS), which has called for the "*urgent development of innovative new treatments that achieve rapid, complete and sustained cerebral reperfusion whilst minimizing the risk of hemorrhagic transformation*".

With this in mind, the overarching aim of this project is to develop novel therapies to improve clot lysis and prevent rethrombosis during thrombolytic therapy for acute ischaemic stroke.

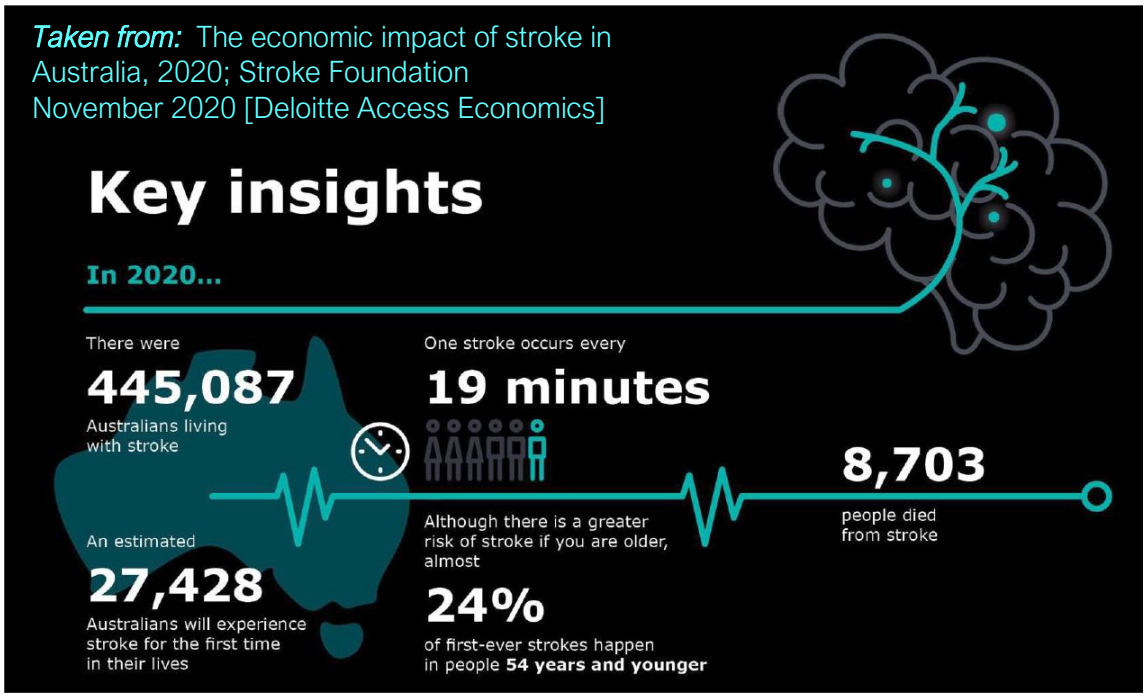
Limitations of thrombolysis: Drugs that degrade the blood clot (for example - recombinant tissue plasminogen activator or rtPA) are currently used to reopen blood vessels, and referred to as "*Thrombolytic therapy*". However, rtPA has significant limitations which undermine its clinical effectiveness. These include a low success rate in lysing clots in larger arteries, a tendency to promote clot reformation and vessel reocclusion, limited capacity to prevent obstruction in the cerebral microcirculation, and increased risk of life-threatening bleeding in the brain (intracerebral haemorrhage-ICH). Due to these limitations and strict eligibility criteria, rtPA is only administered in 13% of all stroke patients, and less than half of these achieve a good therapeutic outcome.

Addressing a significant limitation of thrombolysis: A significant problem associated with the administration of thrombolytic therapy is that, as a result of breaking down the blood clot, rtPA causes the release of clot-bound thrombin. Thrombin is a potent instigator and amplifier of clot formation, and plays a major role in promoting rethrombosis (reformation of dangerous blood clots) following successful thrombolytic therapy. This counterproductive action of rtPA is deemed responsible for the failure of tPA to achieve complete lysis, along with an unacceptable rate of re-thrombosis - both major factors limiting the success of thrombolytic therapy in coronary and cerebral vessels. While an obvious countermeasure for this would be the addition of an anticoagulant, all approved anticoagulants suffer from significant bleeding complications. To date, there are no thrombin inhibitors that can be safely used in stroke, due to their propensity to increase haemorrhagic transformation in the brain.

As a consequence, there is significant ongoing effort to identify safer anticoagulants that can enhance thrombolysis and prevent rethrombosis, without substantially increasing the risk of life-threatening bleeding.

Stroke "Facts & Figures" a 2020 update:

- Acute Ischaemic Stroke (AIS, stroke) is the third most-common cause of death and a leading cause of disability.
- Up to 85% of strokes are caused by blood clots that reduce blood flow to the brain.
- Thrombolytic therapy (aka –recombinant rtPA/rtPA) represents the mainstay of stroke treatment.
- rtPA is administered in an attempt to rapidly dissolve the clot and restore blood flow to the brain.



Progress Report: Following on from our identification of unique anticoagulant properties in a naturally occurring tick protein (Madanin-like 2, or MDL-2), we have commenced studies to characterise the safety and efficacy of this novel anticoagulant for improving stroke therapy. More specifically our immediate aims have been to determine whether MDL-2 alone and in combination with current standard of care stroke therapy (rtPA), can facilitate blood clot lysis in the absence of bleeding, thereby providing a safe alternative leading to improved stroke outcomes.

To this end – we have found that MDL-2 has the unique ability to block the clotting actions of thrombin without causing significant bleeding. Our initial studies have demonstrated that MDL-2 can be administered in mice *in vivo* (10-20 mg/kg), in vast excess of an effective anticlotting dose, without any significant impact on bleeding. Our recent studies and findings during the current funding period include:

1. Synthesis of a large batch of MDL-2 (our lead molecule) for use in *in vivo* studies. These studies have been performed in collaboration with Dr Xuyu Liu, head of the HRI *Cardiovascular protective signalling and drug discovery Unit*.
2. We have confirmed the inhibitory potency and binding mode of our lead molecule against its target enzume – thrombin – in both human and mouse. Our studies demonstrate a dose–response curve for inhibition of human α-thrombin by MDL-2 at a picomolar range, comparable to hirudin.

3. In further studies performed with Dr Liu, we have examined smaller fragments of MDL-2 to determine the shortest peptide required to retain safe anticlotting activity. These peptides, both full-length and fragments thereof, are in the process of being tested in vitro for anticlotting ability (efficacy), with the shortest fragment with effective anticlotting activity to be characterised for further preclinical development.
4. We have also demonstrated that MDL-2 in combination with current stroke therapy (rtPA) improves clot dissolution in vivo, compared with rtPA alone (see preliminary results in Figure 1 below). Following more extensive confirmation of these studies on clot lysis (thrombolysis), we will examine the ability of MDL-2 and active fragments to improve stroke outcomes, using our novel preclinical mouse model of stroke (recently submitted for publication).
5. We have lodged IP protection for MDL-2 and related molecules (*PCT/AU2017/051405: Thrombin inhibitors for treatment of stroke and related coagulative disorders*).

Outcomes and unanswered questions: Our preliminary findings thus far indicate that MDL-2 shows significant promise as a safer and effective anticoagulant *relative to other anticoagulants used in the clinic. Future studies are planned to confirm these findings in independent in vivo mouse models.* Ultimately, we aim to develop attractive candidate molecule to combine with thrombolytic therapies, to take through to Phase 1 Clinical trials.

Moreover, our ultimate aim will be to examine whether MDL-2 can be combined with our novel safe anti-platelet inhibitor AZD86482, which would represent the first demonstration of a safe triple therapy for use in stroke, with potentially significant implications for improving stroke outcomes.

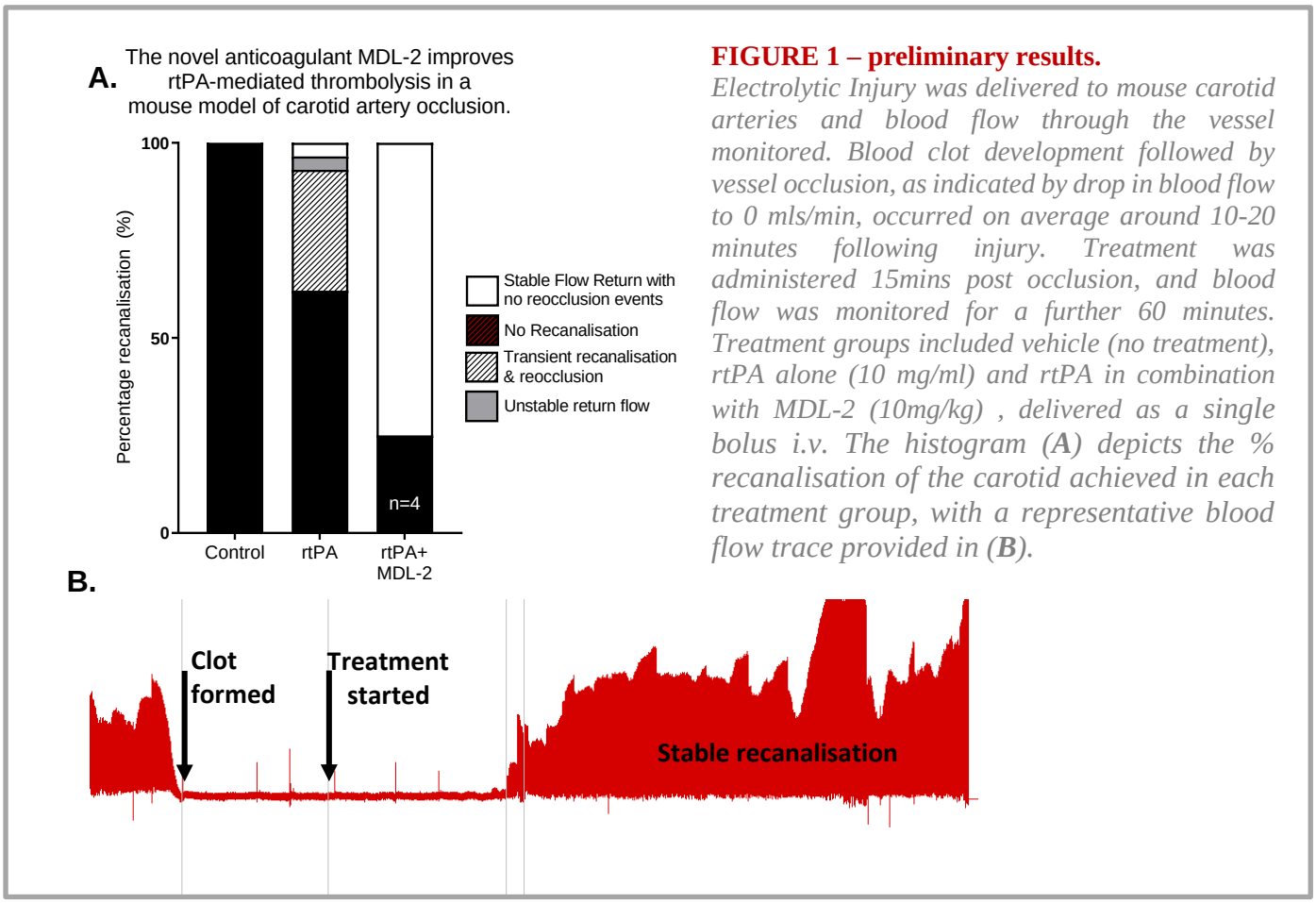


FIGURE 1 – preliminary results.
*Electrolytic Injury was delivered to mouse carotid arteries and blood flow through the vessel monitored. Blood clot development followed by vessel occlusion, as indicated by drop in blood flow to 0 mls/min, occurred on average around 10-20 minutes following injury. Treatment was administered 15mins post occlusion, and blood flow was monitored for a further 60 minutes. Treatment groups included vehicle (no treatment), rtPA alone (10 mg/ml) and rtPA in combination with MDL-2 (10mg/kg) , delivered as a single bolus i.v. The histogram (**A**) depicts the % recanalisation of the carotid achieved in each treatment group, with a representative blood flow trace provided in (**B**).*