PERSPECTIVE

Breaking out from the neuroprotective logjam: combined treatment with remote ischemic conditioning and minocycline in the prehospital setting

The only two treatments effective for acute ischemic stroke are reperfusion therapies. Despite testing of hundreds of neuroprotective agents and treatments, tissue plasminogen activator (tPA), approved by the Food and Drug Administration (FDA) in 1996, remains the only FDA-approved drug for the treatment of ischemic stroke. Recently, the Multicenter Randomized Clinical Trial of Endovascular Treatment for Acute Ischemic Stroke in the Netherlands (MR CLEAN) trial demonstrated that intra-arterial interventions (IA) with clot retrieval devices are effective in acute ischemic stroke patients with large artery occlusions at improving 3 month clinical outcomes if used within 6 hours of symptom onset (Berkhemer et al., 2015). More than ever, adjunctive therapies to these reperfusion strategies are needed to extend the time window to allow more patients to benefit. One way to extend the time window is to induce a protective phenotype in the brain and make the brain more resistant to ischemia. A second way is to augment cerebral blood flow (CBF), increase collateral blood flow and allow more time for large artery recanalization.

Although reperfusion is the goal of acute stroke therapy, reperfusion itself is a double edged sword and carries with it the risk of reperfusion injury. Despite return of blood flow in conducting arteries, the microvasculature may never reperfuse, the "no reflow phenomenon". Moreover, sudden return of blood flow in ischemic tissue increases oxygen free radical production with tissue damage. In the setting of myocardial infarction, reperfusion injury is thought to account for up to 50% of final infarct size. The ideal adjunctive intervention would BOTH extend the time window AND reduce reperfusion injury.

To break the logjam of failed agents and stroke clinical trials, new approaches and shifts in our thinking are needed. First, since "time is brain" and every neuroprotective stroke therapy developed to date is time-dependent, we should begin treatments in the ambulance, in the prehospital setting. The Field Administration of Stroke Therapy–Magnesium Phase 3 (FAST MAG) clinical trial, although negative with respect to the efficacy of magnesium, demonstrated the proof of principle that patients can be consented, randomized, enrolled, and treated in a clinical trial setting within 1 hour of symptom onset in the ambulance (Saver et al., 2014). However, not every intervention is amenable to the ambulance. Interventions to be tested and used in the prehospital setting have to meet certain criteria: they need to be safe and well tolerated, feasible to administer in an ambulance, and safe in both ischemic stroke and intracerebral hemorrhage (Saver et al., 2014). Even with CT scanners and physicians in ambulances (Mobile Stroke Units), the availability of safe and feasible treatments effective in ischemic and hemorrhagic stroke will be a major advantage.

Second, we should *combine agents and treatments* to target multiple pathways and cascades triggered by cerebral ischemia. It is unlikely that targeting one pathway will be effective in acute stroke. The Stroke Therapy Academic Industry Roundtable (STAIR) VII recommended that stroke therapy "focus on drugs/devices/treatments with multiple mechanisms of action and that target multiple pathways" and "target the network modules of integrated signaling pathways that subserve stress tolerance (including ischemic tolerance)" (Albers et al., 2011).

Third, new agents need be tested in combination with the standard of care, IV tPA. It is critical to ensure that that the intervention does not augment or interfere with tPA's fibrinolytic capacity leading to an increase in bleeding risk or resistance to clot lysis. More physiological animal models such as models with autologous clots should be used to test the interventions in combination with tPA (Hoda et al., 2012). In the MR CLEAN trial nearly 90% of the subjects were first treated with IV tPA (Berkhemer et al., 2015).

Fourth, we need safe and feasible interventions that can be used in a variety of clinical settings including the ambulance, in community hospital emergency departments, and in the helicopter during transport to large hub hospitals (**Figure 1**). In the MR CLEAN trial, an organized approach in the Netherlands was needed to funnel patients to larger hub hospitals with the capacity for IA interventions.

In light of these issues, we designed a preclinical study to model the clinical situation where patients are administered agents in the field and receive IV tPA or an IA intervention later, in the hospital (Hoda et al., 2014a). We administered tPA in the late time period of 4 hours, as one of our goals is to extend the window of tPA as few patients currently receive the drug due to late arrival to the hospital. We employed a thromboembolic clot model in middle aged mice with autologous clot that is more physiological than a suture occlusion model and allows testing of agents in combination with IV tPA.

Since the FDA requires each agent needs to be tested alone and in combination, we employed a 2 × 2 factorial design using remote ischemic perconditioning (RIPerC) and minocycline, where each intervention was tested alone and in combination. Remote ischemic conditioning (RIC) is the simple application of a blood pressure to cuff to deliver sublethal ischemia to a limb. Depending on the timing of the limb conditioning with respect to distant organ ischemia, the RIC is referred to as "preconditioning" if applied before ischemia, "perconditioning" during ischemia and "postconditioning" if applied after reperfusion. We also chose two treatments that each target multiple pathways but with minimal target overlap. RIPerC increases CBF and triggers an ischemia-resistant phenotype while minocycline inhibits PARP 1, microglial activation, peroxynitrite and MMP-9 (Hoda et al., 2014a). Minocycline has been shown to extend the time window of IV tPA and to reduce tPA-related hemorrhage, related to its inhibition of matrix metallopeptidase 9 (MMP-9) (Murata et al., 2008).

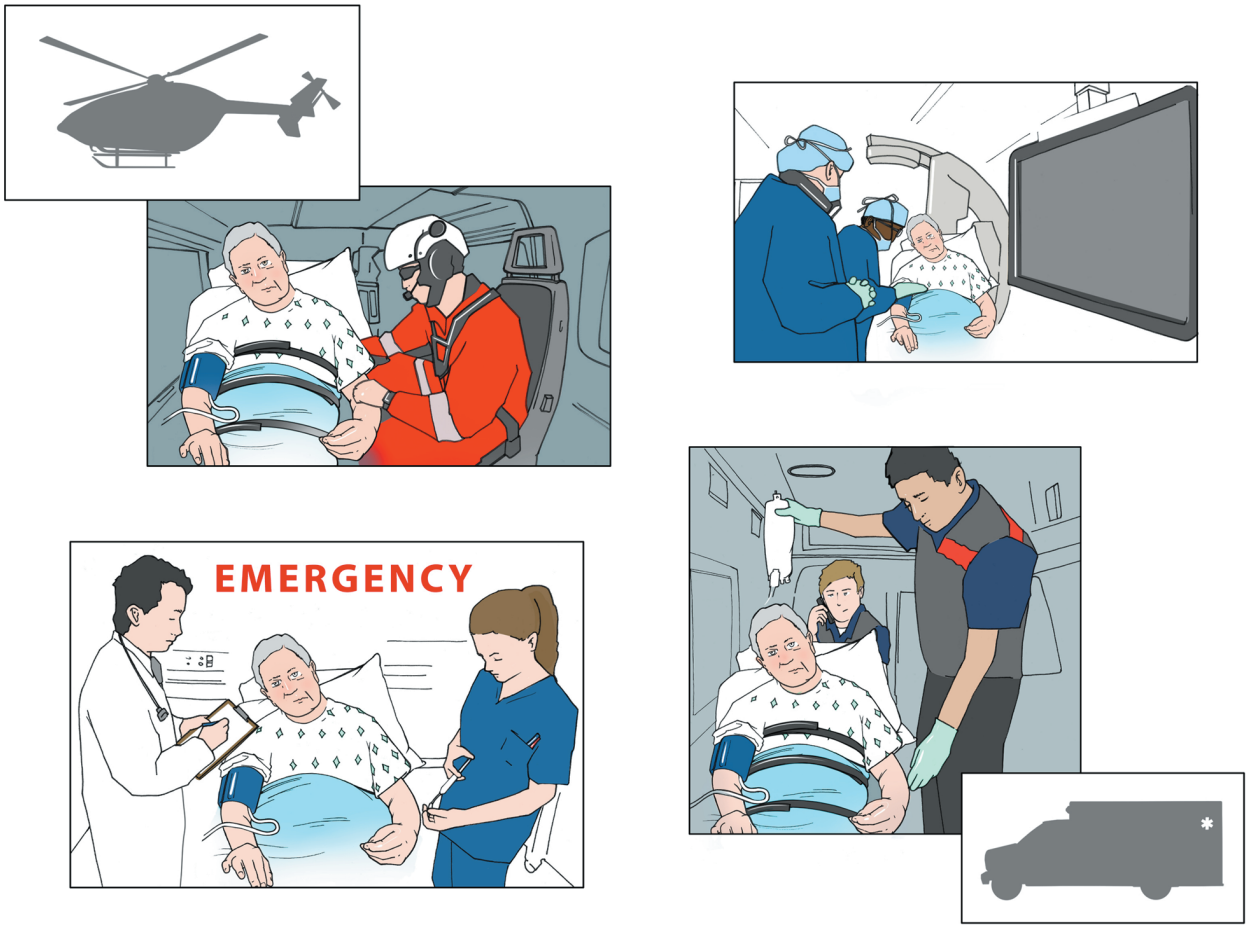

Figure 1 Depiction of use of remote ischemic conditioning in multiple clinical settings.
During transport in the helicopter to a "hub" hospital (top left); during an intra-arterial clot retrieval procedure (top right); in the ambulance (bottom right); and in a community hospital emergency department (bottom left).

We found that minocycline delivered at 1 hour and RIPerC started at 2 hours were effective alone and in combination in a mouse thromboembolic clot model (Hoda et al., 2014a). This efficacy was seen in both groups of mice treated with tPA at 4 hours and mice that did not receive IV tPA. The treatments were additive in reducing infarct size and showed trends to improve short term functional outcome. There was no statistical interaction between minocycline and RIPerC treatments indicating that the effects of RIPerC and minocycline (MINO) were additive; that is, they did not interfere with one another and they were not synergistic on the outcome measures. As we have seen in our other preclinical studies (Hoda et al., 2012, 2014b), RIPerC increased CBF in both animals treated with IV tPA and those not treated with tPA.

Both interventions are safe, feasible, inexpensive and ideal reperfusion agents. With its long half-life of about 24 hours in humans, minocycline was safe and well tolerated in early phase clinical trial in acute ischemic stroke and can be dosed once daily (24-hour intervals) and be given in a one hour infusion in the prehospital setting (Fagan et al., 2010). There are few exclusions for minocycline allowing a wide range of patients to benefit. Minocycline has also been shown to have activity in preclinical studies in intracerebral hemorrhage (ICH) (Zhao et al., 2011). RIPerC has already been shown to be safe and feasible in the prehospital setting in the ambulance in both STEMI and acute stroke trials with a simple blood pressure cuff (Hougaard et al., 2014). In the Danish prehospital stroke trial, RIPerC was safe in patients with ICH (Hougaard et al., 2014). The application of RIPerC is made even easier in the ambulance, emergency departments (ED), and even home setting by availability of an automated cuff (AutoRIC, Cell Aegis, Toronto, Canada; Doctormate, Beijing Institute of Renqiao Cardio-cerebrovascular Disease Prevention and Control, Beijing, China) with the simple push of a button to deliver preset cycles of 5 minute inflations and deflations.

The results of the MR CLEAN trial and the likelihood of success of other trials using the newer stent retrieval devices will lead to a further push for increased IA interventions with the need to select and transport patients for these interventions to regional centers. During the transfer to regional stroke centers, RIPerC can be easily applied. Once the patient arrives to the interventional suite, RIPerC can be applied peri-procedurally (**Figure 1**). RIPerC has been tested with percutaneous coronary interventions (PCI) and coronary artery bypass grafting (CABG) in the cardiology field demonstrating the feasibility and safety of this approach. A

recent meta-analysis of RIC in clinical situations of myocardial ischemia-reperfusion injury shows a benefit at reducing myocardial injury and a significant reduction of mortality and long term major cardiac and cerebrovascular events (Le Page et al., 2015).

It is imperative that RIPerC be tested with pre-clinical rigor. RIPerC is effective in males and females and multiple labs have now shown RIC to be effective in acute stroke (Hoda et al., 2012; Hess et al., 2013). One concern is the use of RIC in patients with diabetes. Both diabetes and drugs used to treat diabetes such as sulfonylurea agents may interfere with RIC (Hess et al., 2013). It is also important to determine whether additional dosing and "postconditioning" with daily or twice a day RIC in the hospital may add further benefit. These issues can be addressed in pre-clinical models and early phase clinical trials. We have only tested PIPerC in combination with tPA out to 4 hours in our preclinical models. Further pre-clinical studies are needed to determine if that window can be extended even longer.

RIC likely works by multiple mechanisms. One of the key effects of RIC is improvement of CBF. In our preclinical studies, we have shown that RiPerC increases CBF (Hoda et al., 2012, 2014b). Imaging biomarkers are important for the development of new stroke therapies. Use of newer imaging modalities such as MRI ASL may serve as imaging biomarkers for the effect of RIC.

Is RIC ready for clinical trial in the prehospital setting in acute stroke? The scientific premise is strong: 1) There is robust pre-clinical data in rodent stroke models; 2) there is extensive safety and tolerability data and hints at efficacy from cardiovascular trials; 3) RIPerC is feasible in the ambulance in acute stroke; 4) it is safe in intracerebral hemorrhage. Hougaard et al. (2014) randomized acute stroke patients in the ambulance in Denmark to RIPerC or no treatment and then performed multimodal MRI imaging with DWI and perfusion on patients receiving IV tPA in the hospital. While the primary outcome of penumbral salvage was not positive, a MRI voxel analysis demonstrated that RPerC during ambulance transportation increased tissue survival, suggesting that pre-hospital RIPerC may be neuroprotective. In this trial, fewer than half the patients randomized to RIPerC received the full conditioning regimen of 4 cycles of 5 minutes and most of the strokes were mild with low baseline median National Institutes of Health Stroke Score (5), making it difficult to show an effect. Using an automated cuff in future clinical trials will make it easier for ambulance and ED personnel.

Some may question whether a 2 × 2 factorial design with RIC and minocycline is a "bridge too far". Certainly, substituting other interventions should be considered. However, with a 2 × 2 design, it will be important to test these agents in combination in a pre-clinical study before they are combined in a clinical trial to determine whether they interact and are safe in combination. An alternative design would be to test multiple agents in the field and use a pooled control group, and not combine the agents together, a design known as multi-agent, multi-stage (MAMS). This would avoid the problem of potential interactions between the treatments. To move the stroke field forward, the time has arrived for innovative designs.

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